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BOOK OF PROCEEDINGS

XV International Scientific Agriculture Symposium
"Agrosym 2024"
Jahorina, October 10-13, 2024

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PREFACE

Dear colleagues,

It is with great pleasure that I introduce the *Book of Proceedings* for the 15th International Scientific Agricultural Symposium "AGROSYM 2024." I trust that you will find it a valuable resource in your work. This year, around 800 contributions have been accepted for inclusion in the *Book of Abstracts*. The themes of AGROSYM 2024 span the full spectrum of agricultural sciences, divided into seven key sessions: 1) Plant Production, 2) Plant Protection and Food Safety, 3) Organic Agriculture, 4) Environmental Protection and Natural Resource Management, 5) Animal Husbandry, 6) Rural Development and Agro-Economy, and 7) Forestry and Agroforestry.

There is growing consensus among scholars and practitioners that technological innovations have the potential to boost agricultural production, enhance supply stability, and reduce the environmental impact of farming. Technology has been especially pivotal in increasing the productivity of annual crops such as maize, rice, soybeans, wheat, and cotton. However, with trees having longer growth cycles, breeding programs for these crops require more time. New plant breeding techniques promise improvements in productivity, but they have sparked an ongoing academic debate regarding their advantages and drawbacks.

While much of the focus in the past has been on the production side of agriculture (e.g., productivity and efficiency), there is an increasing emphasis today on the consumption side and the intermediate stages of the food chain, such as processing and distribution. This shift is driving the transition toward a "farm-to-fork" approach. Globally, consumers are sending clearer signals about their preferences for higher quality, healthier, safer, and more flavorful products. In response, many agri-food companies are exploring innovative ways to gain greater control over production processes and ensure the quality and safety of final products. Furthermore, changes in investment strategies hold the potential to reduce the environmental and social costs of agriculture. It has become increasingly clear to investors that companies that prioritize sustainability and social responsibility tend to yield better long-term returns.

Agri-food systems are at the heart of global discussions surrounding sustainable development and the achievement of the United Nations Sustainable Development Goals (SDGs) by 2030. These systems are deeply connected to numerous global challenges, including climate change, poverty, food insecurity, biodiversity loss, resource depletion, and ecosystem degradation. A key goal of the sustainable agriculture movement is to create farming systems that mitigate or eliminate the environmental harms associated with industrial agriculture. Additionally, it is critical to improve the resilience of food systems to crises, shocks, and pandemics.

I would like to extend my heartfelt thanks to all the authors, reviewers, and colleagues who contributed to the editing of the *Book of Abstracts*. Special thanks are due to our co-organizers and partners for their unwavering collaboration and comprehensive support throughout this endeavor.

Dušan Kovačević

East Sarajevo, 10 October 2024
Prof. Dušan Kovačević, PhD
Editor in Chief, President of the Scientific Committee of AGROSYM 2024

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PLANT PRODUCTION

CROP PRODUCTION: CURRENT STATE AND CHALLENGES, ADVANCES AND PROSPECTS TO BALANCE ENVIRONMENTAL, SOCIAL AND ECONOMIC IMPERATIVES

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Abstract

Although current crop production trends are keeping pace with population growth, climate change challenges agriculture to maintain that food production. This challenges agriculture to develop new varieties whilst balancing yield gains with environmental, social and economic imperatives. Some examples of progress in breeding and technologies are presented here. Last century, AE Watkins collected 827 wheat landraces and old varieties. These are finally all sequenced, revealing around 70% unique haplotypes, which will considerably improve our ability to feed the world as it gets hotter, and agriculture comes under increasing climate stress. The ability now to sequence crop genomes rapidly and generate 'infinite' numbers of DNA markers for genetic maps, makes precise location of genomic regions regulating key genes much easier. A wheat doubled haploid population from Chinese Spring x SQ1, with a dense genetic map has been used to study genetic control of yield and many other traits from multi-year x location trials. We now have a candidate gene (TraesCS2B03G1460600) for yield on chromosome 2B. Novel advances can now make agricultural land more productive by combining crop production with solar panels capturing sunlight, and soil and plant sensors, such as *in planta* nitrate probes, are playing major roles providing farmers with decision-support systems. However, policymakers now have conflicting priorities - what is best for society? Thus, do we need more food or more biogas? We have a new Horizon Europe project Hort2thefuture and to encourage growers to adopt our project innovations, we have a behavioural change Work Package, which targets policymakers.

Keywords: *Crop production, Climate challenges, New technologies, Conflicting priorities.*

Introduction

The world population is currently increasing at around 0.9% per year, equivalent to around 70 million people per year. Although the trend in annual population growth is slowly decreasing, and is predicted to be only 0.47% by 2050, that still represents a population increase from around 8.2 billion in 2024 to 9.7 billion in 2050 (https://www.worldometers.info/world-population). Is crop production currently sufficient to meet this predicted population growth? According to a detailed recent OECD-FAO report (OECD/FAO, 2024), the increase in global food production is predicted to continue exceeding the increase in population for the next 10 years, with overall crop production projected to grow at an annual rate of 1.0%. Examples of trends in crop yields per hectare over the last 60 years are shown in Figure 1 for cereals and for tomato. While increases in cereal yields for developed countries (Fig 1a), such as the United Kingdom, have largely levelled off in recent years, yields for lower- and middle-income countries are continuing to show substantial percentage increases, though starting from a low baseline of only around 1 t/ha. A similar picture is evident for horticultural crops such as tomato (Fig 1b), though the impact of high-intensity production technologies on increasing yields in developed countries, such as the Netherlands and United Kingdom, from the 1980s to the new millennium is strikingly evident.

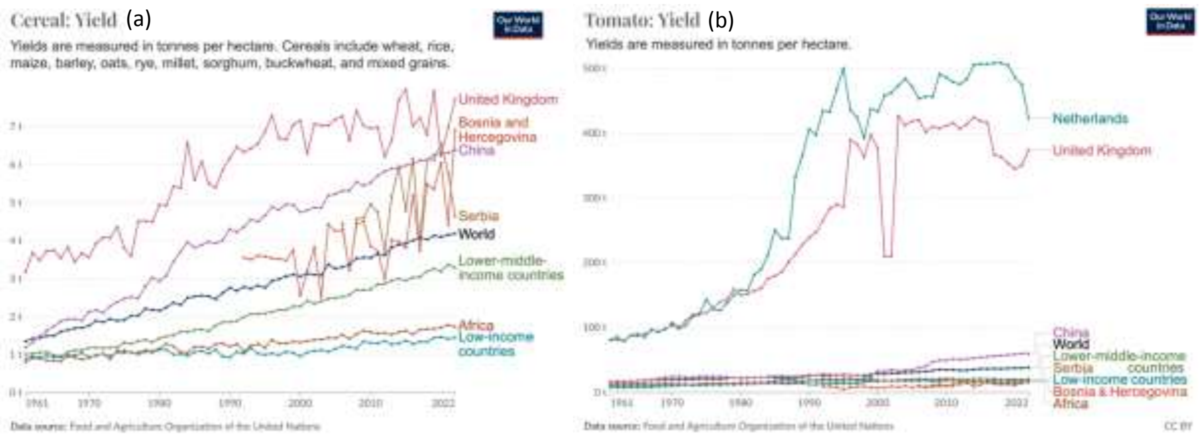

Figure 1. Changes between 1961 and 2022 in cereal yields (a) and tomato yields (b) in tonnes/hectare for selected countries and world production taken from https://ourworldindata.org/crop-yields.

According to the OECD-FAO report, future growth in production is expected to be driven mainly by yield gains in low- and middle-income countries, assuming the continued development of more intensive production systems. For example, while growth in food production in Europe and Central Asia is predicted to be only around 7% over the next 10 years, it is predicted to be over 25% in Sub-Saharan Africa and India. Projections suggest that 80% of global crop production growth will come from improvements in yields, as the long-term decline in agricultural land-use is expected to persist.
Crop yields in a given environment can be described in general terms by Figure 2. Here, traditional varieties give lower yields and respond little to increasing inputs, whereas modern varieties have inherently better yield potential and respond better to inputs.

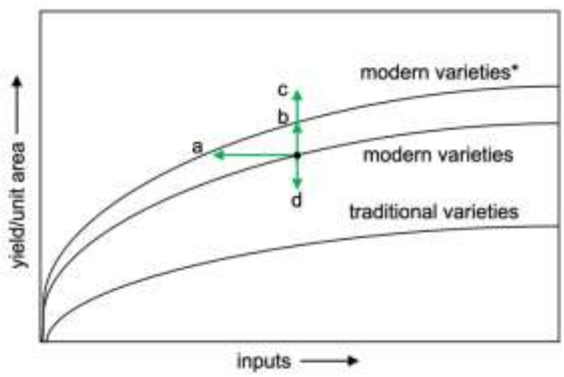

Figure 2. The relationship between crop inputs and yield per unit area for traditional and modern varieties.
* represents maximum yields attainable under current optimum technical and climatic conditions.

The original Green Revolution improved crop production by developing varieties more responsive to inputs, and the following years saw crop production increases mainly through increasing inputs, though with diminishing returns. Crop production now faces four scenarios in the coming years, represented in Figure 2 by the four arrows **a-d**. More recently, the emphasis, especially in the more developed world, has been on improving technical efficiency, replacing yet higher input use with improved information and managerial skills (drone technology to monitor fertiliser needs, for example), thereby moving towards line **b** (yield increases with existing crop varieties and inputs). Alternatively, with appropriate incentives or regulations (e.g. on input pollution) coupled with improved monitoring of input use, farmers may follow line **a**, reducing inputs, but using them more efficiently so that yields are not sacrificed. Thirdly, breeders are constantly striving to improve the yield potential of new varieties (line **c**), allowing farmers to benefit from genetic improvements.

However, aspects of climate change risk imposing serious obstacles on our ability to maintain the necessary increases in crop production, with the risk that farmers' yields in the future will follow line **d**. Climate change will increasingly impact crop yields through increased variability of both temperatures and rainfall. These are likely to lead to lowered efficiency in the uptake of inputs, resulting in lower yields. We are already witnessing an increasing frequency and severity of extreme weather events, such as droughts, floods, heat waves and storms, leading to localised soil erosion and, in some areas, increased soil salinity. While no amount of plant breeding or new technologies can insulate farmers from the devastations of severe flooding events, or soil erosion, predicted changes in crop water availability and temperature can be addressed by the scientific community, to reduce the impact of climate change on line **d**.

Some examples of recent progress with this, considering both genetic gains in future crop varieties and technologies to improve crop production, are described in the next two sections.

Advances in plant breeding

Increases in crop yield per hectare rely on a combination of improved agronomy and improved genetic potential. Traditionally plant breeding relied on 'crossing the best with the best and hoping for the best!'. Since those days, a revolution in molecular genetics has significantly affected the 'hoping for the best' aspect of plant breeding. Breeders are now able to identify relatively easily and quickly specific alleles of specific genes that will help to improve one or other aspect of crop performance, such as disease resistance, or quality.

Thus, the key to being able to exploit the huge wealth of genetic variation amongst agriculture's major crops and wild relatives is the rapid development of DNA sequencing technologies. While it took the International Wheat Genome Sequencing Consortium (IWGSC) 13 years to sequence the large hexaploid wheat genome with a consortium of around 200 scientists from 64 countries (IWGSC, 2018), sequencing wheat varieties has now become routine using long-read sequencing platforms such as the Illumina NovaSeq sequencer. Indeed, long read sequencing has been called the "Method of the year" by Marx (2023): "To large-scale projects and individual labs, long-read sequencing has delivered new vistas and long wish lists for this technology's future."

Long-read technologies are transforming genome studies, with reads considerably over 30 kb now being claimed by sequencing instrument manufacturers. Marx (2023) predicts that this recent revolution in generating long reads of either DNA or RNA could one day lead to routine sequencing of entire genomes, transcriptomes and epigenomes at high throughput rates and accuracy. Furthermore, costs of sequencing are expected to come down. Researchers are progressing towards a "one box" capability, replacing the plethora of bioinformatics tools currently needed to order the reads into the complete genome. Other "wish-list" items being achieved are higher accuracy and saving time. Early sequencers had error rates as high as 30%, but, for example, Oxford Nanopore Technologies (ONT) sequencing platform now regularly achieves 92% accuracy and claim it has achieved close to 99% at a base level. Although it took 13 years to complete the first full sequence of hexaploid wheat, it took the John Innes Centre, UK (Adamski, Simmonds and Uauy, 2023, unpublished information) a matter of weeks to sequence one of the wheat parents of a population my group has used for many years for Quantitative Trait Locus (QTL) analysis (see below).

Here are just a couple of examples of the opportunities this revolution in sequencing technologies offers to plant breeders in the coming years.

The Watkins wheats

During the 1920s and 1930s the British geneticist Arthur Watkins collected 827 old land races and varieties of wheat from around the world, especially Europe, the Middle East, the Indian subcontinent, China, USA and Australia. With the advent of rapid DNA sequencing technologies, these old wheats have now finally been sequenced (Cheng et al., 2024).
They mapped variants in the Watkins wheat collection that are absent from modern wheats, using linkage disequilibrium-based haplotype analysis to identify that of 71,282 haploblocks, almost 70% (49,626) are Watkins-unique haplotypes (Figure 3). From accompanying phenotyping studies, many of the haplotypes restricted to phylogenetically isolated Watkins ancestral groups were found to have beneficial effects on yield potential, adaptation, human nutrition and disease resistance.

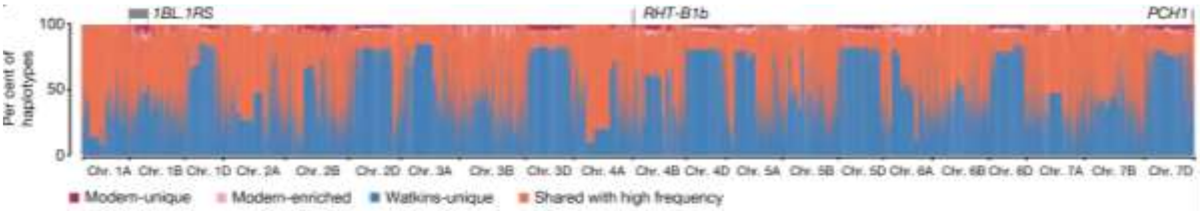

Figure 3. Genomic distribution and comparison of haplotypes between Watkins and modern wheats along the 21 chromosomes, including the proportion of haplotypes that are absent (Watkins-unique), shared with high frequency, modern-enriched or unique to modern wheat. Reproduced from Cheng et al. (2024) with permission.

Overall, their analyses of the genetic makeup of the Watkins collection revealed that modern wheat varieties make use of only 40% of the genetic diversity found in the collection. The Watkins wheats are full of useful variation which is simply absent in modern wheats. These lost traits are now being tested by plant breeders aiming to create new varieties that are made possible only because of the efforts of Arthur Watkins a century ago.
Thus, the results have provided a "gold mine" of opportunities for breeders to exploit the genetic variation revealed amongst these wheats. According to co-author Simon Griffiths "Essentially, we have uncovered a goldmine. This is going to make an enormous difference to our ability to feed the world as it gets hotter and agriculture comes under increasing climatic strain." These Watkins wheat collection resources, including germplasm, genomic and phenotypic data, are now publicly available through the Watkins Worldwide Wheat Genomics to Breeding portal (https://wwwg2b.com/).

Targeting a gene for yield

The ability now to sequence crop genomes rapidly, even the huge wheat genome (16 x rice), and generate an 'infinite' number of DNA markers for genetic maps makes precise location of genomic regions regulating genes of importance much easier. I have worked with a wheat mapping population of 95 doubled haploid lines from the cross Chinese Spring x SQ1 since 1991, and with support from many colleagues, we now have a dense genetic map to study the genetic control of yield and many other traits measured in multi-year and multi-location trials in the field and in pots (Quarrie et al., 2005, Czyczyło-Mysza et al., 2013, 2018, 2019). Nearly 6300 markers with known bp sequences have now been located on both the genetic and physical maps, allowing quantitative trait locus (QTL) analysis to locate genomic regions controlling yield with high resolution. Thus, many genomic regions influencing yield per plant have been identified to have around only 50 high-confidence genes. Furthermore, both parents of the mapping population have now been sequenced (Adamski, Simmonds and Uauy, 2023, unpublished information), and a gene database of SNP polymorphisms between

Chinese Spring and SQ1 has been created. This allows candidate genes for QTL effects to be readily identified.
An example of using the database of SNP polymorphisms to identify a candidate gene for yield per plant is shown in Figure 4. In collaboration with the team of Dragana Rančić at the Faculty of Agriculture, Belgrade University, who studied stem anatomy in the mapping population, a major QTL on chromosome 2B for number of stem vascular bundles was shown to be due to stem cell division traits, and was also coincident with QTLs from several other trials for the following traits: yield/plant, yield/ear, grains/spikelet, biomass/plant, biomass/stem and width of leaves 4, 5 and the flag leaf.

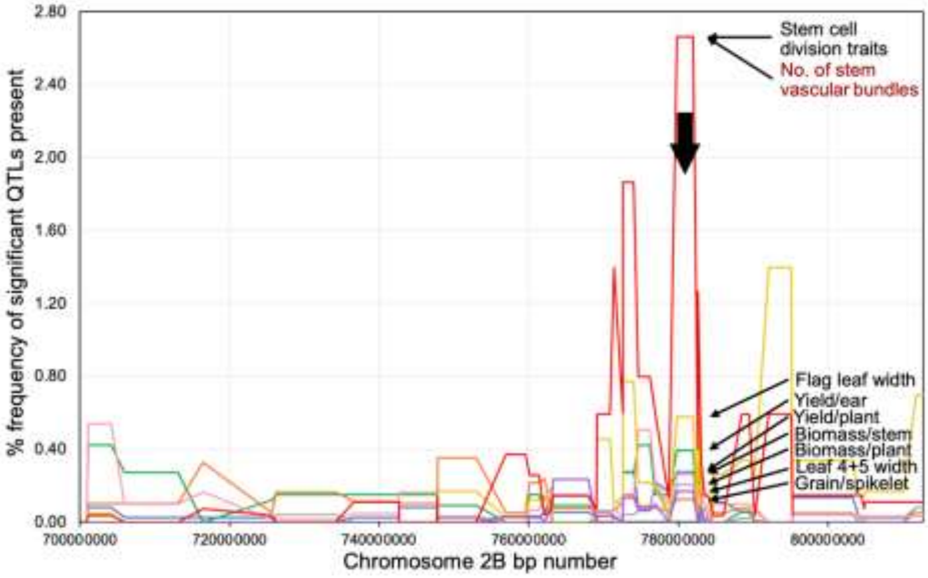

Figure 4. Percentage frequency (calculated as % of all QTLs identified for the trait on the genome) of significant QTLs present on a segment of chromosome 2B long arm (700,000,000 – 813,000,000 bp, Chinese Spring sequence v2.1), for several yield and related traits. Arrows point to the QTL region coincident across all traits.

Thus, the gene(s) responsible for modifying these traits likely affects meristematic cell division leading to differentiation of more leaf and stem vascular bundles, giving wider leaves, more vascular tissue in the stem, hence more biomass per stem and per plant, which would increase assimilate supply to developing ears, giving more grains per spikelet and hence, eventually, more yield per ear and per plant.
Marker polymorphisms on 2B showed this vascular bundle number QTL to be within a 4 MB region from 778594252 to 782573401, which encompasses 48 high confidence genes, of which 30 genes showed SNP polymorphisms between Chinese Spring and SQ1. One of those genes (TraesCS2B03G1460600, equivalent to TraesCS2B02G583800 of Chinese Spring RefSeq v1.1, with Gln→Arg at position 62/354) was identified to be a transcription factor with several gene ontologies consistent with effects on the traits coincident with the vascular bundle number QTL, including yield/plant, namely being located in the nucleus, DNA binding, transcription regulation, cell morphogenesis and differentiation.

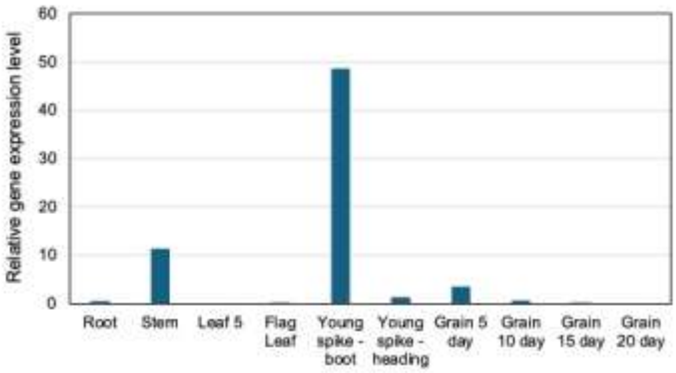

Figure 5. Relative gene expression level in several tissues of wheat at different stages of development.

Furthermore, expression of the gene (TraesCS2B02G583800), measured by Chi et al. (2019), was confined largely to the early stem and young spike at boot (Figure 5), which is consistent with QTL effects we found on the stem (vascular bundle differentiation) and the yield component grains per spikelet (Figure 4). The young spike at boot would be typically around 2 weeks before anthesis, at the time potential number of grains per spike would be determined (Kirby, 1988). Thus, these findings are consistent with TraesCS2B03G1460600 (TraesCS2B02G583800) being the functional gene for this effect on yield and other traits, though much more work would need to be done to confirm this.
The availability now of DNA technologies to sequence large genomes such as wheat relatively quickly, gives molecular biologists and breeders new tools and opportunities to identify genes and their favourable alleles to target yield improvements more effectively in the coming years. So, for wheat, the recent sequencing of the Watkins collection opens the way to more rapid progress in developing varieties which are better adapted to our climate change challenges.

Advances in technologies

Growing crops is becoming ever more sophisticated, with a host of new technologies providing decision support tools and all manner of air, plant, soil and water sensors to give farmers opportunities to increase productivity and reduce inputs. Here are a few recent examples.

Agrivoltaics
The railway from Cambridge to Ely in the UK (bottom right in Figure 6) passes alongside rows and rows of solar panels on what used to be prime agricultural land. These belong to AGR, a renewable energy company specialising in low carbon energy generation. Together with Clarke Energy (a company installing an innovative energy centre for supplying electricity and heat to the project), the glasshouse facility is being used to grow cucumbers. The Energy Centre for the glasshouse combines a heat pump system with a combined heat and power plant (CHP) comprising gas engines with CO_2 recovery. The heat pumps will provide renewable hot water heating to glasshouses and will recover renewable heat from local reservoirs (ponds) on site (Figure 6). The CHP is used to power the glasshouse, the LED grow-lights and various site auxiliaries. The waste heat generated from the engines is used as hot water to heat the glasshouses. The CO_2 recovered from exhaust gases is transferred to the glasshouse to help accelerate the growth of the crop. The glasshouse facility is expected to be able to grow 10% of the cucumbers consumed in Britain using 30% less CO_2 than conventionally heated glasshouses (https://www.clarke-energy.com/2021/agr-glasshouse-chp-heat-pump-project).

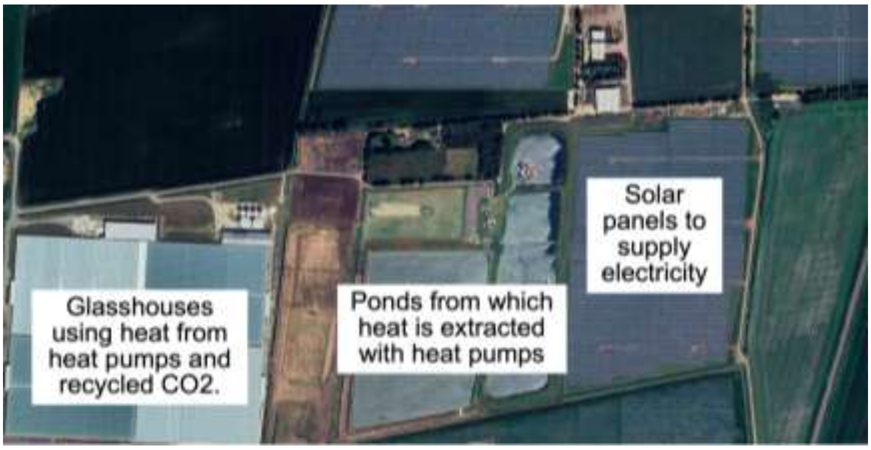

Figure 6. Google Earth image of the AGR facility using solar panels and heat pumps to grow cucumbers in glasshouses.

However, using agricultural land for solar panels does not inevitably mean that the land is lost from agriculture. A solar energy farm in Kosovo is using animals to keep the grass in check. A flock of sheep and goats is brought in twice a week to graze around and under the solar panels.

More sophisticated ways of using land for both solar energy and agriculture (agrivoltaics) are also available. Agrovoltaics is on the rise across France. Research is being carried in the north-east of the country by solar-energy company TSE. Here, solar collectors have been mounted high enough above the field to allow a crop to be grown and combined under the panels. The panels, which normally cover only about half the surface area of the crop, are able to rotate to follow the path of the sun and can also shift to a vertical position to let the rain pass. Horizontally, they can limit the damage of hail or make the ground temperature rise or cool a few degrees depending on the weather. The aim is to allow farmers to keep producing food while providing shade to protect crops from climate change, such as the very hot weather seen this summer across much of Europe, as well as providing renewable energy: (https://www.euronews.com/green/2022/10/19/french-farmers-are-covering-crops-with-solar-panels-to-produce-food-and-energy-at-the-same). A similar system of solar panels fixed at height above the crop is available from Fraunhofer ISE (https://www.ise.fraunhofer.de/en/key-topics/integrated-photovoltaics/agrivoltaics.html) in Germany. Another, less technically complex approach, particularly suited to temperate regions, is in trial by the company Next2Sun (https://next2sun.com/en/agripv) using vertical photo-voltaic panels. In this way, 90% of agricultural land is preserved, yielding more electricity than flat-inclined solar panels.

While agrivoltaics usually lead to slight reductions in crop yield, the economics can still be beneficial, and the topic of agrivoltaics has recently been reviewed extensively by Chopdar et al., 2024 and Garrod et al., 2024.

Sensors and tools

Sensing what is going on in the plant, soil and air and being able to apply that information through decision-support tools (DSTs) make it possible for farmers and growers to optimise input use by applying them where and when they are needed, thereby targeting lines **a** and **b** of Figure 2 (using inputs more efficiently to achieve the same yield and increasing yield towards the theoretical maximum for a crop variety).

More efficient use of fertilisers is a target for the current interest in developing sensors which monitor nitrate uptake and utilisation. Existing tools for probing nitrogen status *in planta* rely primarily on destructive and time-consuming methodologies, with low information content on the temporal characteristics of nitrogen uptake. Literature is accumulating on recent developments with nitrate-selective electrodes, and several sensing materials are summarised by Ibrahim et al., 2022. However, measuring nitrate concentrations continuously *in planta* has only just been demonstrated, using maize as the target crop plant (Ibrahim et al., 2022) and with a needle sensor giving a 200-2000 ppm nitrate concentration range. This allowed 10-h diurnal measurement of nitrate concentrations in a maize stem.

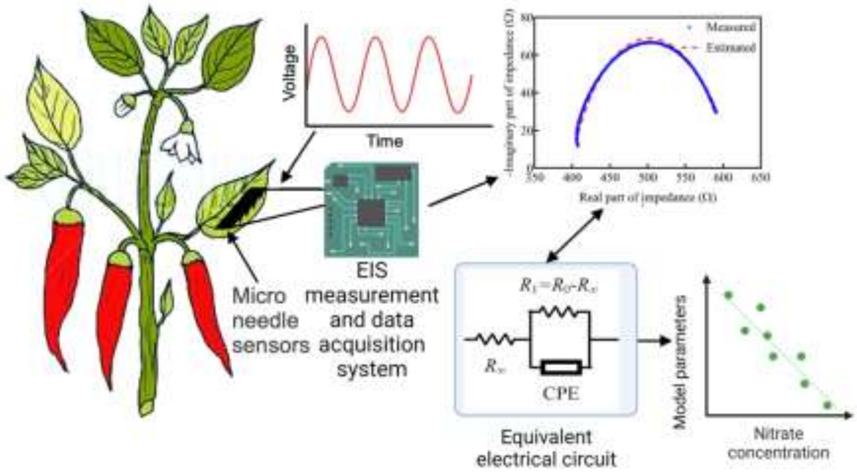

Figure 7. Design elements of an *in planta* probe to measure nitrate concentrations in pepper (*Capsicum annuum*) leaves with measurements based on electrochemical impedance spectroscopy.

Encouraging though this may be, *in planta* nitrate concentrations in horticultural crops, such as tomato and pepper, can exceed 4000 ppm (Magán Cañadas, personal communication), where the nitrate-selective electrode signal of Ibrahim et al. (2022) becomes asymptotic. To address this limitation, a Horizon Europe project (Hort2thefuture, https://hort2thefuture.eu/homepage-hort2thefuture/) plans to develop an *in planta* nitrate electrode allowing nitrate concentrations of pepper plants to be monitored. Design elements for this sensor are illustrated in Figure 7.

Effective use of such sensors requires the parallel development and application of DSTs, such as those recently reviewed by Zhai et al. (2020), Mabhaudhi et al. (2023) and Iakovidis et al. (2024). However, despite the plethora of such tools now available, their uptake by farmers and growers has been limited. Thus, even in the UK, Rose et al. (2016) estimated that of 359 DSTs available to farmers and advisers, only 49% of farmers used some form of DST, compared with 100% of advisers. Farmers found software (28%), paper-based (22%) and apps (10%) most useful, and 15 characteristics were found to influence effective uptake of DSTs (Figure 8).

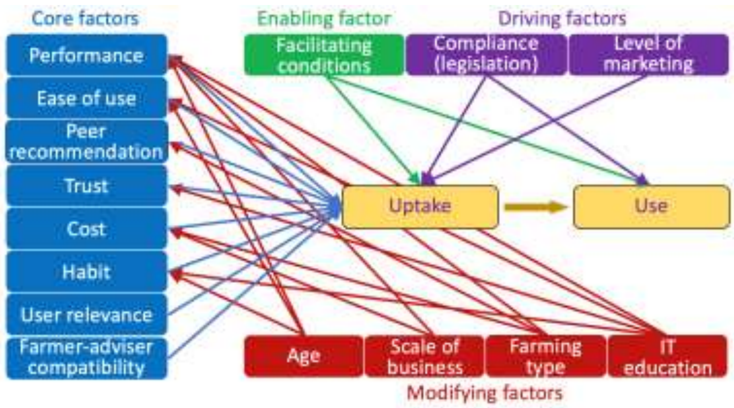

Figure 8. Theory of uptake and use of decision-support tools in agriculture. Redrawn from Rose et al., 2016.

Effective marketing of a new DST was, inevitably, a key determinant of tool uptake, and the authors proposed that instead of just focusing on designing DSTs that are easy to use, other important, but rarely highlighted, factors could be taken into account. Thus, the ability to help users satisfy legislative requirements via a DST was particularly helpful in encouraging uptake.

Balancing environmental, social and economic imperatives

The use of agricultural land by solar panels for the glasshouses growing cucumbers, shown in Figure 6, illustrates the conflict between using land directly to grow food and using that land for other purposes. Farmers will inevitably be tempted to use their land for the greatest economic benefit, so offering them state subsidies and lucrative contracts for anaerobic digestion of maize (https://www.soilassociation.org/media/4671/runaway-maize-june-2015.pdf) are encouraging farmers in the UK to cultivate energy crops, rather than food for either human or animal consumption. While the upward trend in meat eating is stagnating in high-income countries, expansion of maize production for animal feed to meet demand for animal feed is predicted to increase significantly in upper middle-income and lower middle-income countries over the next 10 years (OECD, 2024). Although the OECD report predicts that the intensity of agriculture’s global greenhouse gas emissions will decline overall by 2033, they will rise in lower and lower-middle income countries. This will be due largely to expansion of meat eating, where 1 kg of beef, for example, has a CO_2 footprint about 10 times that of soybean, and a soybean meat substitute (Herrmann et al., 2024). Policy makers need to decide where their country’s priorities lie. What is best for society as a whole? Do we need more food or more biogas, and should policies encourage a reduction in meat consumption? It is a very complex societal issue and every country will have difficult political choices to make in the years ahead, particularly in determining how agriculture could mitigate climate change.

Conclusions

The overall prognosis for crop production during the coming decade or so looks encouraging, with developments in DNA technologies facilitating breeding crop varieties with higher yields and better adapted to climate change, underpinned by an increasingly sophisticated supply of sensors and decision-making tools for farmers. However, the extent to which food production targets are met in the years to come will depend on farmers adopting these new varieties and practices (accepting behavioural change). Thus, projects such as Hort2thefuture (Figure 9) which targets reduced use of peat-based substrates and inputs as well as improved soil health in horticulture, supported by a new *in planta* nitrate probe and decision-support tools, includes activities to target behavioural change amongst its stakeholder groups – not only growers, but consumers and, most importantly, policy makers at the EU, national and local levels.

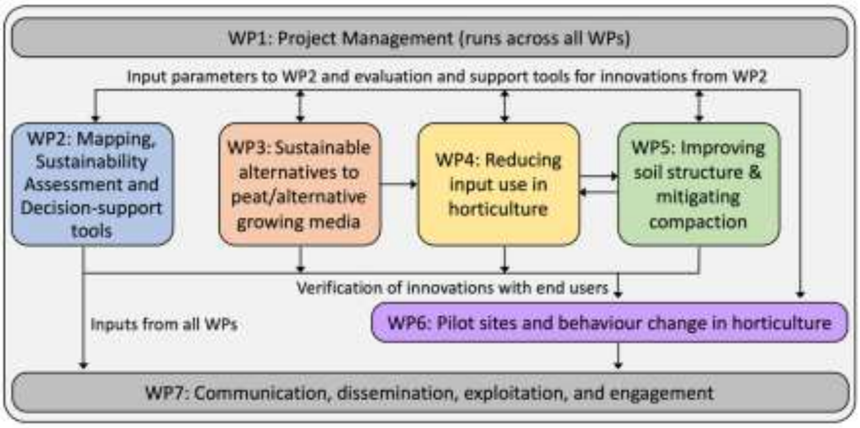

Figure 9. Relationships amongst Work Packages in the Horizon Europe Hort2thefuture project (2024-2028).

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GENETIC IMPROVEMENT OF DATE PALMS - PROTOPLASTS CULTURE OF TEGGAZA, DEGLET NOUR AND TAQUERBUCHT CULTIVARS

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Abstract

Somatic embryogenesis is an efficient method for large-scale date palm propagation, offering high multiplication rates and rejuvenation of plant material, facilitating consistent multiplication or the creation of new varieties. Indirect somatic embryogenesis induces callus formation in Taquerbucht, Teggaza, and Deglet Nour cultivars. This study aims to establish cell suspensions from embryogenic calli in date palms for protoplast isolation, with the goal of obtaining somatic hybrids resistant to Bayoud disease through protoplast fusion.

Embryogenic calli initiated cell suspensions in liquid medium, achieving proliferation within 1.5 months. Calli (100 mg) were fragmented into small pieces, transferred to 25 mL of liquid medium, and stirred at 100 rpm. Subculturing resulted in dissociation of calli and release of variously shaped cells. For protoplast isolation, the highest yield (5.87x106 protoplasts/g) was achieved using a 2% cellulase RS and 6% hemicellulase solution after 24 hours of incubation without agitation. Protoplast yield improved with 45 minutes of stirring and 10 hours of incubation in the enzymatic solution.Protoplast fusion was achieved using 50% PEG (w/v) with a molecular weight of 8000 after 20-25 minutes of incubation. Fused protoplasts were transferred to a regeneration medium to produce callus, somatic embryos, and plantlets.

Key words: *Date palm, cultivars, somatic embryogenesis, cell suspensions, isolation and fusion of protoplasts.*

Introduction

The date palm (*Phoenix dactylifera* L.), named by Linnaeus in 1734, is an ancient fruit tree cultivated for millennia for its highly nutritious and succulent fruit, the date (Abul-Soad, 2012). It was first domesticated around 10,000 years ago in Mesopotamia and Egypt, later spreading to the Maghreb countries by the fourth century (Abul-Soad, 2012).For populations living in Saharan oases, the date palm is a fundamental resource. It not only provides vital products but also conditions the existence of other agricultural resources in the oasis. Date palm cultivation holds significant economic importance in Arab countries, particularly in the Maghreb, where globally renowned cultivars such as „Deglet Nour“and „Madjhool“ are extensively grown.

Algeria's palm-growing heritage spans 169,380 hectares, with over 18 million palm trees producing approximately 1247.403 tonnes annually (FAOSTAT, 2023). Ranking third globally, Algeria contributes 14% of total date production and exports valued at $37 million (FAOSTAT, 2023). These economic figures underscore the crop's significance in both financial terms and for sustaining oasis populations.

However, date palm cultivation faces significant challenges, notably Bayoud disease caused by the fungus *Fusarium oxysporum* f. sp. *albedinis* (Malençon, 1949). Various strategies, including enhanced farming practices and biological, chemical, and genetic controls, are employed to mitigate Bayoud's impact and ensure sustainable date palm cultivation.

Genetic enhancement through biotechnological methods is recognized as the primary approach for conserving and improving the date palm heritage. In vitro enhancement via protoplast fusion of of susceptible and resistant cultivars holds promise as a solution to this challenge. Early advancements in somatic hybridization of date palm were pioneered by Gabr and Tisserat (1984), who successfully isolated protoplasts that formed calli but did not survive. Subsequent efforts by Chabane et al. (2007) achieved viable protoplasts that developed into microcalli and calli, although with a low fusion rate. Yatta et al. (2013) isolated protoplasts from „Deglet Nour“and „Taquerbucht“ cultivars. Badr-Elden et al. (2017) established a protocol for isolating protoplasts from „Medjool“ and „Khalas“ cultivars, regenerating them through somatic embryogenesis. More recently, Yatta et al. (2020) successfully regenerated protoplasts from cell suspensions of 'Deglet Nour', 'Teggaza', and 'Taquerbucht' cultivars.This study aims to establish a preventive strategy against Bayoud disease using chemical fusion facilitated by PEG.

Materials and methods

Plant Material Collection and Dissection

Samples from three cultivars (Deglet Nour, Taquerbucht, and Teggaza) were collected on March 28, 2019, from palm groves in Adrar and Touggourt cities in Algeria, weighing between 2.67 to 4.45 kg. Dissection involved removing the roots and woody bases of the fronds to reveal the white, tender heart.

- Deglet Nour: good date quality, Bayoud susceptible.
- Taquerbucht: average date quality, Bayoud resistant.
- Teggaza: fairly good date quality, Bayoud susceptible.

Callus induction and Cell suspension establishment

Shoot tips were cultured on MS medium supplemented with 45 g l^{-1}sucrose, 1 mg l^{-1}thiamine HCl, 100 mg l^{-1}myo-inositol, 100 mg l^{-1} glutamine, 170 mg l^{-1}monosodium phosphate, 100 mg l^{-1}potassium phosphate monobasic, 200 g l^{-1}ammonium citrate, 7 g l^{-1}agar, 2,4-D, N6-(2-Isopentenyl) adenine, and activated charcoal. Cultures were incubated in darkness at 28 ± 2°C for 16 hours.

For cell suspension initiation, 100 mg of callus was chopped into 1 mm pieces and placed in 100 ml Erlenmeyer flasks with liquid medium containing MS elements, sucrose, thiamine HCl, myo-inositol, glutamine, monosodium phosphate, potassium phosphate monobasic, Picloram, N6-(2-Isopentenyl)adenine, and polyvinylpyrrolidone (PVP). Flasks were shaken at 100 rpm in darkness at 27 ± 1°C for 6 to 10 weeks. Filtration through sterile sieves (380 µm, 250 µm, and 60 µm) was used to establish an embryogenic cell suspension.

Obtaining and Somatic Hybridization by Chemical Fusion with PEG

We used an enzymatic solution (Cellulase Onozuka RS and Hemicellulase) to isolate protoplasts from cell suspensions. For fusion, we employed PEG 8000 at 30% and 50%. PEG solutions were prepared in 100 mL distilled water with 0.5 mM $CaCl_2$, then sterilized by autoclaving and filtration (Table 1).After enzymatic maceration and purification, protoplasts are suspended in 12 mL of fusion solution containing 91 $g.L^{-1}$ mannitol and 74 $mg.L^{-1}$ $CaCl_2 \cdot 2H_2O$. In a sterile 25 mL tube, 5 mL of protoplasts from a Bayoud-resistant cultivar are mixed with 5 mL from a Bayoud-susceptible cultivar. A 300 µL aliquot of this mixture is placed in a 9 cm Petri dish and settled for 10 minutes. In another sterile 25 mL tube, 10 mL of PEG is mixed with 10 mL of the fusion solution. A 300 µL of this PEG solution is applied around the protoplast mixture for 15 minutes at room temperature. Protoplast fusion progress is monitored using an optical microscope.

Table 1.Preparation of PEG Solutions for 100 mL of Water.

PEG (8000)	30%	30%	50%
$CaCl_2$	14.8 g	14.8 g	198 mg
Distilled water	Normal	Lukewarm	Lukewarm
pH	/	5.9	7
Sterilization	filtration	autoclave	autoclave

Culture of Fused Protoplasts

A 0.5 mL aliquot of the fused protoplast suspension is spread onto Petri dishes with a nurse layer and incubated at 28 ± 2°C in the dark. Development is monitored every 24 hours under an inverted microscope (Olympus DP 70) to check survival, cell wall formation, cell division, and microcalli formation.

Results and discussion

Callus induction andCell suspension establishment

The study aims to obtain cell suspensions from embryogenic calli for protoplast isolation to produce somatic hybrids via protoplast fusion. Using 8 offshoots with 8 to 12 explants per cultivar, the explants swell and grow on the culture medium within the first four months, with some developing faster than others.Some explants develop small white nodular calli which, after isolation, become embryogenic strains. The first signs of proliferation are visible after 5 to 7 months. Cultures generally respond within 5 months to a year, producing 164 strains for Deglet Nour, 134 for Taquerbucht, and 159 for Teggaza. Proliferating calli are subdivided during subcultures based on size (0.5 to 4 cm). After 2-3 subcultures, the number of embryogenic strains increases to 180 for Taquerbucht, 212 for Teggaza, and 251 for Deglet Nour. Friable calli grow rapidly, doubling in mass in a month, while compact calli grow more slowly. The initial phase of culturing Deglet Nour, Taquerbucht, and Teggaza cultivars showed callus initiation on medium with 2,4-D and IPA, influenced by growth regulator type and concentration. This supports findings by Abul-Soad (2012) and Yatta El Djouzi et al. (2023) that Murashige and Skoog medium enhances callus initiation and guides morphogenesis. Callogenesis is preceded by significant explant volume increase (Mirani et al., 2021).

For cell suspensions, calli are transferred to a fresh liquid medium with 5 mgL^{-1} picloram and 1 mgL^{-1} N6 -(2-Isopentenyl)adenine (2iP). After one week, embryogenic masses detach from the aggregates, with particles 2-3 mm in diameter. Date palm cells multiply easily in liquid medium, but resulting suspensions have variable regeneration potentials. Subcultures develop bright to pale yellow embryogenic cell aggregates. Viability, evaluated by Fluorescein diacetate (FDA) under an inverted microscope (Fig. 1D), shows isolated cells and variable-sized cell masses. Compact textured calli produce aggregates of dividing amyliferous cells mixed with elongated non-amyliferous cells. These findings align with Al-Khayri (2012) and Yatta et al. (2013), who state that these cells are the starting point of viable suspensions. Embryogenic suspensions typically contain free elongated, oval, or round cells, or cell aggregates (Al-Khayri, 2012). The increase in cell numbers and aggregates, with maintained viability (80-99%), indicates the favorable action of the culture medium.

0,73 cm **A** | **0,90 cm** **B** | **0,91 cm** **C** | **22 µm** **FDA**

Fig. 1:Explant placed in culture on medium (A), Three-month-old explant on medium (B), Embryogenic callus on (C) , Morphology and viability assessment of different embryogenic cells using fluorescein diacetate (FDA) under an inverted microscope.

Isolation and Somatic Hybridization Testing by Chemical Fusion with PEG

To isolate date palm protoplasts, we followed established methods (Assani et al., 2002 ; Chabane et al., 2007; Yatta et al., 2020).Protoplasts were isolated from cell suspensions using an ES enzymatic solution consisting of 2% Cellulase, 6% Hemicellulase, 0.5% CaCl2, 3% KCl, and 0.05% MES. This enzymatic combination effectively removed cell walls, yielding high-quality protoplasts.The combination of 2% cellulase RS and 6% hemicellulase was found to release the highest number of protoplasts, as observed in studies on date palm (Chabane et al., 2007). However, it's noted that concentrations of cellulase exceeding 2% can lead to the digestion of the starting plant material (Badr-Elden et al., 2017).Microscopic observation after purification revealed isolated, perfectly spherical protoplasts of various sizes. These protoplasts were colorless, lacked visible chloroplasts, and had large vacuoles (Fig. 2 A). The highest yields were obtained with the Taquerbucht and Teggaza cultivars, indicating that protoplast morphology depends on both the plant material origin and the cell suspension quality.Protoplast culture requires sufficient isolation, with yield and viability rates depending on the base material (Badr-Elden et al., 2017). High yield and viability rates from cell suspensions make them ideal for protoplast production, aligning with findings by Chabane et al. (2007). Similar results were reported for rice (Jain et al., 1995).For protoplast fusion, we used polyethylene glycol (PEG). We performed heterofusions of:

- Deglet Nour x Taquerbucht protoplasts from cell suspension
- Taquerbucht x Teggaza protoplasts from cell suspension

Under the inverted microscope, PEG caused protoplasts to attract, adhere, agglutinate, and fuse in pairs or groups (Fig.2A-B). The high density and disordered aggregation of fused protoplasts made it impossible to determine the fusion rate.We observed the entire fusion process, which took from a few minutes to half an hour (Tab. 2 and Fig. 3 A-F).

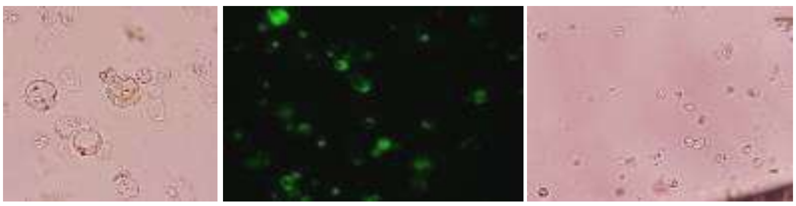

Fig.2:Protoplast suspension of cultivar Taquerbucht (A), Fusion of viable protoplasts stained with FDA using 50% PEG for 25 minutes of incubation (B), Protoplasts burst in 50% PEG after 30 minutes of incubation (C)

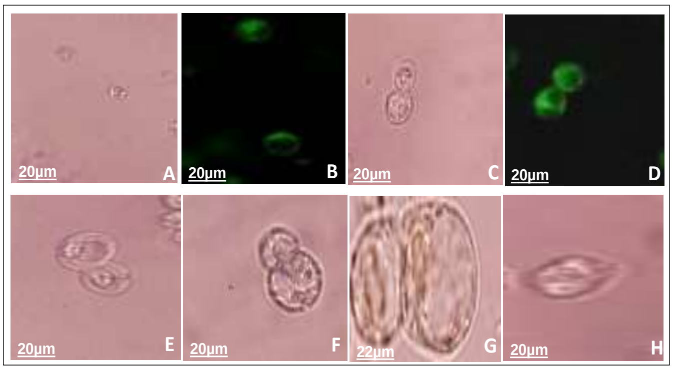

Fig.3. Fusion of TKB x DN cultivar protoplasts with polyethylene glycol: A, B, C, and D: alignment of two different protoplasts; E and F: beginning of one protoplast penetrating the other; G and H: two fused protoplasts.

The results are shown in Table 16. The application of 30% PEG solution did not initiate protoplast fusion. When this 30% PEG solution was adjusted with warm, sterile distilled water at pH 5.9, protoplasts began to align after 15 minutes of incubation. However, the protoplasts remained in this state without adhering or fusing.

Table 2. Effect of different PEG treatments on protoplast fusion (4 repetitions)

Incubation Time	PEG Concentration		
	30% SS pH	**30%, pH 5,9**	**50% pH 7**
15 min	No effect	Alignment	Alignment
20 min	No effect	No adhesion	Adhesion
25 min	No effect	No fusion	fusion
30 min and more	Protoplast death		

Observation of protoplasts treated with 50% PEG at pH 7 showed alignment. After 15 minutes, protoplasts adhered and fused after 25 minutes (Fig. 3 A, B, C). After 30 minutes of incubation, the protoplasts began to burst (Fig. 2C). Beyond this point, PEG becomes toxic, leading to protoplast death (Assani et al., 2002). High density and disordered aggregation made fusion rate evaluation difficult. Multi-fusion was also observed (Fig. 2B).

In Deglet Nour x Taquerbucht heterofusion, 86% of 15 Petri dishes showed no regeneration or embryogenic structures, likely due to aging cell suspensions and PEG treatment effects. Conversely, 33% of Teggaza x Taquerbucht heterofusion cultures developed. After five weeks, yellowish microcalli formed (Fig. 4A, B). The low division rate of protoplasts suggests PEG may disrupt mitotic activity (Assani et al., 2002). Electrofusion, using electric field pulses, yields more embryos and plantlets than PEG fusion (Guo and Deng, 1998).When transferred to germination medium, globular proembryos appeared after 6-8 months (Fig. 4C), developing into embryos. These embryos formed the first green leaf, crucial for developing into plantlets. Some calli necrotized or failed to develop into embryos (Fig. 4B).

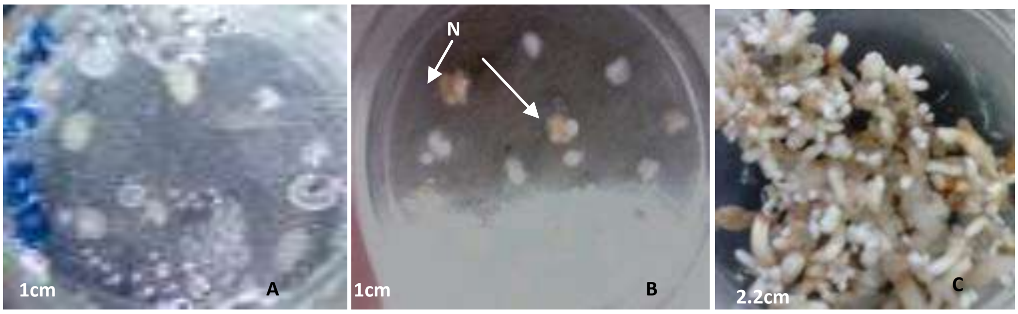

Fig 4. Evolution de la culture de protoplastes fusionnés et la cytologie des microcals issus de fusion. A: microcals issus de l’hétérofusion (Taquerbucht X Teggaza), B: multiplication des microcals issus de l’hétérofusion (Taquerbucht X Teggaza), C: obtention des proembryons.

Conclusion

Indirect somatic embryogenesis in date palm cultivars resistant and susceptible to Bayoud allows for the production of proliferating calli for protoplast isolation, although the PEG used for fusion results in a low rate of cell division. To improve culture and somatic hybridization, alternative methods such as cryopreservation, electric fusion, molecular characterization, and in vitro and greenhouse selection tests are recommended.

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AGRICULTURAL ADAPTATION TO CLIMATE CHANGE IN ALGERIA

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Abstract

Recent data indicates that global cereal production in July was around 7 million tonnes, bringing the total to 2792 million tonnes. This still marks a 0.6% decline from the 2021 figures. Agriculture's susceptibility to climate change is highlighted by the doubling of extreme weather events, such as heatwaves, droughts, floods, and storms, since the early 1990s, averaging 213 incidents annually from 1990 to 2016. These phenomena severely impact agricultural productivity, reducing food availability and income while increasing food prices. Climate change affects crops directly through heat and water stress and indirectly through changes in pest and weed dynamics. This research delves into the impacts of climate change on strategic crop production, with a focus on winter cereals and agricultural adaptation in Algeria. Various adaptation practices, such as new cropping systems, revised cropping calendars, and the introduction of resilient crop varieties, have been successfully implemented to mitigate climate change effects in Algerian drylands. This review synthesizes literature on climate change impacts and agricultural adaptations in Algeria, particularly concerning winter cereals. The insights aim to aid national policymakers in developing strategies for agricultural adaptation and ensuring food security.

Keywords: *climate change, winter cereals, adaptation, arid and semi-arid zones.*

Introduction

Climate change significantly impacts temperature and precipitation patterns, thereby alters the agro-climatic resources critical for crop growth and production (Fischer, 2015). In Algeria, the hot season, lasting about 2.8 months, features daily maximum temperatures exceeding 28°C, with peaks reaching 33°C and lows of 19°C. The cool season spans 4.1 months, with daily highs below 14°C and lows around 9°C (Weather Spark, 2021). Extreme temperatures, more than average temperatures, significantly affect vegetation growth, which only occurs within certain thermal limits—the minimum growth temperature and the maximum growth temperature (Bechikhi Kheira, 2010). Historical data from 1937 to 2009 show an increase in annual mean temperatures by more than 0.5°C, with the 1992-2002 decade being the hottest, witnessing a warming of about 0.7°C (Taibi and Souag, 2012). Additional studies by Nouaceur et al. (2013) identify four distinct periods of temperature trends from 1970 to 2012, characterized by varying incidences of cold and warm years. Regions such as the High Plateaus exhibit significant seasonal and regional temperature variations, with Mediterranean influences moderating temperatures in coastal provinces like Setif, Bordj Bou Arreridj, and Tissemsilt. Conversely, the steppe climate experiences extreme temperature fluctuations, exceeding 40°C in summer and dropping below 0°C in winter, leading to growth slowdowns and even vegetation destruction (Benchrif, 2011). Drought, defined as a significant water deficit compared to the norm for a specific period and region, presents a recurring challenge in Algeria (Benzerti and Habaieb, 2011). Climate constraints, such as water scarcity and uneven precipitation distribution, along with temperature extremes, adversely affect cereal crops. Historical analyses reveal periods of significant dryness in the early 20th century and a pronounced precipitation deficit from 1973 to 1992, intensifying from east to west (Aggoun Zoubida, 2022). Droughts impact crops differentially across developmental stages, delaying germination and potentially preventing crop emergence if water scarcity persists (Ghouer, 2006). During critical growth phases, such as late stem elongation and grain filling, drought severely affects yield components and overall productivity (Ghouer, 2006). Agricultural strategies must therefore address drought proactively, implementing measures to mitigate its impacts. Given these challenges, this paper aims to address the following research questions: What are the specific impacts of climate change on the production of strategic crops, particularly winter cereals, in Algeria? What agricultural adaptation practices can be implemented to mitigate these impacts? This study will explore new cropping systems, adjusted cropping calendars, and changes in crop varieties, assessing their effectiveness in enhancing the resilience of Algerian agriculture to climate change. By providing insights into these adaptation strategies, this review seeks to inform national policymakers and support efforts to ensure agricultural sustainability and food security in Algeria.

Impacts of Climate Change on Algerian Agriculture

Impacts Yield Potential

Climate change directly impacts agricultural yields, particularly for rainfed cereals, which are highly susceptible to drought. Insufficient moisture during germination delays crop emergence, while persistent drought can prevent it entirely. The most drought-sensitive period for Algerian cereals typically occurs between late stem elongation and the milk stage of grains, generally around April-May. During this phase, water deficits can significantly reduce yield components, such as tiller count, ear number, and grain size (Ghouer, 2006).

Simulations by Chourghal and Hartani (2020) on the phenology, water balance, and yields of durum wheat in Algeria reveal that climate warming shortens the vegetative phase by 31 days, negatively impacting dry matter accumulation and reducing yields by 36% under fixed sowing dates. Conversely, dynamic sowing dates, which allow for earlier planting, maintain yields by balancing the vegetative and reproductive phases more effectively.

Impacts of Agricultural Systems

Warming and reduced precipitation are shifting cultivated lands southward in Algeria. The southern limit for winter wheat cultivation has moved due to the presence of aquifers replenished by floodwaters from the Atlas Mountains, where annual precipitation can reach 350-450 mm. Studies indicate that despite limited recharge, these regions can achieve yields of 50 qx/ha compared to 18 qx/ha in the High Plateaus (MADR, 2023). Adopting multiple cropping systems, including no-tillage, direct seeding, and reduced tillage, can enhance total annual production in warmer climates. Transitioning from intensive tillage to simplified cultivation techniques improves soil porosity, structural stability, and water retention capacity (Moussadek et al., 2011). Studies indicate that direct seeding improves organic matter content and soil carbon levels, though the overall soil organic matter remains low, highlighting the need for increased organic input (Lahmar and Ruellan, 2007).

Climate change poses multifaceted challenges to Algerian agriculture, including reduced water availability, increased temperatures, and the frequency of extreme weather events. These factors collectively hinder crop productivity, particularly for water-sensitive crops like cereals. Analyzing historical climate data and crop yield trends reveals a clear correlation between rising temperatures, reduced precipitation, and declining agricultural outputs.

Adaptation Strategies

To counteract the harmful effects of climate change, Algerian agriculture must adopt robust adaptation strategies. These include changing sowing dates, using drought-resistant crop varieties and optimizing irrigation practices. Previously, early sowing could help crops avoid summer heat peaks (Fordyce, 2006), today, with climate change, late sowing of cereals can give satisfactory results. The period of its growth is limited by the water deficit at the beginning and end of the cycle combined most of the time with thermal stress (Chennafi et al., 2008a; Bouzerzour and Benmahammed, 2009). While advanced irrigation techniques can improve water use efficiency, ensuring that crops receive adequate moisture even during dry periods.

Crop Varieties

Developing and adopting new crop varieties that are more resilient to climate stressors is crucial. In northern Algeria, introducing stress-tolerant varieties has shown promise in mitigating yield losses. These varieties are better equipped to withstand high temperatures and water deficits, ensuring more stable production levels despite adverse climatic conditions. Varietal selection has taken on great importance in the cereal growing sector in Algeria, and there is numerous work in this area for the search for durum wheat varieties adapted to the semi-arid climate (Benlaribi et al. 1990). Other studies have focused on the modification of root growth dynamics in relation to the adaptation of varieties cultivated in semi-arid cereal zones (Benlaribi et al. 1990; Khaldoun et al. 1990).

Conclusion

The impacts of climate change on agriculture are essential to food security in Algeria. In this article, we examined the impacts on agricultural production associated with climate change and climatic hazards. We also mentioned recent research advances in Algeria, based on simulations of regional climate and crop models, as well as the exploration of adaptation measures. It is interesting to develop a more comprehensive assessment system for the agricultural impacts of climate change, which integrates cultural aspects, regional specifications and region-specific adaptation measures. Indeed, a system for evaluating the impacts of climate change on multiple aspects of agricultural production (potential productivity, adaptation measures specific to the region and comparative advantages and disadvantages according to crops) has been gradually established in Algeria. Climate change has radically modified the specific and temporal distribution of agro-climatic resources in Algeria. Environmental sustainability is becoming increasingly important, particularly in the arid and semi-arid growing areas of Algeria, due to an increase in potential evapotranspiration under a warmer climate. Weather and climate events have become more frequent and have caused serious damage to agricultural production, but a quantitative assessment is lacking. Most studies focus on a single aspect of an agroecosystem. A more systematic view of agroecosystems is needed to achieve multiple goals in food security and environmental sustainability. Adaptation plays an essential role in mitigating the impacts of climate change on production in the different growing areas of Algeria. Crop calendar, variety, cropping system, agronomic technique and field management must be adjusted to maintain production in the face of climate change. In regions where thermal conditions have improved thanks to warming, there is potential for agricultural intensification. Given the importance of local characteristics in agriculture, a more systematic simulation model of agricultural production and adaptation covering different growing areas across Algeria is crucial. Algeria, faced with the growing challenges of climate change, must implement agricultural adaptation strategies to maintain crop productivity and ensure food security. The combination of improved cropping systems, optimized sowing dates and adapted crop varieties can mitigate the impacts of climate change. The results of this study provide a basis for policy and practical interventions aimed at strengthening the resilience of Algerian agriculture, while providing guidelines for farmers and national policymakers.

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FACILITATING HYSSOP INTRODUCTION TO ARMENIA: ASSESSING GLOBAL GENEBANK RESOURCES AND CULTIVATION POTENCIAL

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Abstract

Climate change presents significant challenges to agricultural sustainability and biodiversity, particularly affecting spice plants valued for their aromatic and honey-producing qualities. Introducing new species and varieties of these plants holds promise for enhancing rural development through their aromatic properties and support for beekeeping. In Armenia, *Hyssopus officinalis* L., commonly known as hyssop, is recognized as a promising yet underutilized species. There is a current imperative to explore and evaluate the adaptive and productive potential of breeding varieties of Hyssopus for introduction in Armenia. To facilitate the adoption of modern varieties, comprehensive research was conducted on seed collections from various regions. This involved assessing available varieties and potential lines, evaluating accessibility to germplasm, and gathering characterization and evaluation data. To locate significant hyssop collections worldwide, and to determine the number of accessions held by various seed banks for facilitating the introduction of hyssop varieties to Armenia for study and cultivation, various online platforms were reviewed. These included international information systems and databases accessible through national gene banks. Additionally, field studies were conducted under valley conditions in the Republic aimed to assess the biological and agronomic characteristics of these plants. These efforts collectively aim to ensure the effective and well-founded introduction of modern breeding varieties.

Keywords: *inventories, origin, distribution, agronomic and biological parameters.*

Introduction

Climate change presents substantial challenges to the sustainability and productivity of agricultural systems and biodiversity, impacting diverse spice plants renowned for their aromatic and melliferous properties (Kumar et al., 2022). Introducing new species and varieties of spice plants can significantly boost rural development by tapping into their potential as aromatic additives and in beekeeping (Torre et al., 2023). One such promising yet underutilized plant in Armenia is *Hyssopus officinalis*, commonly known as hyssop. There remains a gap in understanding the productive and melliferous potential of breeding varieties of hyssop in Armenia, including the analysis of functionally important components under introduction conditions.

Hyssopus officinalis, commonly known as hyssop, is a perennial herbaceous plant belonging to the Lamiaceae family. It is native to the Mediterranean region and parts of Central Asia, but it has been naturalized in various regions worldwide due to its medicinal and culinary uses (Tobyn et al., 2011). Historically, hyssop has been revered for its therapeutic properties and has been mentioned in ancient texts for its medicinal virtues (Bespalyko et al., 2016).

Hyssopus officinalis holds significant importance both culturally and scientifically. It has been traditionally used in herbal medicine for its antiseptic, expectorant, and cough-relieving properties (Fathiazad and Hamedeyazdan, 2011). The herb is also valued in culinary practices, adding a distinctive flavor to soups, salads, and herbal teas. Moreover, *Hyssopus officinalis* is known for its ornamental value, with its attractive purple-blue flowers attracting pollinators, making it a valuable plant in ecological gardening (Maslova et al., 2021).
The chemical composition of *Hyssopus officinalis* highlights its medicinal efficacy, containing bioactive compounds like volatile oils (e.g., pinocamphone, isopinocamphone, β-pinene), flavonoids (e.g., hesperidin, diosmin), tannins, and phenolic acids such as rosmarinic acid (Baj et al., 2018). These compounds contribute to its antioxidant, antimicrobial, and anti-inflammatory properties crucial for therapeutic applications. In traditional medicine, Hyssopus officinalis treats respiratory ailments like bronchitis and asthma due to its expectorant and bronchodilator effects, and its antimicrobial activity supports its use in wound healing and as a natural antiseptic.
Hyssopus officinalis also serves as a nectar source for honeybees, producing abundant nectar for hyssop honey (Antonie, 2019). This honey is prized for its distinct flavor and potential health benefits derived from hyssop's medicinal properties. In Armenia, *Hyssopus officinalis* grows on dry stony slopes and rocks across various floristic zones, including Shirak, Lori, Ijevan, and Zangezur (Flora of Armenia, 1987). Despite its cultural, medicinal, and ecological importance globally, cultivation of Hyssopus officinalis is currently absent in Armenia. To facilitate the introduction of modern varieties of this plant the study of seed collections across various regions were studied in terms of available varieties and prospective lines, accessibility for getting germplasm and catheterization and evaluation data to make the introduction of modern breeding varieties effective and well-grounded.
The research aimed to gather detailed information on available varieties and prospective lines through identification of existing suitable germplasm as well as on accessibility of germplasm through assessing the ease of obtaining seeds or plant material from different sources to support introduction and cultivation efforts. Based on the gathered information, accessions of hyssop varieties were obtained, and a field study was conducted under valley conditions in the Republic to assess the biological and agronomical characteristics of these plants. By conducting this field study, the goal was to provide valuable insights into selecting hyssop varieties that can thrive and contribute effectively to agricultural practices in Armenia, aligning with local farming conditions and economic interests.
By addressing these aspects, the study aimed to establish a robust foundation for the introduction of modern breeding varieties of *Hyssopus officinalis*, ensuring that future cultivation efforts are both effective and scientifically grounded.

Material and Methods

To identify major hyssop collections globally, assess the number of accessions held by various seed gene banks to facilitate the introduction of hyssop varieties to Armenia for further study and cultivation, different web-based platforms, including international information systems and national gene bank's internet available databases were studied. These included:

- EURISCO (the European Search Catalogue for Plant Genetic Resources) being maintained at the Leibniz Institute of Plant Genetics and Crop Plant Research on behalf of the Secretariat of the European Cooperative Programme for Plant Genetic Resources (https://eurisco.ipk-gatersleben.de/apex/eurisco_ws/r/eurisco/home),
- GENESYS portal, a collaborative initiative involving Bioversity International on behalf of the CGIAR System-wide Genetic Resources Program, the Global Crop Diversity Trust,

and the Secretariat of the International Treaty on Plant Genetic Resources for Food and Agriculture (https://www.genesys-pgr.org/).
- Gene Bank of the Slovak Republic, Genetic Resources Information System of Slovakia, https://griss.vurv.sk/,
- Genebank Information System of the IPK Gatersleben, https://www.ipk-gatersleben.de/en/research/genebank,
- Institute of Biosciences and Bioresources, Italy, Mediterranean Germplasm Database, https://www.ibbr.cnr.it/mgd/
- MSBKew Millennium Seed Bank Kew- through Millennium Seed Bank Seed List, https://apps.kew.org/seedlist/SeedlistServlet
- The NARO Genebank, Research Center of Genetic Resources, Japan, https://www.naro.go.jp/english/laboratory/ngrc/database/index.html
- USDA ARS, U.S. National Plant Germplasm System, https://www.ars-grin.gov/Collections#plant-germplasm, and others.

Data from national databases were compared with information obtained from international databases like EURISCO and GENESYS to prevent the double-counting of accessions and to provide a clear understanding of the global distribution and availability of hyssop varieties for potential introduction and cultivation in Armenia.
During the field study, the biological characteristics that include studying the growth habits, flowering patterns, and overall adaptation to local climatic conditions were investigated. In addition, agronomical characteristics were studied through evaluating parameters such as yield potential, resistance to pests and diseases prevalent in the region.

Results and Discussion

The data were compiled from national inventories and genebank’s databases, detailing the availability of *Hyssopus officinalis* L. accessions as of 2024. The countries included in the Figure 1 are encompassing major regions where *Hyssopus officinalis* L. is conserved *ex situ* in seed collections.

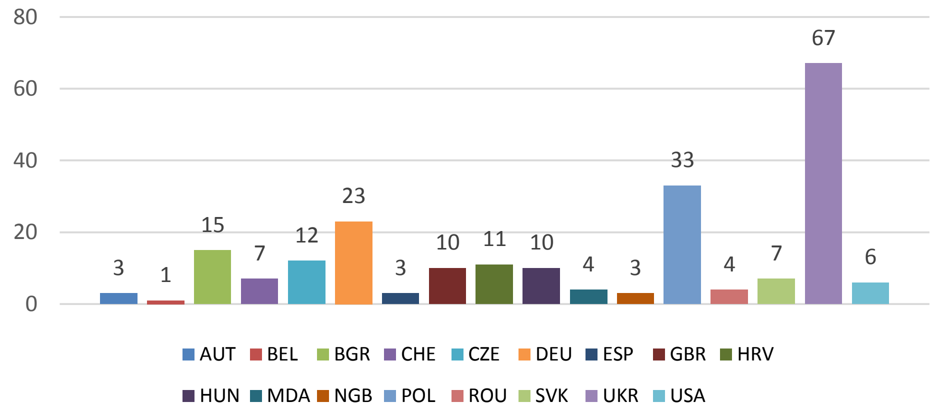

Figure 1. Distribution of *Hyssopus officinalis L.* accessions per national inventories
The Figure 1 illustrates the distribution of *Hyssopus officinalis* L. accessions across various countries' genebanks worldwide. Key findings from the figure include that the countries such as Ukraine, Polland, Germany have a substantial number of accessions, indicating a strong presence of genetic diversity for *Hyssopus officinalis* L. including varieties, prospective lines and wild forms. Conversely, some countries, such as Austria, Belgium, Czech Republic show lower numbers of accessions, suggesting potential gaps in conservation efforts or research focus.

Understanding the origin of *Hyssopus officinalis* L. accessions plays a crucial role in decision-making for selecting varieties to introduce in Armenia. Knowledge of their geographic and environmental adaptations helps assess potential for acclimatization and suitability to local growing conditions, ensuring successful cultivation and sustainable utilization in Armenian agriculture. The Figure 2 displays the distribution of *Hyssopus officinalis* L. accessions across various countries.

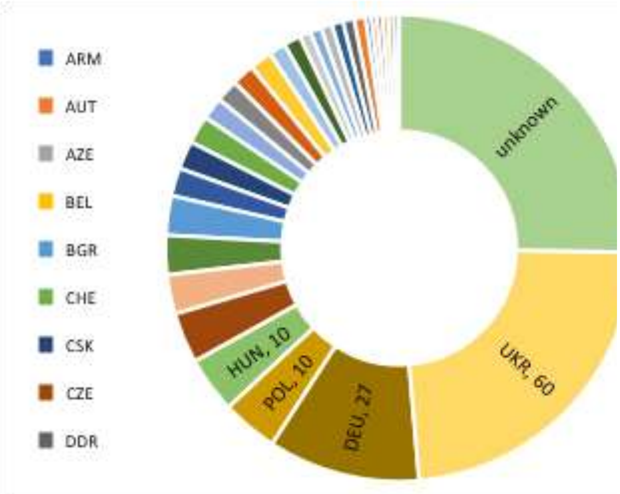

Figure 2. Distribution of Hyssopus officinalis L. accessions per origin

The distribution pattern underscores the Central European region as a primary focal point for research and conservation efforts in the global conservation of *Hyssopus officinalis* L. genetic resources.

Other criteria used to evaluate hyssop accessions availability in genebanks was accessions status. Accessions categorized as "Wild" refer to those collected from wild populations of *Hyssopus officinalis* L. make up 39 accessions. "Weedy" accessions, found in spontaneous or disturbed habitats, account for 1 accession. The category of "Traditional cultivars/landraces" comprises 63 accessions. The category of "Advanced or improved cultivar" includes 24 accessions crucial for our research, although absence on names of varieties for big number of accessions posed challenges during selection. A significant challenge in our research stems from the 84 accessions categorized as “Other” and "Unknown" lacking clear classification information. These figures underscore the difficulty in selecting appropriate varieties due to inadequate passport data on accessions stored in seed collections globally.

Table 1. Hyssopus officinalis accessions number per status

Hyssop accessions status	Number of accessions
Wild	39
Weedy	1
Traditional cultivar/landrace	63
Breeding/research material	8
Advanced or improved cultivar	24
Other	19
Unknown	84

Understanding the distribution of *Hyssopus officinalis* L. accessions per MLS (Multilateral System of the International Treaty of Plant Genetic Resources for Food and Agriculture) status is crucial because it ensures compliance with the international Treaty on plant genetic resources for food and agriculture. This knowledge helps in facilitating responsible sharing and promoting sustainable use of genetic resources under the Treaty's framework. The data presented in Figure 3 illustrates that while there are a sufficient number of accessions included in the MLS, a significant number of accessions have an unknown MLS status. This uncertainty hinders the exchange of data and germplasm, highlighting the importance of clarifying the MLS status for effective international cooperation and resource sharing.

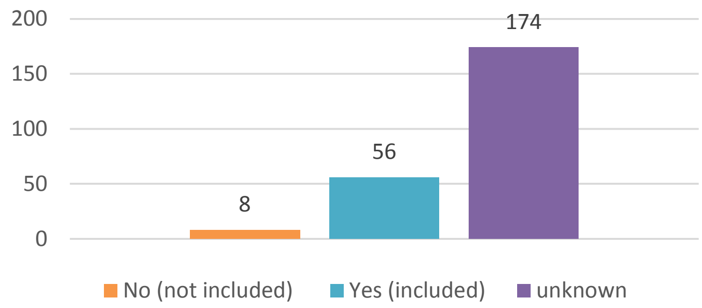

Fig. 3. Distribution of *Hyssopus officinalis* L. accessions per MLS status

The selection of varieties for introduction in Armenia was prevented by absence of characterization and evaluation data both in online available databases. Based on analysis of the data available the following varieties were preliminary selected for introduction: 'Lekar', Inej, 'Lazur', 'Accord', 'Belyj', 'Rozovyj', 'Otradnyj Semko'. To evaluate the adaptability of the selected hyssop varieties to local conditions the biological and agronomic indicators were used. Specifically, emphasis was placed on assessing green mass, measuring green mass yield and 1000-seed weight. Throughout the growing season, biometric measurements, morphological studies, and phenological observations will be conducted to monitor the progression and duration of the main developmental stages (Table 2).

Table 2. Agronomic and biological parameters of different hyssop varieties

Hyssop variety	Variables	DF	Agronomic indicators		Biological indicators	
			Green mass yield (kg/m^2)	1000 seeds weight (g)	flowering state duration (days)	ripening
'Lekar'	Samples	30	0.51*	1.19*	150*	mid-ripening
	Errors	105	0,89	0.04	0.96	
	CV%**	-	4.31	4.83	4.82	
'Inej'	Samples	30	0.58*	1.21*	160*	early-ripening
	Errors	104	0.41	0.06	0.89	
	CV%**	-	6.28	4.65	3.83	
'Lazur'	Samples	30	0.41*	1.24*	153*	mid-ripening
	Errors	104	0.92	0.04	0.87	
	CV%**	-	*5.64*	3.68	4.68	
'Accord'	Samples	30	0.48*	1.19*	155*	late-ripening
	Errors	106	0,79	0.03	0.91	
	CV%**	-	4.34	3.97	5.28	
'Belyj'	Samples	30	0.47*	1.14*	158*	early-ripening
	Errors	104	0.86	0.04	0.89	
	CV%**	-	5.47	4.58	4.88	
'Rozovyj'	Samples	30	0.42*	1.12*	152*	mid-ripening
	Errors	104	0.92	0.04	0.97	
	CV%**		5.03	4.29	4.96	
'Otradnyj Semko'	Samples	30	0.33*	0.99*	148*	mid-ripening
	Errors	105	0.88	0.18	0.87	

	CV%**	-	4.39	3.96	4.96	

*correlation is significant at the 0.01 level (2-tailed)
**ratio of the standard deviation to the mean

Conclusions

Based on the findings, the varieties ‘Inej’, ‘Lekar’, ‘Accord’, ‘Belyj’ that demonstrate optimal performance and potential for commercial cultivation in Armenia, considering both agricultural productivity and environmental sustainability.
The conducted gap analysis underscores the importance of international collaboration in the conservation and utilization of plant genetic resources, particularly for medicinal and aromatic plants like *Hyssopus officinalis* L.
The next step of the research will be quality assessment, in particular, the analyzing the chemical composition of essential oils and other bioactive compounds, which contribute to the plant's aromatic, medicinal and melliferous properties.

Acknowledgments

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A STUDY OF THE EFFECT OF BIOSTIMULANT ON THE YIELD AND QUALITY OF CUCUMBER (CUCUMIS SATIVUS)

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Abstract

In today's time, with the aim of reducing chemization and increasing yield, the improvement of plant production is moving towards the introduction of biostimulants. Many biostimulants help the plant to withstand the effect of biotic and abiotic stresses, increasing quality and yield, and stimulating physiological processes in the plant. The purpose of this study, improvement nutritional conditions of cucumber and production with maximum yield by using biostimulants so if possible recommend this biostimulants asreplacement of current chemical fertilizers.The experiment was conducted in 2021.year in a greenhouse as a two-factorial experiment with a completely randomized block design in four replications on two cucumber varieties. Treatments included three concentrations of 0 (control), 0.5%, 1%, 1.5% by foliar application in three phases.The best treatment in this study was the biostimulant at a concentration of 1.5%, which led to a statistically significant increase in yield. The highest yield in all tested variants was recorded in the Besta variety (14.64 kg m^{-2}) with nutrition with a biostimulant at a concentration of 1.5%, which is statistically significantly higher compared to the same variety fed up with a biostimulant at a concentration of 1% (7.33 kg m^{-2}) and 0.5% (8.13 kg m^{-2}) .

Keywords: *cucumber, biostimulant, yield, quality.*

Introduction

Cucumbers are important for human nutrition are their consumption is a guarantee of health. Cucumbers have about 96-97 percent water content, are low in calories and are an essential part of a healthy diet. Many studies indicate that cucumbers contain a significant amount of vitamins and minerals necessary for human health (Daneshvar, 2004; Peyvast,2009). In nutrition of vegetable, organic and mineral fertilizers are used. Organic fertilizers play a major role in plant growth and developmentas a source of all necessary macro and microelements, where during mineralization they improve the physical and chemical properties of the soil. However, according to many studies (Ogbonna, 2008; Ndaeyo et al., 2005; Makinde et al., 2007) fertilizingthe soil with only organic fertilizers for needs of vegetable nutrition does not give good results in achieving high and stable yields. Therefore, these types of organic fertilizers should be used in combination with mineral fertilizers achieve the best yields (Rašević, 2017).Applying mineral fertilizers achieves high yields, but their application can have a harmful impact on plant quality, reduce dry matter content, increase erosion, and instability of soil aggregates (Adeoluwa and Adeogun, 2010). In order to reduce chemization, increase yield, and improve plant production, the introduction of biostimulants is necessary.

Biostimulants with compared to chemical fertilizers have enormous economic and environmental advantages. Now, biological fertilizers have special significance as a suitable replacement for chemical fertilizers, aiming to improve of soil fertility and ensure plant health (Shahdi Komalah, 2010), environmental safety, improved agricultural crop quality, economic benefits, soil resources stability , long-term production potential protection, and on the other hand, the production of food crops for raising consumer quality(evaluation of biological fertilizers) and consumer health protection(Wu et al.,2005). Adediran and Akande (2005) state that there is a significant difference in the yield growth and plant efficiency due to the use of biostimulants and mineral fertilizers.
The goal of this research was to improve cucumber nutrition and achieve the highest yield by applying biostimulants in order to recommend these fertilizers as a substitute for chemical fertilizers.

Materials and methods

The experiment was conducted in the experimental greenhouse of the Faculty of Agriculture, University of East Sarajevo (City of East Sarajevo, Entity of Republic of Srpska, Bosnia and Herzegovina) in 2022. The area where the greenhouse is located is at an average altitude of about 500m (18^{o}22'59.45" longitude and 43^{o}49'36.95" latitude).Cucumbers were planted et the beginning of April. The plant spacing was 50 cm, the row spacing was 60 cm. The total area of the experiment was 100 m^2. Cultivation and care were conducted according to standard method.The study was conducted with completely randomized block design at four replicates.
The biostimulant "Humistar" was used in concentrations: 0 (control),0.5%, 1%, 1.5%. When cucumber had 7 to 8 leaves conducted the First stage of spraying, and the second stage was after 15 days of first stage and the three stage of spraying conducted when cucumber in flowering stage.Two varieties of cucumber were analyzed: Monolit F_1 and Besta F_1. In during period of harvest were measured the characters like length of fruit, diameter of fruit, mean of weight single fruit, yield per m^2. Were measured the qualitative characters like of the content of: dry matter content, total sugars, vitamin C.
The data were processed by the method of analysis of variance of the two-factorial experiment (ANOVA) using SPSS 4.5 software.We tested the significance of differences between individual means using the LSD test for general means and interaction.

Results and discussion

The morphometric properties of the plant and variance analysis for the cucumber plants grown using different concentration biostimulant are given in the Table 1. The effect of biostimulants on the characteristics of fruit length, fruit diametar average individual fruit weight was not significant. The findings of the present study were in line with the results of the studies carried out by Raeisi et al., (2013) in which he was reported that the diferent doses of biostimulant had insignifikant effect on the number of fruit, diameter of fruit, weight of single fruit, length of internode and diameter of stem cucumber.
The highest yield was recorded in the Besta variety (14.64 kgm^{-2}) when plant nutrition with a biostimulant in a concentration of 1.5%, which is statistically significantly higher compared to the same variety nutrition with a biostimulant in a concentration of 1% (7.33 kgm^{-2}) and 0.5% (8.13 kgm^{-2}) (tab.2). The qualitative characters like of the content of: dry matter, total sugars, total acids, vitamin C are given in the Table 3 The average percentage of dry matter in cucumber was in the range of 3.41% to 4.19%. The highest percentage of dry matter was in the control variant at the variety Monolit (4.19%), which is statistically highly significant compared of the biostimulant at a concentration of 0.5% (3.69%),concentration of 1%

(3.75%) and concentration of 1.5% (3.76%).The percentage of dry matter in cucumber fruits was not directly dependent on the biostimulant. Besides,many researchers have reported that the use of biostimulants does not change the chemical characteristics of the fruit (Hassan et al., 2021; El-Ghadban et al.,2006).

Highest content of total sugar was when using the biostimulant at a concentration of 1.5% (4.09 g $100g^{-1}$), which is statistically significantly higher compared of the biostimulant at a concentration of 0.5% (3.56 g $100g^{-1}$) and concentration of 1% (3.24 g $100g^{-1}$).

The variety Besta variety had the highest total acids content in the control variant of 1.227 g $100g^{-1}$, which is statistically significantly higher compared to the same variety fed with a biostimulant at a concentration 1%(0.920 g $100g^{-1}$) and biostimulant at a concentration 0.5% (1.167 g $100g^{-1}$). However, the variety Monolit had the highest content of total acids when using the biostimulant at a concentration of 1.5% (1.277 g $100g^{-1}$), which is statistically significantly higher compared to the application of the biostimulant at a concentration of 0.5% (1.022 g $100g^{-1}$).

Cucumber provides a large amount of vitamin C (mg $100g^{-1}$ of fresh base), and in this experiment the treatments with the highest content were when using biostimulant concentration 0.5% (4.81 mg $100g^{-1}$) and concentration 1% (4.79 mg $100g^{-1}$).The lowest vitamin C content was in the control treatment (3.70 mg $100g^{-1}$). Similar results were reported by Radames et al.,(2018).

Table 1. Variance analysis of the morphometric properties

Different concentrations of biostimulants	variety Monolit			variety Besta		
	fruit length (cm)	fruit diametar (cm)	individual fruit weight (g)	fruit length (cm)	fruit diametar (cm)	individual fruit weight (g)
0 (control)	13.64	4.33	168.13	33.48	4.02	287.93
0.5%	12.36	4.48	177.61	18.24	4.30	237.22
1%	12.66	4.33	179.89	17.20	4.06	223.28
1.5%	11.67	4.09	148.97	21.86	4.33	316.96

LSD		fruit length	fruit diametar	individual fruit weight
A	0.05	7.31	0.29	34.40
	0.01	10.39	0.42	48.91
B	0.05	10.34	0.41	48.68
	0.01	14.70	0.59	69.20
AxB	0.05	14.60	0.59	68.84
	0.01	20.76	0.84	97.85

Table 2. Variance analysis of the yield (kg m^{-2})

Different concentrations of biostimulants	Variety	
	Monolit	Besta
0 (control)	5.02	11.10
0.5%	8.44	7.82
1%	7.22	7.45
1.5%	5.98	14.68

LSD		
A	0.05	2.47
	0.01	3.52
B	0.05	3.50
	0.01	4.98
AxB	0.05	4.95
	0.01	7.05

Table 3. Variance analysis of the qualitative characters

Different conc of biostimul	variety Monolit				variety Besta			
	dry matter (%)	total sugars (g $100g^{-1}$)	total acids (g $100g^{-1}$)	vit. C (mg $100g^{-1}$)	dry matter (%)	total sugars (g $100g^{-1}$)	total acids (g $100g^{-1}$)	vit. C (mg $100g^{-1}$)
0 (control)	4.19	3.65	1.115	3.45	3.77	3.87	1.227	3.04
0.5%	3.69	3.69	1.022	5.08	3.50	3.43	1.167	4.54
1%	3.75	3.48	1.150	4.29	3.41	3.01	0.920	5.30
1.5%	3.76	4.09	1.277	4.96	3.67	3.50	1.045	2.88

LSD		dry matter	total sugars	total acids	vit. C
A	0.05	0.15	0.74	0.021	0.20
	0.01	0.21	1.05	0.031	0.29
B	0.05	0.21	0.03	0.030	0.28
	0.01	0.30	0.04	0.043	0.41
AxB	0.05	0.30	0.04	0.043	0.41
	0.01	0.43	0.06	0.062	0.58

Conclusion

According to the experimental data obtained, authorscan conclude that the use of biostimulants can reduce the irrational and ecologically harmful use of mineral fertilizers, and that quality of the fruits will not be reduced.The biostimulant applied in a concentration of 1.5%may be a viable alternative to the conventional fertilizer to increase cucumber productivity.

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INTERDEPENDENCE OF PRODUCTIVE CHARACTERISTICS AND FORAGE QUALITY OF RED CLOVER

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Abstract

Red clover is an important plant species due to the possibility of cultivation on poor quality soils, it gives high yields of bulky fodder that are characterized by good nutritional values. The goal of this research was to determine the correlation between yield, productive traits and yield quality of eight genotypes of red clover grown on acidic soil in the hilly area of the city of Banja Luka. Field trial were carried out in the first cutting of 2010 and the first cutting of 2011. In both season, 13 characteristics of yield and forage quality were analyzed. In the first cutting of the first year, the highest correlations was found between green mass yield and hay yield (0.95**) and between leaf width and leaf and flower content (0.87**). In the first cutting of the second year, statistically significant ($p<0.01$) positive correlations were found, which was the highest between tree thickness and NFE (0.94**), followed by high correlation values between green mass yield and hay yield (0.90**), fat content and protein content (0.88**), number of trees per plant and tree thickness (0.86**), leaf and flower content and protein content (0.85**). A high negative correlation was found for the number of stems per plant with leaf and with flower content (-0.90**), as well as between ash content and NFE (-0.89**). Knowledge of the positive correlative relationships of certain productive and nutritional traits is desirable for breeding programs and production practices in order to produce high-quality animal feed.

Key words: *Red clover, correlative relationships, yield, nutritional value*

Introduction

Red clover is a perennial leguminous plant important for fodder production. It can be successfully cultivated in hilly and mountainous areas, namely on: acidic, poor, shallow, soils above 250 m above sea level and on soils where alfalfa does not give satisfactory results (Osztoics et al., 2005). In the Republic of Serbia, the production of coarse fodder on soils with poor chemical composition and in hilly and mountainous areas is mainly realized by growing red and white clover, bird foot trefoil and sainfoin (Radović et al., 2010). The height of the red clover plant is mainly determined by the genetic characteristics of the variety and environmental factors (Leto et al., 1998). The width and length of coot red clover was investigated by Primorac et al. (2008), Gatarić et al. (2010), Popović et al. (2011), Radinović et al. (2022a). Red clover forage is of good quality with high nutritional value, which favors the diet of ruminants, especially cattle (Lee et al., 2009). By growing red clover, high yields of green mass and hay can be achieved, the most productive being the first cutting in the second year of life (Petković et al., 2021). Hay from the first swath is richer in proteins compared to the second swath from the same production year (Petković et al., 2020). In Europe, due to the high yield of digestible organic matter, protein content and metabolic energy, red clover is grown on large areas for the production of animal feed (Staniak, 2019). In the Republic of Serbia, in two-years studies of the correlation between morphological traits, yield and quality of red clover the highest correlation was found between the yield of

green mass and the yield of dry matter were were found in the second year of the study (Radinović et al., 2022b). Differences in morpho-agronomic traits and forage quality are conditioned by genetic variability, but are also influenced by production conditions, stage of maturity at the time of mowing, height and frequency of mowing (Swarup et al., 2021).
The aim of this study was to determine the correlation between yield, productive traits and yield quality of eight genotypes of red clover grown on acidic soil in the hilly area of the city of Banja Luka.

Material and methods

For the purpose of these research, the field experiment was carried out at an altitude of 527 m (44°39' N, 17°00' E). The research was carried out in the first swath of 2010 and the first swath of 2011. Four genotypes of domestically selected red clover from the breeding program of the Public Institution Agricultural Institute of the Republic of Srpska (G-1, G-2, G-3 and G-4) and four varieties of red clover (G-5 Nike, G-6 Viola, G-7 Kolubara and G-8 Start). All genotypes were sown in four repetitions. Sowing was done (manually) at a depth of 0.015-0.02 m. The sowing rate was 17 kg ha^{-1}, the distance between the rows was 0.2 m. The plot on which the field tests were carried out was well supplied with humus (5.2%), the phosphorus content was low (7.0 mg P_2O_5 in 100 g of soil) and well supplied with potassium (30.0 mg K_2O in 100 g of soil. The following parameters were analyzed: yield of green mass (t ha^{-1}), plant height (cm), number of trees per plant, tree thickness (mm), length and width of coot (mm), hay yield (t ha^{-1}), leaf and flower content (%). The yield of green mass was determined by measuring immediately after mowing on a field scale. Samples of 1 kg of green mass were taken from the mowed green mass, which were weighed on a Fisher scale to determine the yield of hay. The height of the plant (cm) was determined by measuring (five plants per repetition), the number of stems per plant (pieces) by counting on (five plants per repetition), the thickness of the tree (mm) was determined by a sliding scale (vernier), thickness measurement (five stems per repetition), the length and width of the trefoil (mm) was determined with a sliding scale, by measurement (five trefoils per repetition). Samples for testing morphological characteristics were taken from the middle rows of plots. Hay yield was determined from the ratio of the weight of 1 kg of fresh and dried sample. The leaf and flower content was determined by manually separating the leaf and flower from the stem by weighing the separated mass and the total mass - in the dry state.
The chemical analysis of hay was performed in the Animal feed quality control laboratory at the Faculty of Agriculture in Banja Luka, where the proportions of the following parameters were determined, expressed on the basis of absolute dry matter: dry mass (%), moisture (%), crude proteins (%), crude fats (%), crude cellulose (%), crude ash (%) and nitrogen-free extractives (%). After determining the percentage content of leaves and flowers, samples were prepared for chemical analysis of hay in such a way that the leaf and flower mass was mixed with the stems and ground on a mill. Chemical analyzes of hay were performed according to the methodology: crude proteins, using the micro-Kjeldahl method, modified according to Bermner (1960), i.e. crude proteins by multiplying by a factor of 6.25, crude fats in plant material, using the Soxhlet method, crude cellulose content in plant material, by the Henneberg-Stohman method, raw ash content in plant material, by annealing at 550^0C until constant mass. The measurement results were processed in the program version 22, Statistical Package for Social Sciences and Excel. The results of the investigated properties were processed by analysis of variance (ANOVA). The significance of the differences between the tested genotypes, and their ranking for the r=0.05 significance level, was determined by Duncan's multiple range test (Duncans Multiple Range Test-DMRT). Correlative relations between the analyzed traits were calculated as Pearson's correlation coefficients and significance was determined.

Weather condition

Meteorological data: mean monthly air temperatures and precipitation amounts were taken from the Hydrometeorological Institute of the Republic of Srpska, from the measuring station in Banja Luka - located in the Lazarevo settlement. Mean monthly temperatures in both years of the study were higher than the multi-year average. In relation to the multi-year average (1960-2010), in the first year, a higher amount of precipitation was found, and in the second, a lower amount. In the first year of research (2010) compared to the second (2011), a higher amount of precipitation was recorded (807.6 mm).

Results and Discussion

The obtained results showed differences in the mean values for most of the tested traits which are statistically significant. However the mean values of plant height in both year were not significant different. The lowest coefficient of variation in both years was found for NFE (Table 1).

Table 1. Average values of the examined traits in the first cutting in 2010 and 2011.

Genotype	1	2	3	4	5	6	7	8	f-test/CV %
				The first swath in 2010					
Green mass	28.38^{abc}	30.75^{ab}	30.63^{ab}	29.75^{ab}	27.00^{bc}	24.13^{cd}	33.00^{a}	21.88^{d}	**
Dry mass	6.94^{b}	8.06^{a}	7.44^{ab}	6.95^{b}	6.91^{b}	4.95^{c}	8.31^{a}	5.13^{c}	**
Plant hight	80^{a}	79^{a}	80^{a}	80^{a}	80^{a}	75^{a}	78^{a}	78^{a}	ns
Number of plants	7.15^{ab}	7.45^{ab}	7.55^{ab}	7.45^{ab}	7.00^{ab}	6.20^{b}	7.90^{a}	6.30^{b}	**
Steam of tickness	3.87^{a}	3.89^{a}	3.9^{a}	3.78^{a}	3.75^{a}	3.44^{b}	3.67^{ab}	3.88^{a}	**
Leaflet length	46.60^{a}	45.67^{a}	43.75^{ab}	44.45^{ab}	43.26^{ab}	31.26^{c}	40.35^{b}	40.20^{b}	**
Leaflet width	23.11^{a}	21.73^{a}	22.98^{a}	22.82^{a}	23.09^{a}	22.21^{a}	19.98^{a}	21.76^{a}	ns
Leaf and flower	46.77^{ab}	45.11^{ab}	44.53^{ab}	45.22^{ab}	46.26^{ab}	48.93^{a}	45.30^{ab}	40.32^{b}	**
Proteins	16.25	13.90	19.30	13.74	13.50	16.31	14.87	16.04	11.64
Fats	2.11	2.19	2.20	1.92	1.89	1.92	1.85	1.88	6.86
Cellulose	26.89	30.60	24.56	31.05	30.90	27.03	29.56	26.29	8.16
Ash	9.64	8.16	8.76	8.98	8.50	12.64	10.73	10.73	14.45
NFE	45.11	45.25	45.18	44.30	45.21	42.10	43.62	45.16	2.37
				The first swath in 2011					
Green mass	43.38^{a}	46.75^{a}	47.63^{a}	44.88^{a}	42.13^{a}	41.50^{a}	49.38^{a}	47.50^{a}	ns
Dry mass	8.70^{a}	9.13^{a}	9.15^{a}	8.51^{a}	7.89^{a}	7.86^{a}	9.23^{a}	8.74^{a}	ns
Plant hight	82^{a}	81^{a}	82^{a}	81^{a}	77^{a}	78^{a}	80^{a}	79^{a}	ns
Number of plants	10.35^{a}	10.00^{ab}	10.40^{a}	10.20^{ab}	10.30^{a}	8.90^{b}	10.10^{ab}	9.75^{ab}	**
Steam of tickness	3.79^{a}	3.71^{a}	3.67^{a}	3.60^{a}	3.77^{a}	3.39^{a}	3.65^{a}	3.65^{a}	ns
Leaflet length	48.20^{a}	44.72^{a}	46.78^{a}	48.03^{a}	46.85^{a}	45.47^{a}	47.93^{a}	46.22^{a}	ns
Leaflet width	27.55^{ab}	25.29^{ab}	25.66^{ab}	28.28^{a}	26.60^{ab}	28.94^{a}	24.22^{b}	28.93a	**
Leaf and flower	43.83^{ab}	42.13^{b}	41.77^{b}	41.13^{b}	41.14^{b}	49.03^{a}	41.74^{b}	44.55^{ab}	**
Proteins	15.24	14.75	13.19	14.75	14.37	17.81	14.50	14.29	8.36
Fats	1.85	1.74	1.82	1.84	2.31	2.09	2.38	2.69	15.21
Cellulose	27.03	28.97	29.69	28.31	28.46	26.45	28.86	29.14	3.62
Ash	9.02	8.51	8.39	8.98	9.04	10.59	9.35	9.18	6.88
NFE	46.86	46.03	46.91	46.12	45.82	43.06	45.09	44.70	2.61

* ($p<0.05$), ns- no significant,[a,b,c,d..] Values denoted by the same letter are not significantly different at the $p<0.05$ level of probability (*Duncan's Multiple Range Test*). *NFE* - nitrogen-free extract. Genotypes of domestically selected red clover (G-1, G-2, G-3 and G-4), varieties of red clover (G-5 Nike, G-6 Viola, G-7 Kolubara and G-8 Start).

Statistically significant differences in the yield of green mass of red clover were also obtained by Leto et al. (2004), Mihovsky et al. (2011), Tucak et al. (2013) and Radinović et al. (2022a). The presented data of these studies show that in the first cut in 2011, the length of the coot was in the range from 44.72 to 48.2 mm. Primorac et al. (2008) measured the middle coot of the second year of life in three mean values on 12 populations of red clover, and determined that its length was from 42.31 to 48.51 mm, in the same research the coot width was in the range from 21.62 to 27, 74 mm. In the first year (2010), the domestic genotype (G3) had the highest protein content of 19.3%, in the second (2011) 17.81% of the Viola variety (G6), in the research of Leto et al. (2004) cultivar Viola had a lower protein content.
Table 2 shows the correlation coefficients of the analyzed traits in the first cut of the first year. The highest positive correlation was between green mass yield and hay yield (0.95**) and between leaf width and leaf and flower content (0.87**). A high positive correlation (0.95**) in the first year of testing between green mass yield and red clover hay yield was obtained by Radinović et al. (2022b), Tucak (et al., 2013) (0.93**) and Radić et al. (2024) between green mass yield and fenugreek hay yield (0.81**).

Table 2. Correlation coefficients between the tested traits in the first cut in 2010

	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.95**												
3	0.46	0.58											
4	0.50	0.45	0.58										
5	0.21	0.39	0.80*	0.59									
6	0.62	0.56	0.14	0.16	-0.16								
7	-0.26	-0.26	0.45	0.41	0.26	-0.08							
8	-0.48	-0.47	0.13	0.35	0.15	-0.13	0.87**						
9	-0.35	-0.48	-0.48	-0.26	-0.40	-0.63	-0.28	-0.25					
10	-0.21	-0.13	-0.23	-0.25	-0.26	-0.27	-0.24	-0.04	0.40				
11	-0.05	-0.28	-0.25	0.03	-0.23	-0.37	-0.17	-0.29	0.80*	-0.19			
12	-0.11	-0.30	-0.61	0.07	-0.28	-0.37	-0.42	-0.22	0.70	0.01	0.74*		
13	0.20	0.44	0.45	0.00	0.65	-0.20	-0.15	-0.26	-0.42	-0.17	-0.42	-0.36	

$p<0.05$*; $p<0.01$**; N=8; Properties: 1-green mass, 2-dry mass, 3-plant hight, 4-number of plants, 5-steam of thickness, 6- leaflet length, 7-leaflet width, 8-leaf and flower, 9-proteins, 10-fats, 11-cellulose, 12-ash, 13-NFE.

The data obtained in the second year (table 3) show that the correlation coefficients between the 13 analyzed parameters were highly significant between stem thickness and NFE (0.94**), green mass yield and hay yield (0.90**), fat content and protein content (0.88**), number of stems per plant and stem thickness (0.86**), leaf and flower content and protein content (0.85**). A high negative correlation was found between the number of stems per plant and the content of leaves and flowers (-0.90**) and the content of ash and NFE (-0.89**).
On the base of the obtained results on the plots was established, a high positive correlation coefficient between the yield of green mass and the yield of hay (0.90**) (table 3) Similar values of correlation coefficient between the yield of green mass and the yield of red clover hay was found (0.86**) in second year of two year study by Radinović et al. (2022b) and as well as in study (0.93**) Tucak et al. (2013).

Table 3. Correlation coefficients between the tested traits in the first cut in 2011

	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.90**												
3	0.45	0.74*											
4	0.33	0.47	0.51										
5	0.20	0.36	0.30	0.86**									
6	0.08	0.11	0.31	0.57	0.33								
7	-0.57	-0.64	-0.29	-0.51	-0.43	-0.06							
8	-0.45	-0.46	-0.33	-0.90**	-0.72*	-0.41	0.61						
9	-0.64	-0.61	-0.39	-0.84**	-0.70	-0.29	0.50	0.85**					
10	0.18	-0.18	-0.65	-0.25	-0.07	0.03	0.18	0.16	-0.04				
11	0.79*	0.65	0.23	0.54	0.41	-0.06	-0.53	-0.70	-0.89**	0.15			
12	-0.49	-0.63	-0.59	-0.86**	-0.77*	-0.13	0.50	0.83*	0.88**	0.35	-0.75*		
13	0.23	0.48	0.69	0.94**	0.81*	0.43	-0.41	-0.77*	-0.74*	-0.52	0.43	-0.89**	

p<0.05*; p<0.01**; N=8; Properties: 1-green mass, 2-dry mass, 3-plant height, 4-number of plants, 5-steam of thickness, 6- leaflet length, 7-leaflet width, 8-, leaf and flower, 9-proteins, 10-fats, 11-cellulose, 12-ash, 13-NFE.

In our research, protein content had high positive correlation with leaf and flower (0.85**), with ash (0.88**) and negative correlations of protein content with number og plants (-0.84**), with cellulose content (-0.89**) and with NFE (-0.74*) table3. However, the authors of Tucak et al. (2013) did not establish high correlative relationships of protein content with analyzed characteristics of red clover. Radić et al. (2024) determined positive correlations between yield and compost yield of green mass and fenugreek seeds.

Conclusion

Positive correlations were established between yield, productive traits and forage quality of red clover. Leaf width was positively correlated with leaf and flower content, and leaf and stem with protein content, which indicates that if we have plants with broad leaves and a higher leaf and flower content in hay, the hay is richer in proteins. A greater number of trees per plant is positively correlated with NFE and stem thickness, but also negatively correlated with leaf and flower, protein and fat content. Genotype 7 (Kolubara) had the highest yields of green mass and hay in both years, domestic genotypes have good forage production potential, and genotype 6 (Viola) high quality forage.

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ECOLOGICAL CHARACTERISTICS OF MACROPHYTES OF THE PROTECTED HABITAT "GROMIŽELJ" (BOSNIA AND HERZEGOVINA)

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Abstract

The paper presents the ecological characteristics of the macrophyte flora of the protected habitat "Gromiželj". The protected habitat "Gromiželj" is located in the far northeast of Bosnia and Herzegovina in the Republic of Srpska, within the municipality of Bijeljina. The protected habitat covers an area of 8.31 km². In the studied area, the presence of 55 species of macrophyte flora was recorded. Ecological indices are provided according to Ellenberg, while data on life forms are taken from Oberdorfer. The analysis of ecological indices for temperature indicates that the macrophytes in the studied area are predominantly mesothermal species. In terms of the moisture index values, the most prevalent are species of wet habitats, while in terms of soil chemical reaction, there is a dominance of plants that mainly grow on alkaline soils. Regarding nutrient relationships, mesotrophic habitat species dominate, and in terms of the light regime, these are predominantly full daylight species that are tolerant to shade. The most present in the studied area are sub-oceanic and oceanic-sub-oceanic species. According to the ecological index for salinity, most macrophytes in the studied area are halophobic. The analysis of the biological spectrum of macrophytes determined the dominance of hydrophytes, followed by hemicryptophytes. Out of the total number of confirmed macrophyte species in the studied area, 6 species are listed on the Preliminary List of Species for the future Red Book of Bosnia and Herzegovina. According to the IUCN list, of the 55 identified macrophytes, 41 species are in the category of least concern (LC), while for *Urtica kioviensis*, there is insufficient data (DD).

Key words: *Macrophytes, Gromiželj, ecological indices, life forms.*

Introduction

The protected habitat "Gromiželj" is located in the far northeast of Bosnia and Herzegovina in the Republic of Srpska, within the municipality of Bijeljina (Map 1).

Map 1. Location of the Protected Habitat "Gromiželj" (1:200 000)

The protected habitat extends from the southwest to the northeast, spanning 5.5 kilometers and covering an area of 8.31 km². The Gromiželj wetland has an elliptical shape, 1600 meters in length (southwest-northeast direction) and 750 meters in width (northwest-southeast direction). The primary natural values of the "Gromiželj" protected area include the Gromiželj wetland, located in the central part of the reserve, and the partially channelized Prugnjača stream. The remaining areas are predominantly intensively cultivated, with occasional forest complexes: Mladenovača, Taborišta, Toplik, Pištalovac, Čitluk, and Tukar.
Until 2009, floristic research in this area was sparse. In the Flora of Bosnia and Herzegovina, Beck, Maly, and Bjelčić (1927, 1950, 1967) reported the presence of *Utricularia vulgaris* and *Hottonia palustris* in Bijeljina, and *Glycyrrhiza echinata* near Bijeljina and Rača. Šumatić (1997) conducted research on weed flora and vegetation in the Pannonian Basin of the Republic of Srpska, noting the presence of 85 species of weed plants in the Bijeljina area. Interest in the Gromiželj wetland was sparked by the discovery of the relict fish species *Umbra krameri*, prompting the Republic Institute for the Protection of Cultural, Historical, and Natural Heritage of the Republic of Srpska to initiate multidisciplinary research from 2009 to 2010. During this period, the vascular flora was extensively studied. Petronic et al. (2011) analyzed the weed flora of the protected habitat Gromiželj. Petronic et al. (2014) studied the floristic diversity and biodiversity conservation of Gromiželj. Habitat types of European significance in the Gromiželj area were documented by Petronic et al. (2017).
The protection of the area was carried out based on the Nature Protection Law and the Study for the Proclamation of the Protected Habitat "Gromiželj," with the declaration act issued by the Government of the Republic of Srpska ("Official Gazette of the Republic of Srpska" No. 19/18).

Materials and methods

The study utilized existing literature data from Petronić (2017), which presented the diversity of vascular flora. The conservation status and protection level were provided according to the Preliminary Red List of Endangered Plants of Bosnia and Herzegovina (Šilić, 1996) and the IUCN (2024). Ecological indices were given according to Ellenberg (Borhidi, 1993), while data on life forms were taken according to Oberdorfer (2001). Indicator values of plant species according to Ellenberg (Borhidi, 1995) were represented by indices for basic ecological parameters, such as temperature, moisture, soil reaction, nitrogen content in the soil, light intensity, continentality, and salinity. Ecological indices according to Ellenberg are given on scales of 1 to 9, or 1 to 12 (moisture index). Species names were adjusted according to the nomenclature from Euro+Med PlantBase (2006–2024).

Results and discussion

Previous research on the flora (Petronić, 2017) of the protected habitat "Gromiželj" recorded 55 macrophytes.

Table 1. Overview of macrophytes within the protected habitat "Gromiželj"

No.	Species	Ecological indices							Life forms
		TB	WB	RB	NB	LB	KB	SB	
1.	*Alisma plantago-aquatica* L.	5	10	6	8	7	4	0	Hyd
2.	*Caltha palustris* L.	5	9	5	5	7	4	0	H
3.	*Cardamine dentata* Schult.	4	7	6	5	6	4	0	Hyd
4.	*Carex elongata L.*	5	9	6	6	4	3	0	H
5.	*Carex pseudocyperus* L.	6	10	6	5	7	3	0	Hyd (H)
6.	*Carex acuta* L.	5	9	6	4	7	7	0	G (H, Hyd)
7.	*Carex brizoides* L.	5	7	5	4	6	4	0	G
8.	*Carex elata* All.	5	10	6	4	8	2	0	H (Hyd)

9.	*Carex flava* L.	5	9	8	4	8	2	0	H
10.	*Carex pendula* Huds.	6	8	6	5	4	2	0	H
11.	*Carex remota* L.	5	8	6	6	4	3	0	H
12.	*Carex riparia* Curtis	7	10	7	4	7	3	0	Hyd (H)
13.	*Ceratophyllum demersum* L.	7	12	8	8	6	4	0	Hyd
14.	*Chrysosplenium alternifolium* L.	5	7	7	4	4	4	0	H
15.	*Cirsium palustre* (L.) Scop.	5	8	5	3	7	3	0	H
16.	*Deschampsia caespitosa* (L.) P. Beauv.	6	7	6	3	7	5	0	H
17.	*Eleocharis palustris* (L.) R. Br.	6	10	7	5	8	5	1	Hyd
18.	*Epilobium hirsutum* L.	5	9	8	7	7	5	0	H
19.	*Glyceria fluitans* (L.) R. Br.	5	9	8	5	7	3	0	Hyd
20.	*Hottonia palustris L.*	6	11	5	4	6	5	0	Hyd
21.	*Hydrocharis morsus-ranae* L.	6	11	7	7	7	4	0	Hyd
22.	*Impatiens noli-tangere* L.	5	7	7	6	4	5	0	T
23.	*Iris pseudacorus* L.	6	9	6	7	7	3	0	Hyd (H)
24.	*Juncus articulatus* L.	5	8	6	2	8	3	1	H
25.	*Juncus conglomeratus* L.	5	8	5	5	8	7	0	H
26.	*Juncus effusus* L.	5	9	6	3	8	3	0	H
27.	*Lemna minor* L.	5	11	7	6	7	3	0	Hyd
28.	*Lemna trisulca* L.	5	11	7	6	8	3	0	Hyd
29.	*Lycopus europaeus* L.	6	9	6	7	7	5	0	Hyd
30.	*Lycopus exaltatus* L.	6	9	6	6	7	6	0	H, Hyd
31.	*Lysimachia nummularia* L.	6	7	8	4	5	4	0	Ch
32.	*Lysimachia vulgaris* L.	5	8	6	4	6	5	0	H
33.	*Lythrum salicaria* L.	5	9	7	4	7	5	1	H
34.	*Mentha aquatica* L.	5	9	7	4	7	3	0	H (Hyd)
35.	*Myriophyllum verticillatum* L.	5	9	7	4	7	3	0	Hyd
36.	*Myriophyllum spicatum* L.	5	12	8	5	5	4	0	Hyd
37.	*Nuphar lutea* (L.) Sm.	6	11	7	8	8	4	0	Hyd
38.	*Oenanthe aquatica* (L.) Poir.	6	10	7	5	7	5	0	Hyd
39.	*Persicaria amphibia* (L.) Delarbre	5	11	7	7	7	4	0	Hyd, H
40.	*Persicaria hydropiper* (L.) Delarbre	5	9	7	5	7	4	0	T
41.	*Persicaria lapathifolia* (L.) Delarbre	6	8	6	8	6	4	0	T
42.	*Phragmites australis* (Cav.) Steud.	5	10	7	5	7	4	1	Hyd, G
43.	*Potamogeton natans* L.	4	12	7	4	6	5	0	Hyd
44.	*Ranunculus fluitans* Lam.	-	-	-	-	-	-	-	Hyd
45.	*Ranunculus repens* L.	5	8	6	6	6	4	1	H
46.	*Ranunculus trichophyllus* Chaix	-	-	-	-	-	-	-	Hyd
47.	*Rorippa amphibia* (L.) Besser	6	10	8	8	7	7	0	Hyd
48.	*Rumex hydrolapathum* Huds.	7	10	7	7	7	3	0	Hyd
49.	*Sium latifolium* L.	6	10	7	8	7	4	0	Hyd
50.	*Thelypteris palustris* Schott	4	9	5	5	7	4	0	G
51.	*Urtica kioviensis* Rogow.	6	9	7	7	7	6	0	H
52.	*Utricularia vulgaris* L.	6	12	7	5	7	5	0	Hyd
53.	*Veronica anagalis-aquatica* L.	6	9	7	7	7	3	0	Hyd, H
54.	*Veronica beccabunga* L.	5	10	7	6	7	3	0	Hyd
55.	*Zannichellia palustris* L.	6	12	8	8	6	5	5	Hyd

The ecological analysis of macrophytes in the studied area was conducted based on ecological indices for temperature, soil moisture, soil reaction, mineral nitrogen content in the soil, light regime, continentality and salinity (Table 2).

Table 2. Indicator values of ecological indices for the macrophyte flora of the studied area

Indicator values	Ecological indices													
	TB		WB		RB		NB		LB		CB		SB	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0	-	-	-	-	-	-	-	-	-	-	-	-	47	88,7
1.	0	-	0	-	0	-	0	-	0	-	0	-	5	9,43
2.	0	-	0	-	0	-	1	1,89	0	-	3	5,66	0	-
3.	0	-	0	-	0	-	3	5,66	0	-	16	30,2	0	-
4.	3	5,66	0	-	0	-	13	24,5	5	9,43	17	32,1	0	-
5.	28	52,8	0	-	6	11,3	13	24,5	2	3,77	12	22,6	1	1,88
6.	19	35,9	6	11,3	17	32,1	8	15,1	9	17	2	3,77	0	-
7.	3	5,66	0	-	22	41,5	8	15,1	29	54,7	3	5,66	0	-
8.	0	-	8	15,1	8	15,1	7	13,2	8	15,1	0	-	0	-
9.	0	-	17	32,1	0	-	0	-	0	-	0	-	0	-
10.	-	-	11	20,8	-	-	-	-	-	-	-	-	-	-
11.	-	-	6	11,3	-	-	-	-	-	-	-	-	-	-
12.	-	-	5	9,43	-	-	-	-	-	-	-	-	-	-
∑	53	100	53	100	53	100	53	100	53	100	53	100	53	100
$\overline{x}$	5,42		9,23		6,6		5,43		6,62		4,06		0,19	

The analysis of ecological indices for temperature indicates the dominance of species with an ecological index TB5 (52.8%). These are plants of the montane mesophilous broad-leaved forest belt. Species of the submontane broad leaved forest belt (TB6) also have significant representation (35.85%). Species of the montane needle-leaved forest or taiga belt (TB4) and species of the thermophilous forest or woodland belt (TB7) are not significantly represented (5.66%). The mean value of the ecological index for temperature is 5.42, indicating that the macrophytes in the studied area are predominantly mesothermal species.

Regarding the moisture index values, the most prevalent are plants of wet, not well aerated soils (WB9) (32.1%). The second most common are species with an index of WB10 (20.8%), which are plants of frequently flooded soils. The third place (15.1%) is occupied by plants of moist soils tolerating short floods (WB8). Plants of fresh soils with an index of WB6 and plants with floating or partly emergent leaves with an index of WB11 are represented by 11.3%. Plants most wholly submersed in water (WB12) are present with 9.43%. The mean value of the moisture index is 9.22. This value indicates that macrophytes in the studied area develop in wet habitats, which is expected given that they are species of aquatic habitats.

Regarding the soil's chemical reaction, there is a dominance of basifrequent plants, mostly on basic soils with an index RB7 (41.5%) and neutrophilic plants with an index RB6 (32.1%), which grow mostly on neutral soils but also in acid and basic ones, more or less indifferent plants. Plants of slightly acid soils with an index RB5 are represented by 11.3%, while plants of basiphilous sites with an index RB8 are present with 15.1%. The average value of the ecological factor for soil acidity is 6.6. Based on this, we can say that the macrophytes of the studied area predominantly inhabit basophilic habitats.

Regarding the nutrient content, the most represented are plants of mesotrophic habitats with an index NB5 (24.5%) and plants of submesotrophic habitats with an index NB4 (24.5%). Plants of moderately nutrient rich habitats (NB6) and plants of soils rich in mineral nitrogen (NB7) are present with 15.1%. N-indicator plants of fertilized soils (NB8) are present with 13.2%. Plants of habitats very poor in nitrogen with an index NB2 and plants of moderately oligotrophic habitats with an index NB3 are not significantly represented (1.89% and 5.66%). The average value of the ecological index regarding nutrient content is 5.43. This indicates that the macrophytes of the studied area are predominantly mesotrophic.

In terms of light as an ecological factor, halflight plants, mostly liying in full light but also shadow tolerants (LB7) have significant representation (54.7%). The second most common are light plants with photosynthetic minimum above 40% relative light intensity, less only in

exceptional cases (LB8) (15.1%). Shadow-halfshadow plants with photosynthetic minimum between 5 and 10% relative light intensity (LB4), are represented by 9.43%. Halfshadow-halflight plants with photosynthetic minimum between 10 and 40% relative light intensity (LB6), are present with 17%, while halfshadow plants receiving more than 10% but less than 100% relative light intensity (LB5), are represented by 3.77%. The average value of ecological indices for light intensity is 6.62, indicating that the macrophytes of this area are predominantly full daylight species that are tolerant to shade.
Regarding the continentality index, species with indices CB4 (32.1%) and CB3 (30.2%) dominate. These are suboceanic species that predominantly inhabit Central Europe but reaching to East and oceanic-suboceanic species these are in whole Central Europe. Species of the intermediate type with slight suboceanic-subcontinental character (CB5) are present with 22.6%. Oceanic species with an index CB2, mainly found in Western Europe and the western part of Central Europe, and continental-subcontinental species with an index CB7 are represented with 5.66%. Subcontinental species (CB6) are not significantly represented (3.77%). Regarding the ecological index for salinity, most macrophytes in the studied area are halophob species (SB0) (88.7%) not occurring in salty or alkalic soils.
The analysis of the biological spectrum of macrophytes established the dominance of hydrophytes (52.7%), which is expected since macrophytes are plant species of aquatic habitats. Hemicryptophytes (34.6%) are the second most common, their significant presence corresponding with the climatic conditions of the studied area, the biological spectrum of Bosnia and Herzegovina, and the Balkan Peninsula. Geophytes (5.5%), therophytes (5.5%), and chamaephytes (1.8%) are insignificantly represented.
Of the total number of macrophyte species recorded in the studied area, 6 species are listed on the Preliminary List of Species for the Future Red Book of Bosnia and Herzegovina (Šilić, 1996), categorized as vulnerable species (VU). These are: *Thelypteris palustris*, *Nuphar luteum*, *Hottonia palustris*, *Zanichellia palustris*, *Hydrocharis morsus-ranae* and *Utricularia vulgaris*. According to the IUCN list, of the 55 identified macrophyte species, 41 species are in the least concern category (LC), while for *Urtica kioviensis* there is insufficient data (DD). This research recorded the tertiary relict species *Urtica kioviensis* for the first time in the flora of Bosnia and Herzegovina.

Conclusion

The protected habitat "Gromiželj" is located in the far northeast of Bosnia and Herzegovina, in the Republic of Srpska, within the municipality of Bijeljina. The protected habitat covers an area of 8.31 km². Previous research on the flora of the protected habitat "Gromiželj" has recorded 55 macrophytes. In the macrophyte flora of the studied area, mesothermal, basic, mesotrophic, halophobic, suboceanic, and oceanic-suboceanic species dominate. Also, the most prevalent are species of wet habitats as well as species that thrive in full daylight but are tolerant of shade. The analysis of the biological spectrum of macrophytes established the dominance of hydrophytes, with hemicryptophytes being the second most common. Of the total number of macrophyte species recorded in the studied area, 6 species are listed on the Preliminary List of Species for the Future Red Book of Bosnia and Herzegovina. According to the IUCN list, 41 species are in the least concern category (LC), while for *Urtica kioviensis*, there is insufficient data (DD).

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ORGANIC PLANT BIOSTIMULANTS AND TOMATO FRUIT QUALITY

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Abstract

The goal of the present experiment was to evaluate the effect of organic plant biostimulant application on the quality parameters of tomato grown under greenhouse conditions. This study was undertaken to determine the effects of humic acid on the ascorbic acid and total acids content of two tomato cultivars Rally F1 and Pink Rock F1. The experimental site was set up in the greenhouse of the Faculty of Agriculture in East Sarajevo, in 2022. Treatment with biostimulant "Humistar" was started with concentration 0, and 50, 100, 150ml L^{-1} were applied into soil every 15 days starting with transplanting in greenhouse and during of the growing seasons. In general,soil application of humic acid presented positive effects on physicochemical quality of tomato fruits irrespective of the dose of humic acid and the cultivar investigated. Soil application of humic acid treatment with 50mlL^{-1} al showed positive effects on the vitamin C of tomato fruits harvested from the cultivar Pink Rock F1. Higher dose of biostimulant of 100ml L^{-1} showed better results in increasing the content of total acids in cultivar Pink RockF1, and a lower dose of 50mL^{-1} in increasing the content of vitamin C in RallyF1 cultivar. Regarding the application of different concentrations of biostimulators, the highest content of vitamin C was recorded on the variant 50mlL^{-1},and total acids in variant with 100mlL^{-1} and that is statistically significant compared to the other investigated variants. A high level of negative correlation was determined between the parameters: Vitamin C - Total acids (- 0.8176).

Keywords: *tomato, cultivar, humic acid, fruits quality.*

Introduction

Plant biostimulants are natural products used to stimulate nutrient uptake and efficiency by crops, increase tolerance to abiotic/biotic stress and improve quality without negative impacts on the environment if obtained from renewed sources. Humic substances are some of the most used plant biostimulants in agriculture and play a central role in plant adaptation. The definition of plant biostimulants is strongly linked to changes in the physiological functions of plants (Ricci et al., 2019). The origin of plant biostimulants is diverse, being obtained from different organic sources, such as microbial fermentation of animal or vegetable raw materials, humic substances, algae extracts, protein hydrolysates, industrial residues, beneficial fungi and rhizobacteria that promote plant growth (Nardi et al., 2015; Rouphael et al., 2015). Humic acid is one of the types of plant organic fertilizer that has a great role in plant nutrition and soil fertility; in addition, produce higher yields, improved fruit quality and healthier plant (Pettit, 2004). Humic acid is particularly used to decrease the negative effects of chemical fertilizers and could have beneficial effects on the nutrition of the plant (Martinez et al., 1983). It is recognized as a major determinant of soil fertility, which covers physical-chemical and biological activities (Bronick, 2005). Also, Farahi et al. (2013) suggested that foliar application of humic acid led to improvement of quantitative and qualitative characteristics of strawberry (chlorophyll content, fruit number,total yield of plant, TSS,total acids, vitamin C, and fruit firmness).

Tomato (*Lycopersicon esculentum* L.), is one of the leading vegetable specie in protected areas and in the open field, belongs to the Solanaceae family. Tomato is an annual dicotyledonous plant in the continental climate. The fruit is used in botanical maturity. The fruit is rich in potassium, phosphorus, magnesium and iron. It also contains vitamins C, B1, B2, and vitamin PP. The color of tomatoes is given by lycopene, but it also contains other dyes, carotenoids (carotene and xanthophyll). It also contains glycoalkaloids which give it a characteristic smell and bitter taste. The taste of tomatoes is the first and most important step by which consumers decide for a given product. The leaf and stem contain tomatin, which has a fungicidal effect and is toxic to some insects. It has a high potential for yield, and the organization of seedling production is very important. The effects of humic acid on plant growth and quality of fruit are very complex and can be change depending on many factors. In this study two tomato varieties were evaluated on influence of humic acid to produce acids, special attention giving to ascorbic acid as one of the most abundant in tomatoes and very important for human health.

Material and methods

Plants of two tomato cultivars with indeterminate growth pattern, namely Rally F1and Pink Rock F1, were transplanted into prepared soil in greenhouse during the third decade of April in 2022. Rally F1 is an early hybrid, tall, characterized by a semi-open habitus with a very strong root, and is highly valued for its ease of cultivation. Pink Rock F1 is an indeterminate pink tomato with short internodes. An early hybrid, very productive, high resistance to diseases.The experimental site was set up in the greenhouse of the Faculty of Agriculture in East Sarajevo (Republic of Srpska, Bosnia and Herzegovina). The greenhouse was maintained at 23 ± 1 °C day temperatures with 59.05 ± 2% RH during the course of crop growth. Average and maximum daily temperatures, air humidity and soil moisture values in the greenhouse during the experimental period are given in Table 1. A recent analysis reveals that the soil (pH 7.38) contains <1% of $CaCO_3$, 6.00% of organic matter, 0.40 mg kg^{-1} of nitrogen, >40 mg kg^{-1} of phosphorus, and 19.60 mg kg^{-1} of potassium. Plants were grown upright and trained onto a single stem. They were supported by iron wires fixed to their main stems. The experiment was set-up as a Randomized Complete Block Design arranged in a split plot system replicated four times. There were 8 combinations with 12 plants per variant. The plants are planted at a distance of 0.6x0.5 m, which makes an assembly of 333 plants/100m^2.

Table 1. Average and maximum daily temperatures, air humidity and soil moisture values in the greenhouse during the experimental period

Month	Greenhouse daily temperature (°C)		Relative air humidity (%)		Soil daily moisture (%)	
	Average	max.	Average	max.	Average	max.
April	13,45	23,2	59,05	99,9	48,87	78,70
May	28,96	43,6	50,79	90,0	36,54	80,80
June	34,89	44,0	38,15	99,2	40,04	79,80
July	32,68	42,7	44,13	95,5	46,46	78,20

Humistar is a special liquid preparation, which contains a high amount of humic acids. The product was obtained by extraction from leonardite, without heavy metal content. Humistar promotes more intensive absorption of all forms of nutrients from the soil, affects the initial development of roots in plants (after transplanting), improves the structure and water - air properties of the soil, and promotes the development of beneficial microbiological fauna. In addition to humic acids, Humistar contains a high amount of other organic compounds (amino acids, peptides, peptones, proteins, biostimulators, etc.) that the plant can quickly and

efficiently absorb through the roots. Composition - Total humus supplement: 15% w/w Humic acid: 12% w/w, Fulvic acid: 3% w/ w.
The following variants are represented within the second tested factor (B) biostimulator:
B1-control, B2-50 ml L^{-1} (0.5%), B3-100 ml L^{-1} (1%), B4-150 ml L^{-1} (1.5%).
The first application of Humistar was well incorporated into the soil before planting. The second soil drench was applied under the growing plants, about 10 cm around their main stems (Salman et al., 2005) using a back-held spray pump and a spade. The thirdtreatment was in the phenophase of tomato flowering, then after 15 days in the phenophase of fruit formation, the fourth treatment in the phenophase of fruit ripening and the fifth application of Humistar in the phenophase of full fruit ripening. The control treatment consisted of soil applications of water only. Harvesting started in mid Julyand continued periodically, depending on fruits ripening. Each lot (5 fruits) assigned for analysis of fruits quality was homogenized in a kitchen blender. Vitamin C content were expressed as mg ascorbic acid 100 g^{-1} fresh weight (FW). The content of total L-ascorbic acid was determined by titration with the Tillman solution (Tillman's Method). Plant material (50 g) was mixed with 200 cm^3 citric acid, and after 30 min the extract was titrated with the Tillman's reagent (2,6-dichloro-indophenol). Content of total acids ($g100g^{-1}$) was determinated using metod (Turhan and Seniz, 2009), titration with sodium hydroxide solution in the presence of phenolphthalein as an indicator . A total of 10 ml of tomato juice is put in to a 100 ml measuring flask, diluted to a tera mark with aquades. Filtered sample, take 20 ml of obtained filtrate and inserted in to Erlenmeyer. The sample was added 2 drops of Phenolphtalein and titrated with 0.1 N NaOH until pink. Also, the microclimatic conditions in the greenhouse were monitored using a digitalization system. The following parameters were monitored: air and soil temperature, relative air and soil humidity. We monitored all of the above via an application on a mobile device (Virtuino 6). Through this application, the experimental greenhouse was regularly ventilated. Identification of significant differences between the studied varieties for the investigated quality parameters showing a normal distribution was evaluated using the one-way one-factor analysis of variation (ANOVA) followed by the least significant difference (LSD). The mean value within each group with different letters showed significant differences with $P < 0.05$.

Results and discussion

Application of humic acid has generally been suggested to improve the nutrient-uptake efficiency of tomato plants from the soil/environment. Atiyeh et al. (2002) reported that following incorporation of humic acid into the soil-less container medium, tomato seedlings showed better vegetative growth. They ascribed it to their improved nutrient uptake efficiency. Similarly, Yildirim (2007) and Ebrahim et al. (2012) found that foliar sprays of humic acid increased vegetative growth of tomato plants as a result of increased nutrient uptake. These nutrients are involved in plant bioactivities and plant growth induction (Abdel-Mawgoud et al., 2007). In addition, humic acid plays a vital role in the uptake and transport of nutrients as a result of increased cell permeability (Dursun et al., 2013). Since humic acid seems to have an ability to interact with various structures of phospholipid in cell membranes thereby serving as carriers of nutrients it might have a role in transport and availability of both micro- and macro-elements in plants (Asri and Demirtas, 2015). Fruits have the characteristics and levels of maturity vary so that the content of substances contained there in also varies. Types of acids are found in many types of plants, especially fruits. These acids are present in small amounts which are the intermediate results in metabolic processes in the Krebs cycle of the tri carboxylic acid, the glycoxylic acid, and the shikimic acid cycle. Citric acid is the most dominant organic acid in tomatoes. In addition to citric acid, malic acid is the most organic acid that contributes to the taste of tomatoes. Some authors suggest that biostimulants improve stress tolerance due to a greater production of anti -oxidants (Ertani et al., 2013). The present data showed that biostimulant application positively affected lycopene, but negatively affected b-carotene and vitamin C contents, thus the influence on total antioxidant activity was not significantly different among the experimental treatments. Ascorbic acid content also known as vitamin C, is a non-enzymatic antioxidant that possesses beneficial effects for the human body. According to Malacrida et al. (2006), a higher AA content in tomatoes improves the postharvest fruit quality. Alenazi et al.(2020) found that ascorbic acid is responsible for a better plant olerance of biotic and abiotic stress. Thus, varieties with a high ascorbic acid content are of particular importance for the breeding programmes and the fresh produce market.

Table 2. Effect of cultivars and humic acid application on vitamine C content mg $100g^{-1}$ FW

Treatments (ml L^{-1})	Cultivars		Average for biostimulant
	Rally F1	Pink Rock F1	
Control	9,32a	9,26b	9,29ab
50	9,09bc	9,57a	9,33a
100	8,89c	8,97c	8,93c
150	9,14b	9,29b	9,21b
Average for cultivars	9,11	9,27	9,19

Means followed by the same letter in each season are not significantly different at the 0.05 level

In the present study, the ascorbic acid content differs significantly among the studied varieties (Table 2.). Regarding the application of different concentrations of biostimulators, the highest content of vitamin C was recorded on the variant $50mlL^{-1}$ and was $9.33mg100g^{-1}$, which is statistically significant compared to the B3 (100 ml L^{-1}) and B4 ($150mlL^{-1}$) variants. The variety Pink RokF1 had a statistically higher content of vitamin C 9.27 $mg100g-^{1}$ compared to the other tested variety ($9.11mg100g^{-1}$). The highest vitamin C content were recorded for the fruits harvested from the plants of Pink Rock F1 cultivar, in treatment with 50ml L^{-1} compared to the other tested concentrations of humic acid and that was statistically significant. Radzevičius et al. (2013) suggested that environmental conditions and plant cultivar have major influences on vitamin C content in tomato fruits. Similar results to ours were obtained by (Zodape et al., 2011) with the foliar application of Kappaphycus alvarezii sap (seaweed) in tomatoes. There was an increase of more than 20% in the levels of ascorbic acid, acidity and total soluble solids, in addition to the levels of N, P, K, Mn and Zn compared with the treatment control. Padem and Ocal (1999) showed that just a single application of humic acid during the plant growth cycle was sufficient to increase vitamin C content in tomato fruits grown for processing industry.

Table 3. Effect of cultivar and humic acid application on total acids content $g100g^{-1}$FW

Treatments (ml L^{-1})	Cultivars		Average of biostimulant
	Rally F1	Pink Rock F1	
Control	5,96b	5,92b	5,94b
50	6,11a	5,53c	5,82c
100	6,09ab	6,20a	6,14a
150	5,74c	5,89bc	5,81c
Average for cultivars	5,97	5,88	5,92

Means followed by the same letter in each season are not significantly different at the 0.05 level

In the present study, the total acids content differs significantly among the studied varieties (Table 3.). Regarding the application of different concentrations of biostimulators, the highest content of total acids was recorded on the variant $100mlL^{-1}$and was $6,14g100g^{-1}$. The Rally F1 variety had a statistically significant higher average content of total acids ($5.97g100g^{-1}$) compared to the Pink Rock F1 variety ($5.88\ g100g^{-1}$). The highest content of total acids in the Rally F1 variety was recorded in the $50mlL^{-1}$($6,11g100g^{-1}$), which is statistically significant compared to the other varieties. The content of total acids in the variety Pink RockF1 was recorded at the variant $100mlL^{-1}$ ($6,20g100g^{-1}$) which is statistically significant compared to the control and the treatment B4 with $150mlL^{-1}$. A high level of negative correlation is determined between the parameters: Vitamin C – Total acids (- 0.8176). The correlation analysis showed that the vitamin C content was negatively correlated with the total acids content. One possible theoretical explanation of this result is that the vitamin C content in tomato fruit increases till beginning of ripening and then begins to decrease due to an increase in the ascorbate oxidase activity (Yahia et al.,2001). Asri and Demirtas (2015) recorded that total acids of tomato fruits has been increased with increasing doses of humic acid application, though remained in the range of 0.3-0.5% citric acid. It was found that water stress increased the sugar and acid contents (ascorbic, malic and citric acid) of the tomato fruits and thus improved the fruit quality. During the experimental period, there were not favorable conditions when it comes to soil moisture and air temperatures. The relative soil moisture ranged from 40,04 to 79,80% in June and 46,46 to 78,20% PVK in July, which is not suitable for successful greenhouse production. Unfavorable conditions were as far as the air temperature is concerned, they were over max. 44,0 ˚C in June and 42.7˚C in July, which is not favorable for growth and development of plants.

Conclusion

Tomato contains several health-beneficial compounds and has high commercial importance throughout the world. Nowadays, water shortage is a challenge for agricultural production, especially for vegetable crops, and long-term and sustainable strategies should be adopted to enhance productivity without compromising the quality of fruits and vegetables. Our results indicate that soil drenching with different concentrations of humic acid influenced to increases the efficiency of both cultivars to produce of quality fruits under protected environment.Soil drenching of humic acid 50 and 100mlL-1 increase the efficiency of both cultivar to produce higher quality fruits under protected environment. A high dose of biostimulator $150mlL^{-1}$ did not affect the improvement of the tested quality parameters of tomatoes in both tested varieties. The occurrence of a negative correlation between total acids and ascorbic acid in tomato fruit should be investigated in further studies. When analyzing the results, it is necessary to take into account the characteristics of the variety for the given tested parameters as well as its resistance to lack of water for a certain period and high temperatures in the greenhouse. Differences in temperature and soil water content between June and July can be one of the causes of the low content of vitamin C in the RallyF1 variety and total acids in the tomato fruit at single treatments in both cultivars.Therefore, further research is needed to fine-tune the application of those formulations of humic acid that had beneficial effects in order to suggest an application protocol that may improve processing tomato fruit quality under water limitation condition, high temperatures or choose a different cultivar. Biostimulants offered advantages for greenhouse tomato through protection of plants against abiotic stresses and have a potential to improve yield quality.

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FLOWERING AND FRUIT SET OF CULTIVARS OF SWEET CHERRY IN AGRO-ECOLOGICAL CONDITIONS OF SARAJEVO

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Abstract

Phenological traits and initial and final fruit set of five sweet cherry cultivars (Burlat, Schneider's Spate Knorpelkirsche, Kordia, Karina and Regina) were studied in the region of Sarajevo (Bosnia and Herzegovina) over one-year period (2024). The beginning of flowering of the tested cultivars was at the end of the third decade of March, respectively at the beginning of the first decade of April. The phenophase of full flowering was in the interval from April 1 to April 9. The end of flowering of all cultivars was in the second decade of April. The shortest duration of flowering was 8 days, and the maximum was 12 days, while the abundance of flowering was in the interval 2.8–4.5. The initial fruit set in open pollination was in the interval from 15.2% to 53.2%, while the final fruit set in open pollination was in interval from 0% to 2.6%. The cultivar Burlat was statistically significantly different from other cultivars according to the initial and final fruit set in open pollination.

Key words: *sweet cherry, phenological properties, initial fruit set, final fruit set.*

Introduction

Flowering represents one of the most important phenophases and simultaneously the most critical phase on which the yield largely depends. The time of flowering varies from year to year and is conditioned by agro-ecological conditions before and during the flowering. The start and duration of flowering, depends on the hereditary basis of the variety, but also on the temperature or the sum of the active temperatures after the end of the dormancy period. A significant problem in studying the phenology of sweet cherry flowering and its impact on the proper formation of cultivar composition is determining the temporal overlap of different cultivars in the full flowering phenophase, which is a prerequisite for potential pollination. The interval between the flowering of the earliest and the latest sweet cherry cultivar in our agro-ecological conditions ranges from 8 to 15 days, while in warmer areas, this range is greater, extending from 15 to 20 days. The flowering of one cultivar on average lasts 12 to 15 days. Cultivars with longer flowering periods have a greater chance for successful pollination and fertilization. They also have a lower chance of being damaged by late spring frosts. Observing the flowering time of sweet cherries over a longer period, a tendency for its advancement can be noticed, which is associated with the phenomenon of global warming. Fertilization is a process that occurs after pollination and is a necessary condition for the development of the sweet cherry fruit. This physiological phase is conditioned by biological factors and ecological conditions. The success of sweet cherry fertilization depends on three factors: stigma receptivity, the growth rate of pollen tubes, and the vitality of the embryo sac. The receptivity of the sweet cherry stigma lasts 1 to 7 days depending on weather conditions. It has been established that at a temperature of 10 ˚C, sweet cherry stigma receptivity lasts up to nine days, at 20 ˚C, five days, and at 30 ˚C, two to three days (*Milatović et al.,* 2011).

Pollen germination does not occur at temperatures lower than 5 ˚C. The optimal temperature is between 20–25 ˚C, and under these conditions, the period from pollination to fertilization is 2–4 days. In sweet cherry, pollen tubes reach the base of the style in 2–3 days, and it takes 6–8 days for most pollen tubes to reach the embryo sac. The vitality of the embryo sac primarily depends on temperature, as low temperatures prolong, while high temperatures shorten the period of functional ability.
Various biological and ecological factors affect the percentage of initial and final fruit set in open pollination in sweet cherry. The percentages of initial and final fruit set in open pollination can serve as indicators of yield, the degree of self-fertilization, the quality of fertilization, and the suitability of the cultivar as a pollinator. The cause of poor fruit set can be the absence of pollinators (insects) as well as poor weather conditions during the flowering phenophase. *Glišić et al.* (2012) state that the percentage of final set fruits indicates the suitability of pollinators, as the percentage of initial set fruits includes those that are degenerated and defective. Fruit set depends on the compatibility and vitality of the pollinator's pollen, temperature, and insect activity. Low temperatures during the winter period can result in damage to flower buds, indicating potentially lower yields. Environmental factors influence different fruit set rates between cultivars and within a cultivar over the years (*Nava et al.,* 2009). The same authors note that the genetic factor has the greatest impact on the sensitivity of fruit trees to higher temperatures during the flowering phenophase. In sweet cherry, higher temperatures during the flowering phenophase can significantly reduce fruit set (*Hedhly et al.,* 2007). The aim of the study was to examine the flowering phenophases, as well as the initial and final fruit set in open pollination of sweet cherry cultivars under the agro-ecological conditions of Sarajevo.

Materials and methods

The research was conducted in 2024, in the area of Sarajevo, within the experimental sweet cherry orchard of the Federal Institute of Agriculture of Bosnia and Herzegovina, which was established in the spring of 2007 at an altitude of 600 meters. The rootstock for all the studied cultivars (Burlat, Schneider's Späte Knorpelkirsche, Kordia, Karina and Regina) was the vegetative rootstock Gisela 5. The phenological characteristics of flowering and the percentage of initial and final fruit set were analyzed. Flowering was recorded by recommendations of the International Working Group on Pollination: start of flowering – 10% open flowers, full bloom – 80% open flowers, end of flowering – 90% of the petal fall (*Wertheim,* 1996). The duration of flowering was determined by the number of days from the beginning to the end of flowering, while abundance was assessed on a scale from 1 to 5 (1–trees without flowers, 5–abundant flowering). Fruit set was determined based on open pollination on six selected branches (two repetitions with three branches each) from each cultivar. The counting of initial fruit set was done three to four weeks after pollination, and final fruit set at the beginning of the fruit ripening phenophase. The fruit set expressed as a percentage was calculated by the ratio of the number of set fruits to the number of flowers. The obtained data for the percentage of initial and final fruit set were statistically processed using Fisher's model of analysis of variance (ANOVA) for a one-factor experiment, applying the F-test (*Fisher*, 1953) for $R \leq 0.05$ (using the statistical package IBM SPSS Statistics 20 (SPSS Inc, Chicago, IL, USA)). Differences in the means of the observed traits were determined using *Duncan's* multiple range test for a significance level of $P \leq 0.05$. Correlation analysis between individual phenological traits and fruit set was performed using IBM SPSS Statistics 20 (SPSS Inc, Chicago, IL, USA).

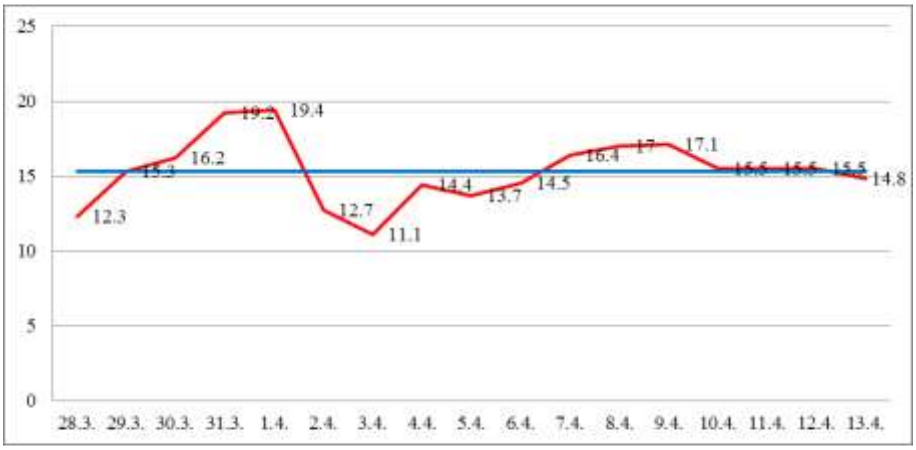

Chart 1. Dynamics (red line) of mean daily air temperatures during the flowering phenophases. The blue line inside the graph represents the average value of the average daily air temperature for the duration of flowering.

Air temperature–the area where the experimental sweet cherry orchard is located is characterized by a distinctly continental climate, with an average annual air temperature for the period 1961–1990 of 9.8 ˚C (http://www.fhmzbih.gov.ba/). According to the same data source, the average annual air temperature for the period 2000–2018 was 11.6 ˚C. For the first five months of 2024., when the research was conducted, the following air temperatures were recorded: January (0.7 ˚C), February (6.5 ˚C), March (9.5 ˚C), April (12.4 ˚C), and May (16.1 ˚C). Observing the flowering period, it is characteristic that the average daily air temperatures during the first five days were stable, showing a gradual increase, while from the sixth to the tenth day, slightly lower values than the established average for the flowering period this year were recorded (Chart 1). After the tenth day, an increase in daily air temperature was observed. The average value of the mean daily air temperature during the flowering period in 2024. was 15.3 ˚C.

Results and discussion

The results of the study of the start, full, end and duration of flowering are presented in table 1.

Table 1. The flowering phenophase of sweet cherry cultivars in 2024

Cultivar	Flowering dates			Duration of flowering (days)	Abundance of flowering
	Start	Full	End		
Burlat	28.3.	2.4.	9.4.	12	4.5
Schneider's Spate Knorpelkirsche	29.3.	1.4.	10.4.	12	4.3
Kordia	1.4.	6.4.	11.4.	10	2.8
Karina	2.4.	9.4.	13.4.	11	4.0
Regina	3.4.	7.4.	11.4.	8	4.1

The earliest flowering start date of the studied cultivars was for the Burlat cultivar (March 28), while the latest flowering start date was recorded for the Regina cultivar (April 3). The difference between the earliest and latest flowering start dates was small, amounting to six days. The other flowering phases were consistent with the flowering start dates. For the trees of the Schneider's Späte Knorpelkirsche cultivar, the smallest number of days (three days)

was needed for 80% of the flowers to open (April 1). A day later, on April 2, the full flowering occurred for the Burlat cultivar. For the other cultivars, the full flowering dates were April 6 (Kordia), April 7 (Regina), and April 9 (Karina). The end of flowering date was correlated with the flowering start date, with the earliest being for the Burlat cultivar (April 9), and the latest end of flowering was in the Karina cultivar (April 13). *Milatović et al.* (2020) consider that longer flowering duration is a desirable trait as it increases the possibility of successful pollination. The average duration of flowering was 10.6 days, with variations ranging from 8 to 12 days (Table 1). The average flowering abundance ranged from poor (2.8) to excellent (4.5) (Table 1). *Hodun* and *Hodun* (2002), examining the characteristics of 80 cultivar, state that the average duration of the sweet cherry flowering phenophase is 8–10 days. *Nyéki* and *Soltész* (1996), dealing with the characteristics of the sweet cherry flowering phenophase as a species, note that the sweet cherry is a species with a short full flowering period, lasting up to three days. The flowering dates recorded in our studies under the agro-ecological conditions of Sarajevo were consistent with the dates recorded for the conditions of the Belgrade (*Milatović et al.,* 2013), as well as for the agro-ecological conditions of the Sub-Mediterranean part of Herzegovina (*Badžak et al.,* 2021).
Analyzing the data on fruit set under open pollination presented in Chart 2, we can state that there are variations in initial and final fruit set among the studied sweet cherry cultivars.

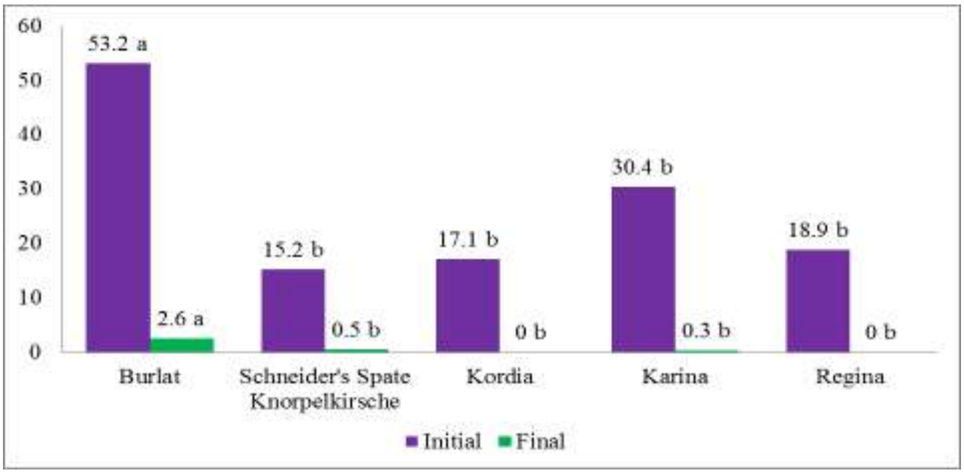

Chart 2. Fruit set of sweet cherry cultivars in 2024
Different letters for individual factors indicate significant differences for R≤0.05 using Duncan's test of multiple intervals

The highest initial and final fruit set in open pollination was in Burlat cultivar (53.2%), respectively 2.6% (Chart 2). On the other hand, there were no statistically significant differences in the percentage of initial and final fruit set between the Schneider's Späte Knorpelkirsche, Kordia, Karina, and Regina cultivars. The percentage of initial fruit set for the studied sweet cherry cultivars followed this trend: Karina (30.4%) > Regina (18.9%) > Kordia (17.1%) > Schneider's Späte Knorpelkirsche (15.2%). However, what can be concluded from the results of the final fruit set is that this percentage was significantly low, with the Kordia and Regina cultivars having a final fruit set of 0%. *Nyéki et al.* (2003) classified sweet cherry cultivar into three groups based on the degree of fruit set: low fruit set (below 20%), medium fruit set (20–30%), and high fruit set (above 30%). According to this classification, most of the studied cultivars in our research had low fruit set. For high sweet cherry yield, it is necessary to achieve a fruit set of 20–30% during abundant flowering (*Milatović et al.,* 2011). In our research, the abundance of flowering ranged from poor to excellent, but the percentage of fruit set was recorded as low. The reason for this can be found

in the air temperatures. During the period from the beginning to the end of flowering (a total of 17 days), the average air temperature was 15.3 °C (Chart 1), which is one of the potential reasons for poor fruit set of cultivars. *Postweiler et al.* (1985) note that higher flowering temperatures shorten the lifespan of the seed embryos of some sweet cherry cultivars, and at a constant temperature of 20 °C, their vitality is lost within one to two days. Additionally, during the second half of April, lower air temperatures were recorded, which could have led to the dropping or rejection of set fruits of sweet cherry cultivars since early degeneration of primary seed embryos is strongly influenced by environmental factors (*Lillecrapp et al.*, 1999).
The results shown in Table 2 indicate the correlation relationships between the number of days from January 1 to full bloom, the duration of flowering in days, the average daily temperature from January 1 to full bloom, the average daily temperature during the flowering phenophase, and the final fruit set.

Table 2. Correlation matrix among the examined indicators of flowering, fruit set and air temperature between sweet cherry cultivars (2024)

Traits	FD	TFB	TFP	FFS
NoD	-0.62	0.72	-0.11	-0.58
FD	1	-0.92**	-0.06	0.61
TFB		1	-0.10	-0.52
TFP			1	-0.72
FFS				1

NoD – number of days from January 1 to full bloom; FD – flowering duration in days; TFB – average daily temperature from January 1 until full bloom; TFP – mean daily temperature during the flowering phenophase; FFS – final fruit set.

**Correlation coefficients are statistically significant for R≤0.01 based on the t-test.

Analyzing the correlation between the number of days from January 1 to full bloom and the duration of flowering, a negative correlation (r=-0.62) was observed, without statistical significance. The studied cultivars with a greater number of days from January 1 to full bloom (i.e., later flowering time) had a shorter flowering duration. This phenomenon can be explained by the influence of air temperatures in the period from January 1 to full bloom. A positive, but not statistically significant, correlation was found between the number of days from January 1 to full bloom and the average daily temperature from January 1 to full bloom (r=0.72). Also, a negative correlation without statistical significance was found between the sum of days from January 1 to full bloom and the average daily temperature during the flowering phenophase (r=-0.11), as well as with the percentage of final fruit set (r=-0.58). The duration of flowering had a highly significant negative correlation with the average daily temperature from January 1 to full bloom (r=-0.92**), indicating that flowering duration was shorter at higher air temperatures.
Based on the presented results of the correlation analysis, a positive correlation coefficient is observed between the duration of flowering and the percentage of final fruit set, meaning that longer flowering resulted in a higher percentage of final fruit set (r=0.61). Negative correlation coefficients were found between the average daily air temperature from January 1 to full bloom and fruit set (r=-0.52), as well as between the average daily temperature during the flowering phenophase and fruit set (r=-0.72). Higher air temperatures resulted in a lower number of final fruit set, as they led to various disturbances in the pollination and fertilization process.

Conclusions

Based on the flowering time and the overlap of individual flowering phenophases, we can conclude that the sweet cherry cultivars are suitable as mutual pollinators and can be recommended for production orchards. However, meteorological conditions during the flowering period resulted in poor fruit set. The different behavior of cultivars in terms of final fruit set is a consequence of genotypic characteristics influenced by ecological factors, primarily air temperature. One of the possible reasons for the poor fruit set in the studied year is the air temperatures during the flowering phenophase. Specifically, the somewhat earlier occurrence of high temperatures in 2024, which led to flowering, created stressful conditions for the flowers, resulting in poor fruit set. Additionally, another reason that could have led to a significantly lower percentage of initial and final fruit set in the studied year is precisely the higher air temperatures during the period of flower bud differentiation.

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PROSPECTS OF CORN CULTIVATION AND PRODUCTION IN THE SEMBERIJA REGION IN BOSNIA AND HERZEGOVINA

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Abstract

Corn is one of the most important agricultural crops, with a wide range of uses. In the Bosnia and Herzegovina, Semberija holds a significant position in the production and cultivation of corn, owing to its natural characteristics. Previous research has demonstrated that Semberija possesses all the necessary conditions for high levels of corn production. However, the question arises whether this undeniable potential of the area has been fully utilized. The goal of this paper is to address that question. For this research, 50 corn producers from the Semberija area were included. The study hypothesized and confirmed that corn cultivation in Semberija is predominantly geared towards meeting the personal needs of producers and the domestic market rather than the export market. The findings are crucial for assessing the current status and future prospects of corn cultivation in this region. Additionally, they provide recommendations for further developing corn production in this naturally fertile area.

Keywords: *corn, agriculture, production, export, market research.*

Introduction

Corn is utilized for human and animal nutrition, as well as in industrial applications across the food, pharmaceutical, textile, and chemical sectors. Globally, approximately 889 million tons of corn are produced annually, equating to 129 kilograms per person. The leading producer of corn worldwide is the United States of America, accounting for 36% of global production (an average of 1,020 kilograms per capita), followed by China (22%), Brazil (7%), Argentina (3%), and Mexico (2%). The United States is also the largest exporter of corn, responsible for 46% of global exports. Other major exporters include Argentina (13%), Brazil (9%), and France and Ukraine each with 6% (Zlatanović, 2017).

In the Bosnia and Herzegovina, Republic of Srpska, according to data from the Republic Institute of Statistics in 2022, corn production totaled 372,083 tons (Institute of Statistics RS, 2023). The territory of Republic of Srpska is divided into two main agricultural production regions based on relief and climatic conditions. The first region includes plains along rivers, undulating coastal areas, and lower to medium-high mountain areas (such as Banjalučka Krajina, Posavina, Semberija, and Birač). The second region comprises mountainous areas with karst fields (including the Sarajevo-Romanija region and Herzegovina). In the first region, the primary agricultural crops are corn and wheat (Milić et al., 2016). At the level of the Republic of Srpska, the Semberija area holds particular significance, rich in soil and climatic conditions conducive to the cultivation of corn.Corn is the predominant and most significant crop in agricultural production in the Semberija region. Other crops such as wheat, barley, oats, and rye are cultivated to a much lesser extent. The average annual corn production from 1997 to 2017 was 114,950.7 tons, while wheat averaged 47,187.4 tons and barley 6,721.1 tons annually. Corn achieves slightly higher yields per hectare (4.48 tons/ha) compared to wheat (3.81 tons/ha) and barley (4.03 tons/ha) (Popov and Delić, 2019). Despite being a staple food in many parts of the world, corn serves various purposes including animal feed, biofuel, and sweeteners (Chavas and Mitchell, 2018). Global forecasts indicate that by 2025, corn will surpass all other crops, with demand doubling in developing countries (Nedeljković, 2019). Globally, corn is grown on over 160 million hectares across 166 countries, under diverse soil, climate, biodiversity, and management conditions. Maize contributes 40% annually to global food grain production (Kumar et al., 2012). Maize thrives in varied climatic and ecological conditions, favoring warmer climates. The optimal planting time is mid to late April, as corn is resilient to colder temperatures (Kazerooni et al., 2019). In the period from 1997 to 2017, Semberija has experienced a downward trend in corn production (decreasing by 1,148.3 tons annually) and a minor, insignificant decline in corn yield by 17.2 kg/ha per year. When a small number of plants survive with poor health conditions, it becomes impractical to continue producing the same crop due to insufficient returns to cover costs. Reseeding, if feasible under agroecological and organizational conditions, is advisable (Simić and Prodanović, 2018). These trends in corn yield and production are heavily influenced by temperature and moisture availability during critical development stages. Corn yield is particularly sensitive to weather conditions between 90 to 110 days after planting, coinciding with tasseling and silking stages. High temperatures, low humidity, and inadequate rainfall during this period adversely affect corn growth. Precipitation, especially during summer months, significantly impacts annual yield variability. Unfavorable conditions for corn development include temperatures exceeding 30°C and relative humidity below 60% (Popov and Delić, 2019). The production of hybrid corn seeds is crucial, requiring substantial investments in labor and professional expertise (Lopandić et al., 2007). Given corn's importance to Semberija agriculture, this paper aims to explore its cultivation prospects, analyzing results obtained from surveys of local corn producers. These findings will assess the extent to which Semberija's natural potential for corn production is utilized for export markets. The hypothesis explored in this paper is that corn produced in Semberija is primarily intended for domestic consumption rather than export.

Material and Methods

The paper is based on the analysis of data collected through a survey questionnaire regarding corn cultivation practices, the purpose of production, and the market orientation of producers. The research was conducted among corn producers in the Semberija region, Bosnia and Herzegovina. The survey involved 50 corn producers. The survey questions covered, among other things, the amount of corn yield per hectare, the intended market orientation of the corn, and the producers' plans for the future. Data analysis was performed using descriptive statistics.

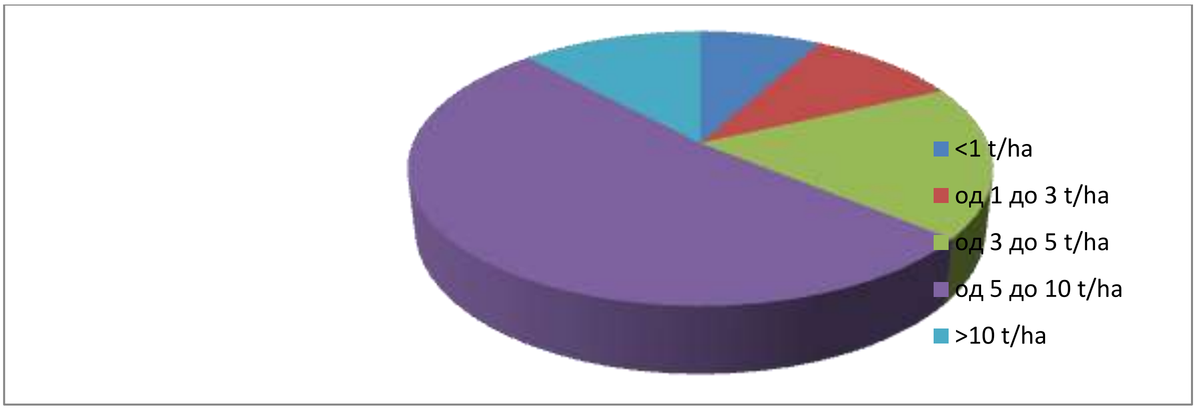
Results and Discussion

The results regarding the areas planted with corn by the companies that participated in this study are presented in the following table:

Table 1. Corn areas in surveyed companies

Companies that grow corn in the area of Semberija	
Number of companies surveyed	50
Areas of planted corn	Up to 27 hectares individually

*Source: Author’s elaboration based on the questionnaire survey results.



Graph 1. Achieved corn yield

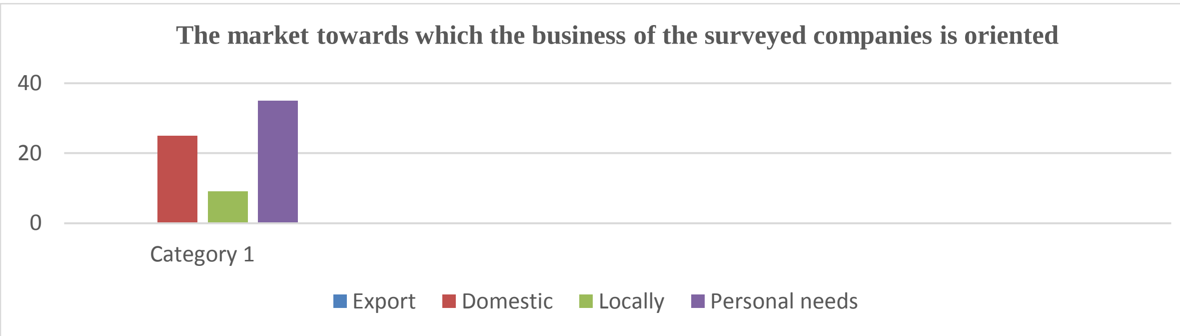
Based on the presented data, it is evident that the majority of surveyed corn producers in the Semberija area derive income from corn yields ranging between 5 and 10 tons per hectare, with 26 producers, representing over 50% of those surveyed. Following this, 9 producers achieve yields ranging from 3 to 5 tons per hectare. In third place, 6 producers achieve yields exceeding 10 tons per hectare. Additionally, 5 producers achieve yields between 1 and 3 tons per hectare, while 4 producers achieve less than 1 ton per hectare. Regarding the target markets of the surveyed companies, the research results indicate the following.

Table 2. Presentation of the market towards which the business of the surveyed manufacturers is oriented.

The market towards which the business of the surveyed companies is oriented			
Export	Domestic	Locally	Personal needs
0	25	9	35

Source: Author's elaboration based on the questionnaire survey results.

The obtained results will be graphically displayed in the following graph:



Graph 2. Presentation of the market towards which the business of the surveyed producers is oriented

Based on the results obtained, it is evident that none of the 50 surveyed producers export corn outside the territory of Bosnia and Herzegovina. Nine producers are oriented towards the local market, 25 towards the domestic market within Bosnia and Herzegovina, and a significant 35 producers cultivate corn primarily for personal consumption. Additionally, it is noteworthy that the surveyed producers expressed their intention to increase corn plantings in the following manner:

Table 3. Presentation of the planning of the increase in the area with corn in the following period by the surveyed producers

Producers who plan to increase corn production in the future period	36
Producers who do not plan to increase corn production in the future period	14

Source: Author’s elaboration based on the questionnaire survey results.

It is very important for the further development of this area and provides opportunities for expanding the placement of this culture.
The presented results confirm the hypothesis that corn cultivation in the Semberija area is predominantly oriented towards meeting the personal needs of producers and the domestic market rather than the export market. Despite historically being known as the breadbasket of Bosnia and Herzegovina, the current situation is concerning. Corn remains the most widely cultivated crop in this region, with production levels of other crops even lower.
While the natural characteristics of the area, particularly the soil and climate, provide ideal conditions for high production yields, this potential has not been fully realized in practice. Factors such as unfavorable weather conditions, drought, and current inflationary trends have hindered optimal production outcomes. Additionally, the cultivation of corn in Semberija faces challenges exacerbated by significant emigration, particularly among the younger population, leading to abandonment of arable lands.
Despite challenges in market orientation reported by surveyed producers, there is some optimism indicated by their plans to expand corn cultivation areas in the upcoming period. Specifically, 36 surveyed producers expressed intentions to increase their corn-growing areas.
To enhance corn cultivation results in the Semberija area, it is essential for state and local authorities to implement measures aimed at increasing subsidies for farmers. Additional funding would enable farmers to invest in improved agricultural machinery and irrigation systems, which can mitigate the adverse impacts of weather conditions on corn production. Previous research indicates that rainfed systems often outperform irrigated systems in terms of energy efficiency, productivity, profitability, and overall environmental impact, including water scarcity and carbon footprint (Mitrović et al., 2023). However, both systems heavily rely on non-renewable energy sources, with inputs like fertilization and mechanization contributing to negative environmental effects. Optimizing these inputs is crucial for reducing environmental impact and promoting sustainability in maize cultivation in Bosnia and Herzegovina. Many small-scale agricultural producers, including those surveyed, lack resources to implement adequate agrotechnical measures that could significantly boost production. Addressing issues such as mycotoxin occurrence through investment in protective treatments is crucial. Furthermore, proper fertilization systems are integral to increasing corn yields and are among the most important agrotechnical measures in agriculture. Rational and adequate use of both organic (e.g., manure) and mineral fertilizers is essential for effective implementation. All the aforementioned measures necessitate substantial financial investments, making the proactive involvement of the state crucial. This is particularly significant considering that such investments would yield returns and positively impact the national economy through increased exports to foreign markets.

Conclusion

The data presented and analyzed in this paper indicate an inadequate level of development in corn cultivation within the Semberija basin, suggesting that the region's soil and climatic potential are not fully utilized. The areas currently planted with corn are insufficient to achieve significant market results. Regarding market orientation, none of the surveyed producers export their corn beyond Bosnia and Herzegovina's borders, while 70% grow corn primarily for personal consumption. Only 50% of the producers operate within Bosnia and Herzegovina, with 18% focusing exclusively on the local market. These findings underscore the insufficient development of corn cultivation in this highly fertile area, historically known as the granary of Bosnia and Herzegovina. Factors contributing to reduced production include adverse weather conditions, frequent droughts, price increases due to current political situations and the multi-year COVID-19 pandemic, and high levels of population emigration.
Improving production levels requires significant financial investments in irrigation systems and higher quality fertilizers to mitigate weather-related impacts. Additionally, greater investments in protective treatments, quality fungicides, and insecticides are needed to minimize damage from pests and diseases affecting corn. Given that many farmers lack the financial means to undertake these measures due to reduced incomes from lower production levels, the role of state and local governments in allocating budget resources for agricultural subsidies is crucial. These subsidies aim to enhance national agriculture development and secure a market position that aligns with the area's natural characteristics.

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YIELD AND CHARACTERISTICS OF BUNCHES OF RED WINE VARIETIES GROWN IN THE AREA OF TREBINJE (BOSNIA AND HERZEGOVINA)

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Abstract

In this study, we present the three-year results of research on the yield and cluster characteristics of four red wine grape varieties (Blatina, Vranac, Merlot, and Cabernet Sauvignon) cultivated in the Trebinje region (Bosnia and Herzegovina). The highest yield per vine was recorded for the Blatina variety (4.70 kg), while the lowest yield was found in the Cabernet Sauvignon variety (2.99 kg). Cabernet Sauvignon stood out with the highest number of clusters per vine (23.5), while Vranac had the fewest (15.0). Vranac led in cluster mass and peduncle weight with the highest values (350.49 g and 12.37 g, respectively), whereas Cabernet Sauvignon showed the lowest values (149.17 g and 6.31 g, respectively). Merlot had the longest and widest clusters and the highest number of berries (19.47 cm, 12.05 cm, 186.9, respectively), while Cabernet Sauvignon had the shortest and narrowest clusters (15.88 cm, 8.23 cm, respectively), and Blatina had the fewest berries per cluster (107.7). There is a very significant difference between the observed varieties for all yield indicators and cluster characteristics. The effect of the year is very significant for yield per vine, cluster length, and cluster width, but not significant for all other indicators. The combined effect of variety and year is significant for cluster length and very significant for cluster width and peduncle weight. For other indicators, the combined effect of variety and year is not significant.

Keywords: *Vitis vinifera, varieties, yield, year.*

Introduction

Grape yield per unit area is a fundamental indicator of a variety's fertility and depends on a multitude of factors. Foremost among these is the variety itself, followed by the climatic and soil conditions of the cultivation area, and finally, the level of applied agronomic and ampelotechnical measures in the vineyard (Jovanović-Cvetković et al., 2008). The fertility of the buds is expressed through the average number of blooms or clusters per pruned bud. The significance of this indicator lies in the fact that, based on it and the average cluster mass, the number of buds that should be left per vine to achieve a specific, desired grape yield is determined. Kojić and Lasić (2002) state that cluster characteristics such as shape, size, and mass largely depend on the biological traits of the variety, and for this reason, these features are used for ampelographic identification of varieties. The mechanical composition of the cluster and berry involves the absolute and relative proportion of individual parts within the cluster and berry (Marković and Pržić, 2020). This composition is an important technological and ampelographic feature of each variety and is of great importance in assessing the quality of grapes as a raw material for processing. Parameters determined during the mechanical analysis of the cluster and berry include cluster length, width, and mass, number of berries in the cluster, peduncle mass, berry mass, skin, mesocarp, and seed mass, and the number of seeds in the berry. Researching parameters such as bud fertility, cluster characteristics, and the mechanical composition of the cluster and berry has multiple significances in optimizing yield, ampelographic identification of varieties, technological quality assessment, and improving agronomic practices. The aim of this study is to compare different grapevine varieties based on yield indicators and cluster characteristics (such as cluster mass, cluster length, number of berries per cluster, and peduncle mass) over various years. The results will help increase productivity and quality in viticulture, enabling producers to better understand which varieties are best suited to specific growing conditions.

Materials and methods

The field experiment was set up in the production vineyards of AD Popovo Polje-Dubljani and AD Agrofin Petrovo Polje, located in the Trebinje area (Bosnia and Herzegovina). The experimental plots belong to the Herzegovina region, specifically the sub-regions of the middle Neretva and Trebišnjica and the Mostar wine-growing region. The material used in these studies included the autochthonous grape variety Blatina and varieties for the production of high-quality red wines: Vranac, Merlot, and Cabernet Sauvignon. All tested varieties were grafted onto the *Vitis berlandieri x Vitis riparia* Kober 5BB rootstock. The study of the Blatina variety's characteristics was conducted in a vineyard established in 2013, while the study of the Vranac, Merlot, and Cabernet Sauvignon varieties was conducted in a vineyard established in 2004. The planting distance for all four varieties was 2.8×1 m. The Blatina vineyard covers an area of 3.16 ha and is the only large vineyard with this variety in the Trebinje area. The study of the characteristics of the examined grape varieties was carried out during the years 2016, 2017, and 2018. The altitude of the plot where the Vranac, Merlot, and Cabernet Sauvignon varieties were grown is 269 m, located at Petrovo Polje, while the altitude of the plot where Blatina was grown is 242 m, located at Popovo Polje. The row direction for all studied varieties is north-south. The number of buds per vine for all studied varieties is 14. During the grape harvest period, the yield (kg/vine) and the number of clusters per vine were determined by measuring the mass of all clusters from each vine, and then the yield per unit area was calculated. In laboratory conditions, the structural composition of the cluster was determined, including the length (cm), width (cm), and mass of the cluster (g), the number, mass (g), and percentage of berries (%), and the mass (g) and percentage of the rachis (%). The mass of the components of the cluster and berry was measured on an analytical scale, ADL 600, with an accuracy of 1/10 g. The length and width of the cluster were measured using a caliper, "Meba" brand.

Results and discussion

The average values of yield indicators and cluster characteristics are presented in Table 1. The highest yield per vine was recorded in the Blatina variety (4.70 kg), while the lowest was in Cabernet Sauvignon (2.99 kg). The Cabernet Sauvignon variety had the highest number of clusters per vine (23.57), while Vranac had the fewest (15.0). In terms of cluster mass and rachis weight, the Vranac variety had the highest values (350.49 g and 12.37 g), while Cabernet Sauvignon had the lowest (149.17 g and 6.31 g). The Merlot variety had the longest and widest clusters and the highest number of berries per cluster (19.47 cm, 12.05 cm, 186.9), while Cabernet Sauvignon had the shortest and narrowest clusters (15.88 cm and 8.23 cm), and Blatina had the fewest berries per cluster (107.7). The Blatina variety showed the greatest variation in yield per vine, cluster mass, and number of berries per cluster (Cv=38.76%, Cv=42.99%, Cv=43.77%), which is considered a consequence of its uneven pollination (due to its functionally female flower type). The greatest variation in cluster length and rachis weight was observed in the Merlot variety (Cv=19.12%, Cv=46.44%). Regarding cluster width, the highest coefficient of variation was found in the Cabernet Sauvignon variety (Cv=29.68%), while Vranac showed the greatest variation in the total number of clusters (Cv=31.56%). In his research, Rogić (2009) reports that the average yield per vine of the

Blatina variety was 1.16 - 2.07 kg, and the average number of clusters per vine was 9.3 - 10.4. Examining six varieties of Cabernet Sauvignon, Matthew et al. (2006) found that the yield per vine varied between 1.8 - 2.3 kg, and Garić and Vukosavljević (2016) report that the average yield per vine for the same variety ranged from 1.81 - 2.85 kg, and the average number of clusters per vine was 21 - 38. Kraljević (2013) in his work states that the average yield of the Vranac variety under different vine load conditions was from 4.63 - 7.03 kg, while Milanov et al. (2014) highlight that the average number of clusters per vine for the Vranac variety was from 10.4 - 20.8, and the average yield per vine was 2.61 - 5.21 kg. Garić et al. (2010) report that the average yield per vine for the Merlot variety was 1.92 kg. The results of our research are consistent with those of Kraljević (2013), Milanov et al. (2014), and Garić and Vukosavljević (2016), while they differ from the values reported by Rogić (2009), Matthew et al. (2006), and Garić et al. (2010).

Table 1. Descriptive statistics for yield and grape cluster characteristics of studied varieties (2016-2018)

Proprety/variety	**Blatina**		**Vranac**		**Merlot**		**Cabernet Sauvignon**	
	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)	$\overline{X}$	Cv (%)
Yield per vine (kg)	4.70	38.76	4.58	31.50	3.91	34.35	2.99	26.79
Total number of clusters	17.33	28.48	15.00	31.56	18.27	29.98	23.57	23.63
Cluster mass (g)	302.52	42.99	350.49	28.35	255.22	40.11	149.17	40.64
Cluster length (cm)	16.43	18.80	18.03	17.19	19.47	19.12	15.88	12.95
Cluster width (cm)	11.44	15.25	10.53	18.95	12.05	24.51	8.23	29.68
Number of berries per cluster	107.70	43.77	147.57	33.81	186.90	42.03	120.47	28.64
Rachis mass (g)	9.43	44.16	12.37	37.28	7.35	46.44	6.31	37.00

Analysis of variance revealed that there is a very significant difference among the observed varieties for all yield indicators and cluster characteristics. The impact of the year is very significant for yield per vine, cluster length, and cluster width ($p<0.01$), but it is not significant for all other indicators ($p>0.05$). The combined effect of variety and year is significant for cluster length ($0.01<p<0.05$), and very significant for cluster width and rachis mass ($p<0.01$). The combined effect of variety and year is not significant for the other indicators (Table 2). The Dunnett test determined that the Blatina variety has significantly higher values for yield per vine, cluster mass, cluster width, and rachis mass compared to the Cabernet Sauvignon variety. The total number of clusters in Blatina is statistically significantly lower than in Cabernet Sauvignon. Comparing the Blatina variety with the Merlot variety, the differences are not significant except for cluster length and number of berries per cluster, where the Blatina variety has significantly lower values for these indicators. The Blatina and Vranac varieties differ significantly in the number of berries per cluster and very significantly in rachis mass. There are no significant differences for the other indicators (Table 2). Cluster mass depends on several factors, the most significant of which are cluster length and width, and the mass and number of berries per cluster. The results of our research for the Blatina variety partially differ from those of Blesić (2001), who reports an average cluster mass of 250.39 g, from Rogić (2009), who studied the impact of defoliation on the quality of Blatina grapes and found that the average cluster mass ranged from 125.00 to 189.88 g, and from Jovanović-Cvetković et al. (2015), who report an average mass range of 171.65 - 307.32 g. Kojić et al. (2010) observed a significant impact of location on the cluster weight of the Blatina variety, with average values ranging between 537.90 and 200.10 g, which is consistent with our results. The results regarding the average cluster mass of the Vranac, Merlot, and Cabernet Sauvignon varieties partially differ from the results of previous studies by Milosavljević (2012), who states that Merlot has an average cluster mass of 40 to 150 g, Vranac 150 to 300 g, and Cabernet Sauvignon 60 to 130 g. They also differ from the results of Avramov and Žunić (2001), who report that the Merlot variety had an average cluster mass of 105.10 – 150.4 g, and Cabernet Sauvignon 90.40 – 130.10 g, and from Matthew et al. (2006), who state that the cluster mass of Cabernet Sauvignon varies from 171.7 g to 295.1 g. The results of our research are consistent with Banjanin et al. (2018), who report that the average cluster mass of the Vranac variety is 351.6 - 359.3 g, and Cabernet Sauvignon 105.1 - 163.1 g. Analyzing the cluster masses of the Blatina, Vranac, Merlot, and Cabernet Sauvignon varieties over the years of study and comparing them with literature data, certain discrepancies can be observed. One reason is the influence of ecological factors of the cultivation site and the response of the varieties to the applied agronomic and ampelotechnical measures.

Table 2. Statistical significance of the impact of variety and year on yield and grape cluster characteristics of the studied varieties

	Yield per vine	**Total number of clusters**	**Cluster mass**	**Cluster length**	**Cluster width**	**Number of berries per cluster**	**Rachis mass**
ANOVA *F.quotient*.							
Variety	10.86^{**}	15.28^{**}	22.89^{**}	10.00^{**}	19.93^{**}	12.71^{**}	17.15^{**}
Year	13.07^{**}	2.57^{ns}	1.32^{ns}	6.73^{**}	8.45^{**}	2.05^{ns}	1.46^{ns}
Variety x year	2.78^{ns}	1.38^{ns}	1.94^{ns}	2.50*	3.97^{**}	1.48^{ns}	3.03^{**}
DUNNETT-test							
Blatina	4.70	17.33	302.52	16.43	11.44	107.70	9.43
Vranac	4.58^{ns}	15.00^{ns}	350.49^{ns}	18.03^{ns}	10.53^{ns}	147.57^{*}	12.37^{**}
Merlot	3.91^{ns}	18.27^{ns}	255.22^{ns}	19.47^{**}	12.05^{ns}	186.90^{**}	7.35^{ns}
Cabernet Sauvignon	2.99^{**}	23.57^{**}	149.17^{**}	15.88^{ns}	8.23^{**}	120.47^{ns}	6.31^{**}

*Not significant for p> 0.05; *for p< 0.05; **for p< 0.01.*

Table 3. Statistical significance of the impact of year on yield and grape cluster characteristics of the studied varieties

Year		**Yield per vine**	**Cluster length**	**Cluster width**
		Mean differences		
2016	2017	-1.05^{**}	-2.16**	-1.16*
	2018	-0.92^{**}	-0.39^{ns}	0.71^{ns}
2017	2016	1.05^{**}	2.16**	1.16*
	2018	0.13^{ns}	1.77*	1.87**
2018	2016	0.92^{**}	0.39^{ns}	-0.71^{ns}
	2017	-0.13^{ns}	-1.77*	-1.87**

*Not significant for p> 0.05; *for p< 0.05; **for p< 0.01.*

The impact of the year on yield indicators and cluster characteristics was analyzed using Tukey's HSD test. The impact of the year is very significant for yield per vine, cluster length, and cluster width, but not significant for all other indicators (Table 3). The yield per vine was significantly lower in 2016 compared to 2017 and 2018, while the differences between 2017 and 2018 were not significant. The cluster length was significantly greater in 2017 than in 2016 and significantly greater than in 2018. There is no significant difference in cluster length between 2016 and 2018. The cluster width in 2017 was statistically significantly greater than in 2016 and very significantly greater than in 2018. There is no significant difference in cluster width between 2016 and 2018.
When observing the combined effect of variety and year on cluster characteristics, it is noted that the cluster length of the Blatina variety in 2018 is significantly shorter than that of the Vranac variety and very significantly shorter than that of the Merlot variety (Table 4). The cluster width of the Blatina variety in 2016 is significantly greater than that of the Vranac variety and very significantly greater than that of the Cabernet Sauvignon variety. In 2017, the cluster width of the Blatina variety is very significantly smaller than that of the Merlot variety, while in 2018, it is very significantly greater than that of the Cabernet Sauvignon variety. The rachis mass of the Blatina variety in 2016 is significantly greater than that of the Merlot variety and very significantly greater than that of the Cabernet Sauvignon variety. The differences in rachis mass in 2017 between the Blatina variety and the other varieties are not statistically significant, while in 2018, the Blatina variety has a very significantly lower rachis mass than the Vranac variety.

Table 4. Statistical significance of the combined impact of variety and year on grape cluster characteristics of the studied varieties

Proprety/variety			**Cluster length**	**Cluster width**	**Rachis mass**
			Mean differences		
2016	Blatina	Vranac	-0.64^{ns}	2.47*	-2.59^{ns}
		Merlot	-0.14^{ns}	1.09^{ns}	4.88*
		Cabernet Sauvignon	0.86^{ns}	5.36**	5.99**
2017	Blatina	Vranac	-0.52^{ns}	-1.01^{ns}	0.34^{ns}
		Merlot	-2.69^{ns}	-3.73**	2.51^{ns}
		Cabernet Sauvignon	2.07^{ns}	0.20^{ns}	3.33^{ns}
2018	Blatina	Vranac	-3.62^{*}	1.27^{ns}	-6.59**
		Merlot	-6.28^{**}	0.83^{ns}	-1.15^{ns}
		Cabernet Sauvignon	-1.27^{ns}	4.09**	0.02^{ns}

*Not significant for p> 0.05; *for p< 0.05; **for p< 0.01.*

Conclusion

For yield indicators and cluster characteristics (yield per vine, cluster mass, cluster width, and rachis mass), the Blatina variety has significantly higher values than the Cabernet Sauvignon variety. The total number of clusters of the Blatina variety is statistically significantly lower than that of the Cabernet Sauvignon variety. Comparing the Blatina variety with the Merlot variety, the differences are not significant except for cluster length and the number of berries per cluster, where the Blatina variety has significantly lower values for these indicators. The Blatina and Vranac varieties differ significantly in the number of berries per cluster and very significantly in rachis mass. For other indicators, the differences are not statistically significant. There is a very significant difference between the observed varieties for all yield indicators and cluster characteristics. Yield per vine was significantly lower in 2016 than in

2017 and 2018, while the differences between 2017 and 2018 were not significant. The cluster length was significantly greater in 2017 than in 2016 and significantly greater than in 2018. There is no significant difference in cluster length between 2016 and 2018. The cluster width in 2017 was statistically significantly greater than in 2016 and very significantly greater than in 2018. There is no significant difference in cluster width between 2016 and 2018. The impact of the year is very significant for yield per vine, cluster length, and cluster width, but not significant for all other indicators. The combined effect of variety and year is significant for cluster length, and very significant for cluster width and rachis mass. The combined effect of variety and year is not significant for the other indicators. The presented results provide grape growers and winemakers with important information that can assist in making decisions regarding the cultivation of different grape varieties, optimizing yields, and improving grape quality, all of which directly impact wine production.

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THE YIELD OF POTATOES TESTED IN MULTIPLE ENVIRONMENTS IN REPUBLIKA SRPSKA (BOSNIA AND HERZEGOVINA)

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Abstract

Potato is one of the main cultivated crops and ranks fourth in terms of sown areas worldwide, after wheat, maize, and rice. By selecting suitable cultivars and applying modern farming techniques, it is possible to achieve high yields.Research on five commercial potato cultivars (Agria, Faluka, Kennebec, Kuroda, and Desiree) was conducted during 2016 and 2017 in the field conditions of three locations (East Sarajevo, Bijeljina, and Rogatica - Borike) in the territory of Republika Srpska. The selected areas are located at different altitudes (from 90 to 1100 meters above sea level). The obtained results were analyzed using Analysis of Variance in the two-factorial design in statistical packages GenStat 12th and Microsoft Excel 10, and significance of differences between the mean values were compared in LSD test. The genotype by environment interaction and the stability were determined using the AMMI model. The cultivars and environment significantly influenced potato yield. Cultivars Agria and Kurada had the highest yields in the Rogatica-Borike area, while cultivars Faluka, Kennebec, and Desiree performed best in the Bijeljina area. Conversely, East Sarajevo showed the lowest yields for all cultivars except cultivars Desiree. In these studies, yield variability was most influenced by the environment (42.67%), followed by cultivars (34.54%), and genotype by environment interactions (22.79%). The first principal component (PCA1) explained 71.75% and the second (PCA2) 27.99% of the interaction. This research provides insights into the stability and adaptability of the tested potato cultivars in our agroecological conditions, suitability of regions for potato cultivation, and potential benefits of exploiting G *x* E interactions to increase tuber yields.

Key words: *AMMI analysis, environment, genotip, interaction, potato, yield.*

Introduction

Thanks to the existence of numerous varieties adapted to different ecological conditions, the potato is a cosmopolitan plant species grown around the world, from 47°N to 65°S latitude, though the most favorable cultivation area is considered to be the temperate zone (40–60°N). In the Republic of Srpska, the average production of commercial potatoes takes place on an area of about 15,000 ha, with an average yield of 11.9 t ha-1 (http://www.rzs.rs.ba). In addition to tuber yield, assessing the stability of varieties is crucial for evaluating their true value, along with other agronomically important characteristics. If the productivity and quality parameters of a cultivars do not change or are approximately the same under different environmental conditions, it can be said that the cultivars is stable. The concept of biological or static stability implies that a stable cultivars has minimal variation across different growing regions (Becker and Leon, 1988). Depending on external environmental conditions, varieties exhibit different reactions, meaning that varieties interact with the environment in which they are grown, and the extent of this interaction is determined by genetic composition and the intensity of environmental factors. Due to the specific combination of predictable and unpredictable factors, it is important to understand the varieties' reactions to environmental conditions (Gehan and Hala, 2013), which is crucial for successful breeding. To assess varieties' adaptability to environmental conditions, many statistical models are applied to analyze genotype-environment interactions (Rodrigues et al., 2008). Multivariate biometric methods (AMMI method) are used for data processing. This method is considered the most suitable model for evaluating genotype-environment interactions because it has significant predictive value when selecting the most stable and adaptable varieties, as well as in identifying mega-environments. Unstable and non-adaptable varieties and environments are excluded from further production, while stable varieties and environments are recommended for continued production. The significance of the AMMI method lies in determining genotype stability across different agroecological regions, grouping genotypes based on adaptability, identifying locations with the most favorable conditions based on average yields, detecting positive and negative specific interactions between genotypes and locations, and facilitating the selection of locations (Perišić, 2016).

Materials and Methods

The research was conducted over two growing seasons (2016 and 2017) at three locations: in the territory of the City of East Sarajevo (experimental field of the Faculty of Agriculture); on a private property (Perković family) in Bijeljina (village of Kojičinovac); and on the property of Solanum Produkt in Rogatica (Borike). The selection of locations was influenced by abiotic factors characteristic of each site: meteorological conditions, significant differences in altitude, differences in soil type and fertility, which can significantly affect the yield components, yield, and tuber quality. The experiment used five commercial potato varieties that are significantly represented in production in Bosnia and Herzegovina: Agria, Faluka, Kennebec, Kuroda, and Désirée. The experiment was set up as a two-factorial design in a randomized block design with four replications, with a layout of 53,333 plants. The area of the basic plot was 15 m² (four rows 5 m long with 20 plants per row; row spacing 0.75 m and plant spacing within the row 0.25 m). For determining tuber yield, samples were taken at the technological maturity of the potatoes, with ten boxes from the middle rows of each replication, and yield was calculated per hectare (t ha^{-1}). Basic data about the studied locations. The experimental field of the Faculty of Agriculture East Sarajevo is located at 550 m a.s.l. (43°49′01″ N, 18°20′57″ E). The ecological conditions in East Sarajevo are characterized by strong continental climate influences. The average annual temperature is 10.2°C, and the average amount of precipitation is around 900 mm. The experiment was set up on alluvial soil (fluvisol). The second location, Kojičinovac, is situated at 44°46′02″ N; 19°14′01″ E at 90 m a.s.l. This location is characterized by a moderately continental climate. The average annual temperature is 11°C, and the average amount of precipitation is around 850 mm. The experiment was set up on semi-gley soil. The company Solanum Produkt grows potatoes at 1100 m a.s.l., 43°51′16″ N; 19°05′57″ E. It is characterized by a mountain climate with heavy snowfalls, which last until late spring. The average annual temperature is 7°C, and the average amount of precipitation is around 1500 mm. The experiment was set up on acidic brown soil (dystic cambisol).

Table 1. Chemical analysis of soil

Locality	Depth (cm)	pH/H_2O	pH/KCl	Humus %	N %	Soluble mg 100 g^{-1} P_2O_5	K_2O
East Sarajevo	0–30	7,16	6,39	4,12	0,27	41	36,41
Bijeljina	0–30	7,67	6,61	4,66	0,3	> 40	36,91
Rogatica - Borike	0–30	5,02	3,85	2,55	0,16	8,98	25,38

The optimal soil pH value for potatoes is slightly acidic, ranging from 5.6 to 6.5 (Morar, 2008). Potatoes require high soil permeability, as tubers become deformed in compacted soils. Potatoes need well-drained soil, as waterlogged soils lead to numerous physiological changes in the tubers, making them watery and difficult to store. For successful potato production, the content of organic matter is crucial, as it improves the quality, structure, and water-holding capacity of the soil (Baniuniene and Zekaite, 2008).
Meteorological Conditions. Based on the data analysis in Table 2, it can be seen that in terms of temperature and precipitation, the year 2016 was considerably more favorable for growing potatoes at all three locations compared to 2017 and the multi-year average.

Table 2. Average Monthly Air Temperatures (°C) and Precipitation Amounts (mm) During the Experiment and Multi-Year Average

	Months / Season		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average/ Sum
E. Sarajevo	2016	°C	1,2	7,4	7,1	12,9	13,9	19,5	21,1	18,6	15,6	9,8	6,1	-0,9	10,9
		mm	46,6	87	131,7	60,5	82,1	96,4	104,5	105,5	71,1	112,8	93,1	16,8	1008,1
	2017	°C	-4,8	5,2	8,5	9,2	15,3	20,3	21,8	22,6	15,5	10,4	5,4	2,2	11,0
		mm	57,9	69,1	43.6	132,4	73,8	55	66,5	38,7	93,2	89,3	74,9	142,9	937,3
	1981-	°C	-0,1	1,4	5,3	9,9	15,0	17,7	19,8	19,7	15,1	10,9	5,2	1,1	10,1
	2010.	mm	67	63	71	78	73	94	72	70	86	85	91	85	932
Bijeljina	2016	°C	2,0	8,1	8,2	13,8	16,6	22,0	23,5	21,2	18,4	10,6	7,0	0,8	12,68
		mm	70,5	50,7	120,5	61,8	86,4	120,7	84,8	66,0	52,3	58,1	64,4	3,2	839,4
	2017	°C	-4,9	5,2	10,6	11,6	17,9	23,2	24,6	24,7	17,0	12,6	7,4	4,7	12,88
		mm	35,3	45,1	42,4	92,8	67,5	39,9	47,3	35,4	102,3	60,9	45,7	64,3	678,9
	1981-	°C	0,3	1,9	6,9	11,8	16,9	19,9	22,1	21,2	17,2	11,6	6,0	2,3	11,5
	2010.	mm	54,1	43,5	59,7	66,1	68,2	100,0	74,6	60,7	55,8	67,6	64,0	63,9	778,2
Borike	2016	°C	-1,6	4,2	3,03	9,9	11,1	16,7	18,0	15,7	12,3	7,1	2,9	-2,7	8,1
		mm	59,1	57,8	186,4	72,7	70,4	158,9	130,3	95,7	67,3	117,7	107,7	10,7	1135
	2017	°C	-8,0	2,1	5,2	6,0	12,7	17,5	18,6	19,2	12,5	7,4	2,2	-0,7	7,9
		mm	50,6	51,2	43,8	165,1	51,7	90,0	97,8	16,6	85,8	121,7	67,9	107,7	949,9
	1981-	°C	-3,5	-1,9	2,4	6,9	11,8	14,7	17,0	16,3	12,5	7,7	1,9	-2,0	7,0
	2010.	mm	52,7	52,9	56,8	65,3	73,9	93,7	74,9	72,1	80,2	78,0	88,6	69,9	859,0

Statistical Analysis. The obtained data were processed statistically, using the method of analysis of variance (ANOVA), for individual comparisons the least significant difference test LSD test (significance level 0.01) was used. The additive main effect and multiplicative interaction (AMMI) method was used, where an integrated ANOVA and principal component analysis (PCA) were applied to analyse the MET (Zobel et al. 1988; Crossa et al. 1991). ANOVA was used to determine the main effects of genotypes and environments and the PCA for the residual multiplicative interaction amongst genotypes and environments. After detecting a significant G x E interaction, the data were graphically analysed by biplot technique to simultaneously classify genotypes and environments (Zobel et al. 1988). Through this method, cultivars with high productivity and wide adaptability, as well as cultivars with specific adaptability, were identified.

Results and Discussion

The tuber yield varied from 31.38 t ha^{-1} (Désirée) to 35.67 t ha^{-1} (Kennebec). The lowest yield was recorded for potatoes at the experimental field in East Sarajevo (31.87 t ha^{-1}), while the differences in yield between the experimental fields in Bijeljina (35.67 t ha^{-1}) and Rogatica – Borike (35 t ha^{-1}) were negligible (Tables 3 and 4).

Table 3. Analysis of variance for the genotype *x* environment interaction for average tuber yield of five commercial potato cultivars in the East Sarajevo, Bijeljina, and Rogatica - Borike trial sites during the 2016/17 potato growing season

	d.f.	Sum Sq	Mean Sq	F value	Pr(>F)
Repetition	3	11.066	3.689	1.56	
Environment	2	164.511	82.256	34.74	<.001
Genotip	4	133.167	33.292	14.06	<.001
Env×Gen	8	87.872	10.984	4.64	<.001
Residual	42	99.459	2.368		
Total	59	496.075			

Table 4. Variation of yield (t ha^{-1}) of potato varieties in different localities

Genotip	Environment			Average
	East Sarajevo	Bijeljina	Rogatica - Borike	
Agria	30.73^{e}	34.51^{bc}	37.84^{a}	34.36^{B}
Faluka	33.08^{cd}	36.60^{ab}	35.79^{b}	35.15^{AB}
Kennebec	33.87^{bc}	37.83^{a}	35.31^{bc}	35.67^{A}
Kuroda	31.34^{de}	35.57^{bc}	36.11^{ab}	34.34^{B}
Desiree	30.34^{e}	33.84^{c}	29.94^{e}	31.38^{C}
Average	31.87^{B}	35.67^{A}	35.00^{A}	34.18

The Désirée cultivars had the lowest yield at all locations, while the highest yield at the East Sarajevo and Bijeljina locations (33.87 t ha^{-1}; 37.83 t ha^{-1}) was achieved by the Kennebec cultivars, and at the Rogatica-Borike location (37.84 t ha^{-1}), the highest yield was achieved by the Agria cultivars. Tuber formation is influenced by temperature, with particularly unfavorable high night temperatures (optimal around 18°C, and unfavorable above 22°C). High temperatures lead to a higher percentage of smaller tubers, which affects potato yield (Kumar et al., 2003). Optimal daytime temperatures and lower nighttime temperatures favored tuber bulking in the Rogatica area, as did the more evenly distributed precipitation at this location during the growing season. Low precipitation amounts during the most critical stages of potato growth and development significantly reduce yield and worsen tuber quality. Considering the high air temperatures during July and August, it can be conclusively stated that, apart from higher air temperatures and soil temperatures, one of the limiting factors for plant growth and tuber bulking in potatoes is the insufficient amount and irregular distribution of precipitation during the growing period. The first growing season had more favorable temperature conditions as well as the quantity and distribution of precipitation during the growing season. In the second growing season, all three locations experienced reduced precipitation, which was unevenly distributed. At the East Sarajevo location, the lack of precipitation was from June to August; at Bijeljina from May to August; and at Rogatica part of July and August, which was reflected in the average potato yield, in agreement with results obtained by Hassanpanah (2011), Hassanpanah et al. (2011), and Siano et al. (2024). Environmental conditions for potato cultivation in certain areas are very diverse, and thus the responses of some varieties under these conditions vary. Breeding can overcome the adverse effects of environmental factors, especially soil quality, water and air regime of the soil, high temperatures, and short growing seasons in mountainous areas. Optimal temperatures for tuber formation and bulking are between 16 and 19°C (Barkley, 2005; Struik, 2007).

Interaction of Genotype x Environment. The environments in which the experiments were conducted were diverse, causing the majority of the variation in average potato yield (42.67% environment; 34.54% genotype; and 22.79% genotype *x* environment interaction).

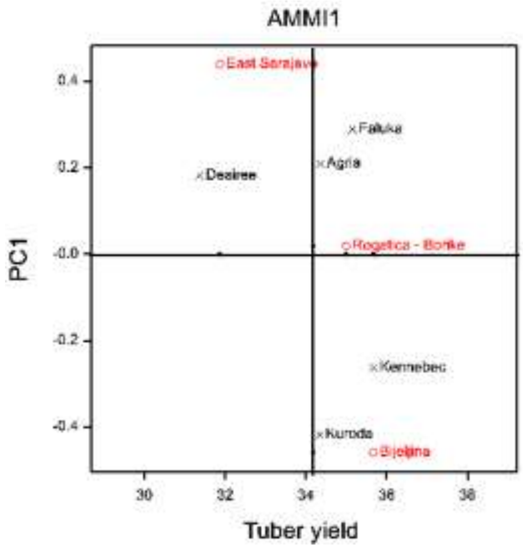

Fig. 1 Additive Main Efect and Multiplicative Interaction (AMMI1) biplot showing the Interaction Principal Component Analysis (PCA1) (Factor1) vs. estimated average tuber yield

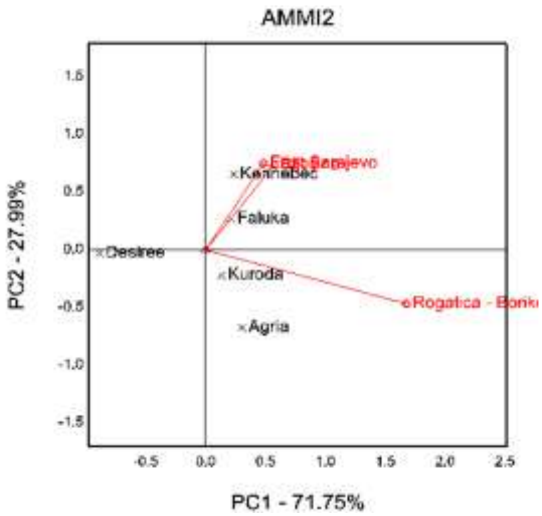

Fig. 2 AMMI2 biplot showing the frst two principal axes of interaction (PCA2 vs. PCA1) of five commercial potato cultivars evaluated in three environments in the Republic of Srpska during the 2016/17 growing season

The significant effect of the G x E interaction showed that genotypes performed differently in various environments. Stable genotypes have a lower impact on the G x E interaction and can be indicated by a lower IPCA1 value (Silveira et al. 2013). The Agria cultivars was ranked third based on average yield but exhibited the highest stability (broad adaptation) compared to other tested varieties. In contrast, Kuroda was identified as an undesirable genotype due to low productivity (ranked fourth) and low stability (IPCA1 values farthest from zero) (Gauch and Zobel 1988). The Désirée cultivars showed medium stability but had below-average yield, while the Faluka and Kennebec varieties had yields above average but displayed high instability. Genotypes and environments that are close to each other in the biplot have positive relationships, allowing for the formation of a group of genotypes within the agronomic zone. Thus, the Kennebec and Faluka genotypes performed better in the Bijeljina and East Sarajevo environments, while the Kuroda and Agria genotypes performed better in the Rogatica - Borike environment. The East Sarajevo environment is characterized by the fact that none of the tested varieties had a yield above the average tuber yield, indicating a stronger presence of abiotic stress. However, the Bijeljina and Rogatica – Borike environments only had below-average yield for the Désirée cultivars, indicating a weaker abiotic stress.

Conclusions

Multienvironmental research has shown that in the examined growing seasons, abiotic stresses associated with climate change, specifically heat and drought stress, have affected the yield of commercial potato varieties. The varieties displayed different sensitivities to abiotic stress. G x E analysis revealed location-specific adaptations among the tested commercial potato varieties. The Agria cultivar was the most stable and adaptable variety across different environments, while the Kuroda cultivar exhibited the lowest stability. The East Sarajevo environment is unsuitable for potato production, and this environment was not suitable for any of the tested cultivars. In the Republic of Srpska, such investigations have not been conducted so far, and there has been no effort to determine the most suitable areas for potato production. Additionally, no research has been done on selecting stable cultivars. Failures in the application of agrotechnical measures, poor choice of potato growing areas, and selection of unstable cultivars have contributed to the low average potato yields.

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APPLICATION OF DIFFERENT VARIANTS OF SUPERABSORBENTS AND THEIR IMPACT ON YIELD OF SELECTED POTATO VARIETIES

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Abstract

The ability to retain water in soil is influenced by a series of agronomic measures: selection of suitable plant varieties of specific genotypes, sowing dates, planting density, fertilization, inter-row cultivation, irrigation, and application of water conservation products. The ultimate goal of such substances, which began to be used in the 1950s, is to improve the soil's water regime. These materials can enhance the physical and chemical properties of soil, affect the content and availability of nutrients in the soil, positively influence soil microorganisms, and soil fertility. Field trials with seven potato varieties (Agria, Faluka, Kennebek, Marabel, Laura, Rudolph, and Memphis) involved six variants based on a hydrophilic superabsorbent: control variant, superabsorbent alone, superabsorbent enriched with growth stimulators, superabsorbent enriched with microorganisms (*Bacillus subtilis*), superabsorbent enriched with micronutrients, and superabsorbent enriched with growth stimulators, microorganisms, and micronutrients at a rate of 20 kg ha^{-1}. The field trials were arranged in three replications with a planting density of 41666 plants per hectare. The main goal of these studies was to determine the impact of superabsorbents of different compositions on the yield of potatoes. Unfavorable climatic conditions, including high average air temperatures and precipitation deficits during critical periods (June – August), resulted in lower average potato yields in 2019 compared to 2018. The highest average yield was achieved by the Rudolph variety (34.35 t ha^{-1}), while the lowest average yields were observed for the Laura (24.72 t ha^{-1}) and Kennebek (24.28 t ha^{-1}) varieties. The lowest yield was recorded in the control variant (24.77 t ha^{-1}), and the highest yield (34.64 t ha-^{1}) was in the variant with superabsorbent enriched with growth stimulators, microorganisms, and micronutrients.

Key words: *potato, variety, superabsorbent, year, yield.*

Introduction

Increasing potato yields can be achieved through the full application of agronomic practices and adherence to optimal agronomic timings, as well as better adaptation of varieties to agronomic and agroecological production conditions (Broćić and Stefanović, 2012). To ensure sufficient food supply for the human population, it is necessary to double the average yields by 2050 (Ray et al., 2013). Climate change has led to increased water demands in agricultural production, influencing the development of various models aimed at preserving soil moisture and reducing water consumption in agriculture. The World Meteorological Organization (Alcamo et al., 2007) notes that climate change is particularly intense in Southern Europe, which includes our country. In addition to the continuous trend of rising air temperatures, further reductions in precipitation, accompanied by lower soil moisture and availability of water resources, significantly impact agricultural production uncertainty and cause substantial reductions in yields per unit area. The issue of rational water use is of great importance, as water increasingly becomes a source of strategic significance (Bates et al., 2008). Water conservation in agriculture can be achieved through the use of various substances (polymers) that alter the properties of water and air, as well as the physical composition of soil. Water-insoluble polymers (hydrogels) were introduced into agricultural production in the 1980s. Hydrogels also increase water use efficiency and reduce the frequency of irrigation (Sivapalan, 2006) and enhance fertilizer use efficiency (El-Hady and Wanas, 2006). Superabsorbents contain biodegradable fragments within a network structure obtained through radical polymerization in the presence of a redox system. Humic acids, microorganisms, and microelements have been incorporated into the superabsorbent matrix. Raw materials containing starch, pectin, chitosan, and other polymer materials that decompose into water, carbon dioxide, and nitrogen during degradation are used to produce superabsorbents. Superabsorbents have demonstrated positive effects on yield increase in many field and vegetable crops in multi-year experiments conducted in Russia (yield differences between the control variant and the superabsorbent variant of up to 2.5 times), and importantly, superabsorbents do not pollute the soil or crops (Lukin and Maraeva, 2018; Lukin et al., 2017a; Lukin et al., 2017b; Kuznetsov et al., 2017; Lalić et al., 2019).

Materials and methods

Over a two-year period, experiments were conducted in the territory of the city of East Sarajevo, at an altitude of 550 m (43°49'01" N and 18°20'57" E). The agroecological conditions in East Sarajevo are characterized by the strong influence of the continental climate. The average annual temperature is 10.2 °C, and the average amount of precipitation is about 900 mm. The experiment was set on alluvial soil (fluvisol). Soil samples were taken from experimental parcels and after laboratory analysis it was established that alluvial soil from East Sarajevo has pH H_2O 6.63, it contains 3.62% humus, 0.23% N, and in 100 g^{-1} soil, it has 14.75 mg of soluble P_2O_5 and 15.59 mg of soluble K_2O. The experiment included seven varieties and applied 6 variants of superabsorbent at a rate of 20 kg ha^{-1}. Field trials were set up using a randomized block design with three replications, with a density of 41,666 plants per ha. The plot size was 15 m^2 (four rows, each 10 m long, with 25 plants per row, a spacing of 0.6 m between rows, and 0.4 m between plants within the row). For the needs of the experiment, classic agronomic measures were carried out: primary tillage (plowing), secondary tillage (disking after plowing), pre-sowing preparation (rotovation), and no mineral fertilizers were used. Primary soil tillage to a depth of 30 cm and secondary tillage were performed during the fall. Protection was applied depending on the appearance of pests and disease pathogens. At technological maturity of the potatoes, samples were taken from each main plot, consisting of ten hills. The average number of tubers, average tuber mass, and average tuber yield were determined, and the yield was then converted to t ha^{-1}. The obtained data were processed statistically, using the method of analysis of variance (ANOVA), for individual comparisons the least significant difference test LSD test (significance level 0.05) was used.

Table 1. Factors covered by field research

Factor	Treatments
Varieties (A)	A_1 – Agria A_2 – Faluka A_3 – Kennebek A_4 – Marabel A_5 – Laura A_6 – Rudolph A_7 – Memphis
Adsorbent (B)	B_0 – control variant B_1 – superabsorbent "Tverdaya Voda" B_2 – superabsorbent "Tverdaya Voda" enriched with growth stimulants B_3 – superabsorbent "Tverdaya Voda" enriched with microorganisms B_4 – superabsorbent "Tverdaya Voda" enriched with microelements B_5 –superabsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms and microelements
Growing seasons (C)	C_1 – 2018. year C_2 – 2019. year

Table 2. Average air temperatures and precipitation sumsfrom April to September at East Sarajevo

Months	Temperature (°C)		Precipitation (mm)	
	2018.	2019.	2018.	2019.
April	14.8	11.4	61.4	98.2
May	16.6	12.3	178.1	102.3
June	17.9	21.1	131.5	97.1
July	19.7	21.0	119.6	67.8
August	20.7	21.9	87.5	69.7
September	16.2	16.5	25.9	44.1
Average/Sum	17.7	17.4	604	479.2

Meteorological conditions during the test period are shown in Table 2. During the potato growing season, the average monthly temperatures in 2018 (17.7 °C) and the amount of precipitation (604 mm) were higher compared to 2019 (17.4 °C and 479.2 mm). In 2019, there was 124.8 mm less precipitation compared to 2018. Although 2018 had a higher average temperature, 2019 was characterized by higher average temperatures in July and August, along with lower precipitation, which affected tuber bulking.

Results and discussion

Number of Tubers: The number of tubers per plant is a productive trait influenced by the choice of variety, agronomic practices applied in potato production, and agroecological conditions. The number of tubers per plant was significantly influenced by the factors examined, as well as by the interactions between variety and superabsorbent application (AB) and variety and growing season (AC). The effects of variety, production conditions, and growing season on potato productive traits have been studied by Razzaque and Ali (2009) and Zhao et al. (2012), who obtained similar results. Genetic factors determine the potential for stolon formation, the number of tubers, and their morphological characteristics (Barčić et al., 2003). Poštić et al. (2015) confirmed that the number of tubers per plant is a varietal characteristic.

Table 3. Influence of Superabsorbent and Growing Season on the Number of Tubers per Plant for the Tested Varieties

Varieties (A)	Growing seasons (C)	Adsorbent (B)						Average
		B_0	B_1	B_2	B_3	B_4	B_5	
A_1	C_1	7,7	7,7	9,0	8,3	8,7	9,7	8,5
	C_2	7,2	7,2	8,5	7,8	8,2	9,2	8,0
Average		7,4	7,4	8,8	8,1	8,4	9,4	8,3
A_2	C_1	7,0	7,7	8,3	7,7	7,7	9,0	7,9
	C_2	6,7	7,4	8,0	7,4	7,4	8,6	7,6
Average		6,9	7,5	8,2	7,5	7,5	8,8	7,7
A_3	C_1	5,7	6,7	7,7	7,3	7,0	7,7	7,0
	C_2	5,9	6,9	7,9	7,5	7,2	7,9	7,2
Average		5,8	6,8	7,8	7,4	7,1	7,8	7,1
A_4	C_1	8,3	9,3	10, 7	8, 7	8,3	10,3	9,3
	C_2	7,7	9,7	11,1	9,1	8,7	10,7	9,5
Average		8,0	9,5	10,9	8,9	8,5	10,5	9,4
A_5	C_1	8,0	8,3	8, 7	7,3	8,3	9,3	8,3
	C_2	7,0	8,0	8,4	7,0	8,0	9,0	7,9
Average		7,5	8,2	8,5	7,2	8,2	9,2	8,1
A_6	C_1	11,0	11,3	12,7	10,7	11,3	13,3	11,7
	C_2	10,4	10,7	12,1	10,1	10,7	12,7	11,1
Average		10,7	11,0	12,3	10,4	11,0	13,0	11,4
A_7	C_1	8,7	8,3	10,3	8,7	10,0	10,0	9,3
	C_2	7,8	7,4	9,4	7,8	9,1	9,1	8,4
Average		8,2	7,9	9,9	8,2	9,6	9,6	8,9
Average	C_1	8,0	8,5	9,6	8,4	8,8	9,9	8,9
	C_2	7,5	8,2	9,3	8,1	8,5	9,6	8,5
Average		7,8	8,3	9,5	8,2	8,6	9,8	8,7

	A	B	C	AB	AC	BC	ABC
$LSD_{0,05}$	0,3155	0,2921	0,1687	0,7729	0,4462	0,4131	1,0930

The variety Rudolph achieved the highest average number of tubers per plant (11.4), followed by Marabel (9.4), Memphis (8.9), Agria (8.3), and Laura (8.1). The lowest number of tubers per plant was found in the varieties Faluka (7.7) and Kennebek (7.1). The variety Kennebek had a significantly lower average number of tubers per plant compared to all other tested varieties. Reduced water use efficiency and the occurrence of water stress affect the reduction of yield components, including the number of tubers per plant, as confirmed by Alva et al. (2012). The use of the superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements achieved the highest average number of tubers (9.8), whereas the control variant had the lowest (7.8). These differences were highly significant, as were the differences between the control variant and all other variants applied in the experiment. The superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements had a significantly higher number of tubers per plant compared to the variants where superabsorbent "Tverdaya Voda" was used alone (8.3), with microorganisms (8.2), with microelements (8.6), and significantly more compared to the variant with superabsorbent "Tverdaya Voda" enriched with growth stimulators (9.5). The number of tubers per plant is also influenced by meteorological conditions, as confirmed by Van Dam et al. (1996). These authors found a higher number of tubers per plant at lower temperatures. Observing by years, the average number of tubers per plant was higher in 2018 (8.9) compared to 2019 (8.5). In 2018, the average monthly temperatures in June (17.9 °C) and July (19.7 °C) were lower than the temperatures in June (21.1 °C) and July (21 °C) of 2019. The varieties Agria, Faluka, Kennebek, and Marabel had the smallest number of tubers per plant in the control variant, while Laura and Rudolph had the smallest number in the variant with the superabsorbent "Tverdaya Voda" enriched with microorganisms. The variety Memphis had the smallest number of tubers per plant in the variant with superabsorbent "Tverdaya Voda." The highest number of tubers per plant in the variant with the superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements was achieved by the varieties Agria, Faluka, Laura, and Rudolph, while Marabel achieved the best results for the number of tubers per plant with the variant using superabsorbent "Tverdaya Voda" enriched with growth stimulators. The variety Kennebek had the highest number of tubers per plant in the variants with superabsorbent "Tverdaya Voda" enriched with growth stimulators and superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements, while the variety Memphis had the highest number of tubers per plant in the variants with superabsorbent "Tverdaya Voda" enriched with microelements and superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements. The varieties Agria, Faluka, Laura, Rudolph, and Memphis had a higher number of tubers per plant in the first growing season, while Kennebek and Marabel had a higher number in the second growing season. For some varieties, higher temperatures can induce branching of primary stolons and create more sites for tuber formation, as confirmed by Struik et al. (1989).

Tuber Mass: Tuber size is a varietal characteristic but is also influenced by ecological factors, agronomic practices, the number of tubers per plant, the formation of hills, and stolon length (Singh and Ahmed, 2008). The average tuber mass was 81.70 g. The varieties Agria (92.71 g) and Faluka (92.97 g) had the highest average tuber masss, while Laura (72.74 g) and Rudolph (71.92 g) had the lowest. The highest tuber mass was observed in the variants with superabsorbent "Tverdaya Voda" enriched with growth stimulators (85.21 g) and superabsorbent "Tverdaya Voda" enriched with growth stimulators, microorganisms, and microelements (85.66 g). All variants with different combinations of superabsorbent "Tverdaya Voda" had significantly higher average tuber masss compared to the control variant. Significant differences were also found between the different combinations of superabsorbent "Tverdaya Voda" in terms of average tuber mass. Application of Superabsorbent Polymers affects the increase in water absorption and retention in the soil, the storage of essential nutrients for plant development, and the improvement of soil structure. This positively impacts plant growth and yield (Nazarli et al., 2010), whereas in the control variant (without the use of superabsorbent polymers), dry years may lead to a reduction in average tuber mass (Liu et al., 2006). In 2018, the average tuber mass was significantly higher compared to 2019. This was influenced by optimal daytime temperatures and lower nighttime temperatures that favored tuber bulking, as well as more evenly distributed rainfall during the 2018 growing season. The obtained results are consistent with findings by Poštić (2013), who established that high temperatures and a deficit of rainfall during critical periods for potato development significantly affect the average tuber mass. The smallest average tuber masss were observed in the control variants, except for the Marabel variety, which had the smallest tubers in the variant with superabsorbent "Tverdaya Voda".

Table 4. Influence of Superabsorbent and Growing Season on the Average Tuber Mass (g) for the Tested Varieties

Varieties (A)	Growing seasons (C)	Adsorbent (B)						Average
		B_0	B_1	B_0	B_1	B_0	B_1	B_0
A_1	C_1	90,90	92,84	95,36	91,75	92,66	98,01	93,59
	C_2	87,65	89,59	95,94	92,33	91,65	93,80	91,83
Average			91,21	95,65	92,04	92,16	95,90	92,71
A_2	C_1	87,95	92,66	97,82	92,37	93,62	101,15	94,26
	C_2	83,74	88,45	95,39	90,83	92,08	99,61	91,68
Average			90,55	96,61	91,60	92,85	100,38	92,97
A_3	C_1	76,82	83,98	88,16	79,68	82,12	86,78	82,92
	C_2	75,28	85,03	89,21	76,39	76,67	81,33	80,65
Average			84,51	88,69	78,03	79,40	84,06	81,79
A_4	C_1	76,53	71,14	83,14	77,83	82,07	84,83	79,25
	C_2	78,74	73,35	85,35	80,04	81,09	83,85	80,40
Average			72,25	84,24	78,93	81,58	84,34	79,83
A_5	C_1	65,20	70,65	77,36	74,65	73,13	77,38	73,06
	C_2	64,93	71,80	78,51	74,46	70,26	74,51	72,41
Average			71,23	77,93	74,56	71,69	75,94	72,74
A_6	C_1	67,93	69,23	72,22	69,58	75,81	78,46	72,21
	C_2	67,63	70,21	72,25	67,71	73,94	78,01	71,63
Average			69,72	72,24	68,65	74,88	78,24	71,92
A_7	C_1	76,26	77,84	81,49	81,72	80,21	81,22	79,79
	C_2	78,67	80,25	80,70	80,93	79,42	80,37	80,05
Average			79,04	81,09	81,32	79,81	80,80	79,92
Average	C_1	77,37	79,76	85,08	81,08	82,80	86,83	82,15
	C_2	76,66	79,81	85,34	80,38	80,73	84,50	81,24
Average			79,79	85,21	80,73	81,77	85,66	81,70
	A	B	C	AB	AC	BC	ABC	
$LSD_{0,05}$	1,860	1,722	0,994	4,557	2,631	2,436	6,444	

The largest tubers were found in varieties Agria, Marabel, and Memphis in the variants with superabsorbent "Tverdaya Voda" enriched with growth stimulants and superabsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms, and microelements. The varieties Kennebek and Laura had the largest tubers in the variant with superabsorbent "Tverdaya Voda" enriched with growth stimulants, while the varieties Faluka and Rudolph had the largest tubers in the variant with superabsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms, and microelements. **Tuber Yield.** Yield is the most important quantitative characteristic and is subject to significant influences from agronomic and agroecological factors, making it variable. One of the primary goals of modern intensive agriculture is to increase yield. Special attention is given to varieties with high and consistent yield potential under different agroecological conditions. Previous research by Momirović et al. (2016) has highlighted the significant impact of variety and production conditions on tuber yield, while Esehaghbeygi (2010), Ayas (2013), Salavati et al. (2018), and Starovoitova et al. (2019) have demonstrated the significant effect of superabsorbent polymers (superabsorbents) on potato yield. Results presented in Table 5 show that the highest average tuber yield was achieved with the Rudolph variety (34.35 t ha^{-1}), followed by Agria (31.93 t ha^{-1}), Marabel (31.29 t ha^{-1}), Faluka (30.08 t ha^{-1}), and Memphis (29.62 t ha^{-1}).

Table 5. Influence of Superabsorbent and Growing Season on the Average Potato Yield (t ha^{-1}) for the Tested Varieties

Varieties (A)	Growing seasons (C)	Adsorbent (B)						Average
		B_0	B_1	B_0	B_1	B_0	B_1	B_0
A_1	C_1	28,99	29,68	35,76	31,88	33,41	39,47	33,20
	C_2	26,13	26,77	33,98	30,16	31,11	35,82	30,66
Average			28,23	34,87	31,02	32,26	37,65	31,93
A_2	C_1	25,71	29,65	33,93	29,50	29,84	37,90	31,09
	C_2	23,43	27,20	31,91	27,88	28,19	35,80	29,07
Average			28,43	32,92	28,69	29,02	36,85	30,08
A_3	C_1	18,11	23,37	28,15	24,39	23,93	27,74	24,28
	C_2	18,37	24,37	29,23	24,00	22,98	26,68	24,27
Average			23,87	28,69	24,20	23,46	27,21	24,28
A_4	C_1	26,58	27,39	36,95	28,14	28,52	36,55	30,69
	C_2	25,13	29,47	39,35	30,27	29,53	37,53	31,88
Average			28,43	38,15	29,21	29,02	37,04	31,29
A_5	C_1	21,77	24,55	27,97	22,82	25,42	30,13	25,44
	C_2	19,00	24,05	27,40	21,85	23,54	28,08	23,99
Average			24,30	27,69	22,34	24,48	29,10	24,72
A_6	C_1	31,12	32,69	38,13	30,92	35,86	43,57	35,38
	C_2	29,34	31,40	36,32	28,39	33,13	41,35	33,32
Average			32,04	37,23	29,65	34,49	42,46	34,35
A_7	C_1	27,58	26,99	35,08	29,56	33,45	33,85	31,09
	C_2	25,50	24,82	31,71	26,24	30,14	30,48	28,15
Average			25,90	33,40	27,90	31,80	32,17	29,62
Average	C_1	25,69	27,76	33,71	28,17	30,06	35,60	30,17
	C_2	23,84	26,87	32,84	26,97	28,38	33,68	28,76
Average			27,31	33,28	27,57	29,22	34,64	29,47
	A		B	C	AB	AC	BC	ABC
$LSD_{0,05}$	1,283		1,188	0,686	3,142	1,814	1,680	4,444

The lowest average yields were observed with the Laura (24.72 t ha^{-1}) and Kennebek (24.28 t ha^{-1}) varieties. The lowest yield was recorded in the control variant (24.77 t ha^{-1}), and the highest yield (34.64 t ha^{-1}) was achieved in the variant with superabsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms, and microelements. In 2018, the tuber yield (30.17 t ha^{-1}) was significantly higher compared to the yield in 2019 (28.76 t ha^{-1}). Adverse climatic conditions, characterized by high average temperatures and a deficit of rainfall during the critical period (June–August) for tuber formation and bulking, led to a lower average tuber yield in 2019, consistent with findings by Lahlou et al. (2003) and Tomasiewicz et al. (2003).

The lowest tuber yields for the Agria, Faluka, Kennebek, Marabel, and Laura varieties were observed in the control variant, while the Rudolph variety had the lowest yield in the variant with superabsorbent "Tverdaya Voda" enriched with microorganisms. The Memphis variety had the lowest yield in the variant with superabsorbent "Tverdaya Voda". All varieties had the highest yield in plots where superabsorbent "Tverdaya Voda" enriched with growth stimulants, microorganisms, and microelements was applied, except for Kennebek and Memphis varieties, which had the highest yield in plots with superabsorbent "Tverdaya Voda" enriched with growth stimulants. In the first growing season (2018), varieties generally had higher yields, except for the Marabel variety, which had a higher yield in the second season (2019).

Conclusions

Meteorological conditions during the trials (2018–2019) varied significantly, which affected the development of the productive traits of potatoes. The effects of variety, superabsorbent application, growing season, and their interactions were found for most of the examined traits. Superabsorbent polymers increase the percentage of water and nutrients in the soil for a longer period, allowing for more uniform and even water use by the crop, and promoting faster and healthier crop growth, especially in very hot and dry areas, as well as on light and sandy soils. Since their application has not been sufficiently studied in our regions, it is necessary to continue trials not only with potatoes but also with other crops, in order to address issues arising in plant production due to soil moisture deficits and irrigation problems.

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ENDANGERED AND PROTECTED PLANT SPECIES OF THE RAMSAR SITES OF BOSNIA AND HERZEGOVINA

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Abstract

The Ramsar sites of Bosnia and Herzegovina, as part of internationally recognized wetland ecosystems, represent vital ecological resources with rich biodiversity. These areas face various threats that endanger their biodiversity. This paper presents the diversity of endangered and protected plant species of the Ramsar sites of Bosnia and Herzegovina. The aim of the Ramsar Convention is to stop the degradation and ensure the conservation and wise use of wetlands worldwide. Bosnia and Herzegovina adopted the convention through succession on March 1, 1993. To date, three sites in Bosnia and Herzegovina have been designated as Ramsar sites: Bardača in the Republic of Srpska, and Hutovo Blato and Livanjsko Polje in the Federation of Bosnia and Herzegovina. In the investigated areas, 161 endangered and protected plant species have been identified. The Livanjsko Polje area has 94 plant species, Bardača 69, and Hutovo Blato 59. The status of endangerment and the degree of protection are given according to the preliminary Red List of endangered plants of Bosnia and Herzegovina and the IUCN list. According to the IUCN list (globally, IUCN G), 112 species are in the category of least concern (LC), 4 species are data deficient (DD), 3 species are near threatened (NT), and 2 species are vulnerable (VU). In Europe (IUCN E), 82 plant species are least concern (LC), 4 are data deficient (DD), 4 are near threatened (NT), and 1 is vulnerable (VU).

Keywords: *Endangered species, protected species, Bardača, Hutovo Blato, Livanjsko Polje.*

Introduction

The aim of the Ramsar Convention (February 2, 1971) is to stop the degradation and ensure the conservation and wise use of wetlands worldwide, both for biodiversity conservation and for securing sufficient natural resources for people who depend on wetland systems (https://www.ramsar.org). Bosnia and Herzegovina adopted the convention through succession on March 1, 1993. In Bosnia and Herzegovina, Ramsar sites include: Bardača (April 12, 2007) in the Republic of Srpska, and Hutovo Blato (September 24, 2001) and Livanjsko Polje (April 11, 2008) in the Federation of Bosnia and Herzegovina. The Ramsar sites of Bosnia and Herzegovina are vital ecosystems that support a wealth of plant life, but they are also exposed to various threats (http://www.mvteo.gov.ba). Bardača (3,500 ha) is located in the northeastern part of Lijevče Polje, in the floodplain between the Sava River and the left bank of the Vrbas River, in the municipality of Srbac. The sub-Mediterranean wetland Hutovo Blato (7,411 ha) is located in the south of Herzegovina, in the karst landscape, in the Neretva River valley, in the municipalities of Čapljina and Stolac. Livanjsko Polje is located in the Cetina River basin and represents an extraordinary natural phenomenon in the karst on 46,000 hectares. Since half of this field is regularly under water, Livanjsko Polje is essentially a combination of wetlands of exceptional value, important bird habitats, and peatlands and meadows inhabited by endemic and rare species (Davidović, 2006).

Materials and Methods

This study used existing literature sources to present the diversity of endangered and protected species in the Ramsar sites of Bosnia and Herzegovina (Cvijić, 1900; Bjelčić, 1954; Riter-Studnička, 1954, 1955; 1972, 1973, 1974; Riter-Studnička and Grgić, 1971; Nedović and Mejakić, 1997; Šumatić et al. 2001; Nedović et al. 2004; Davidović, 2006; Redžić et al. 2008; Redžić, 2012; Milanović and Stupar 2017; Milanović, 2018). The status of endangerment and the degree of protection are given according to the preliminary Red List of endangered plants of Bosnia and Herzegovina (Šilić, 1996) and IUCN (2024). The species names are adjusted according to the Euro+Med PlantBase (2006–2024).

Results and Discussion

Based on the literature data, 161 endangered and protected plant species were identified in the investigated areas. An overview of the protected and endangered species of the Ramsar sites of Bosnia and Herzegovina, according to the preliminary list of endangered species of Bosnia and Herzegovina (PRLBiH) and the IUCN categorization, globally (IUCN G) and for Europe (IUCN E), is shown in Table 1.

Table 1. Protected and endangered species in the area od Bardača, Hutovo blato and Livanjsko polje

Species	Hutovo blato	Livanjsko polje	Bardača	PRLBiH	IUCN (G)	IUCN (E)
Acanthus spinosus L.	+			V		
Acorus calamus L.			+	V	LC	
Agrimonia eupatoria L.		+				LC
Alisma plantago aguatica L.		+	+		LC	LC
Allium carinatum L.		+			LC	LC
Allium tuberosum Rottler ex Spreng.		+				LC
Alnus glutinosa (L.) Gaertn.		+	+		LC	
Alopecurus aequalis Sobol.		+			LC	LC
Anacamptis morio (L.) R. M. Bateman, Pridgeon & M. W. Chase		+				NT
Anacamptis palustris (Jacq.) R. M. Bateman, Pridgeon & M. W. Chase		+			LC	LC
Angelica sylvestris L.			+		LC	
Asparagus tenuifolius Lam.		+			LC	LC
Baldellia ranunculoides (L.) Parl.	+				NT	
Berula erecta (Huds.) Coville	+	+			LC	LC
Berula latifolium L.		+	+			LC
Bidens cernus L.	+				LC	LC
Butomus umbellatus L.	+	+	+	V	LC	
Caltha palustris L.			+	V	LC	
Cardamine pratensis subsp. *atlantica* (Emb. & Maire) Greuter & Burdet		+			VU	
Carex acutiformis Ehrh.		+			LC	LC
Carex distans L.		+			LC	LC
Carex divisa Hudson	+				LC	
Carex elata All.			+		LC	
Carex elata All. subsp. *elata*		+			LC	LC
Carex hirta L.		+				LC)
Carex nigra (L.) Reichard		+			LC	LC
Carex pseudocyperus L.		+			LC	LC
Carex riparia Curtis		+			LC	LC
Carex rostrata Stokes		+			LC	LC
Celtis tournefortii Lam.	+			V	LC	
Centaurea cyanus L.		+				LC
Centaurium erythraea Rafn		+			LC	
Ceratophyllum demersum L.	+		+		LC	
Colchicum autumnale L.		+	+			LC
Convallaria majalis L.			+	V	NT	
Corrigiola litoralis L.		+			LC	
Corylus avellana L.		+	+		LC	LC
Cyclamen hederifolium Aiton	+			V		

Cyclamen repandum Sibth. & Sm.	+			V		
Cyperus flavescens L.	+				LC	
Cyperus fuscus L.	+				LC	
Cyperus longus L.	+				LC	
Cyperus michelianus (L.) Link	+				LC	
Dactylorhiza incarnata (L.) Soó		+				LC
Daucus carota L.		+	+		LC	LC
Eleocharis acicularis (L.) Roem. & Schult.		+			LC	LC
Eleocharis ovata (Roth) Roem. & Schult.	+				LC	
Epipactis palustris (L.) Crantz		+			LC	LC
Equisetum fluviatile L.		+			LC	
Euphorbia palustris L.	+	+	+		LC	
Filipendula ulmaria (L.) Maxim.		+	+		LC	
Filipendula vulgaris Moench		+				LC
Fimbristylis bisumbellata (Forssk.) Bubani	+			V	LC	
Galanthus nivalis L.	+			V	NT	
Galium palustre L.	+	+	+		LC	
Galium verum L.		+	+			LC
Galium uliginosum L.			+		LC	LC
Glechoma hederacea L.			+		DD	DD
Glyceria fluitans (L.) R. Br.		+	+		LC	LC
Glyceria maxima (Hartm.) Holmb.			+		LC	LC
Gratiola officinalis L.			+	V	LC	
Gymnadenia conopsea (L.) R. Br.		+				LC
Hippuris vulgaris L.	+	+		V	LC	
Hordeum geniculatum All.		+				LC
Hordeum murinum L.			+		LC	
Hordeum secalinum Schreber	+				LC	
Hottonia palustris L.	+			V	LC	
Hydrocharis morsus-ranae L.	+		+	V	LC	
Hydrocotyle vulgaris L.	+				LC	
Iris pseudacorus L.	+	+	+		LC	LC
Iris sibirica L.		+		V		NT
Juncus articulatus L.		+	+		LC	LC
Lathyrus palustris L.		+		V	LC	
Lathyrus tuberosus L.		+			LC	LC
Lemna gibba L.			+		LC	
Lemna trisulca L.			+		LC	
Leontodon autumnalis L.			+		LC	
Lolium perenne L.		+				LC
Lolium temulentum L.		+				LC
Lotus pedunculatus Cav.		+				LC
Ludwigia palustris (L.) Elliott	+			E	LC	
Lysimachia vulgaris L.	+	+			LC	LC
Lythrum salicaria L.	+		+			LC
Marsilea quadrifolia L.	+		+	V	LC	
Medicago polymorpha L.		+				LC
Melilotus officinalis (L.) Desrs.		+	+			LC
Mentha aquatica L.			+		LC	
Mentha pulegium L.		+			LC	LC
Menyanthes trifolia L.		+			LC	LC
Molinia caerulea (L.) Moench		+				LC
Myosotis scorpioides L.		+				LC
Myriophyllum spicatum L.			+		LC	
Najas marina L.			+		LC	
Najas minor All.	+				LC	LC
Neotinea tridentata (Scop.) R. M. Bateman, Pridgeon & M. W. Chase		+				LC
Nuphar lutea (L.) Sm.	+	+	+	V	LC	
Nymphaea alba L.		+	+		LC	LC
Nymphoides peltata (S. G. Gmel.) Kuntze	+		+	V	LC	LC
Oenanthe fistulosa L.		+			LC	LC
Onobrychis viciifolia Scop.		+			LC	LC
Ophioglossum vulgatum L.		+			LC	
Orchis simia Lam.	+	+				LC
Pedicularis palustris L.			+	V	LC	
Periploca graeca L.	+			V		
Persicaria amphibia (L.) Delarbre			+		LC	LC
Phalaroides arundinacea (L.) Rauschert		+			LC	LC
Phragmites australis (Cav.) Steud.	+	+	+		LC	LC
Pinguicula vulgaris L.		+		V	VU	
Plantago lanceolata L.		+	+			LC

Plantago maritima L.		+	+		LC	
Platanthera bifolia (L.) Rich.		+				LC
Poa palustris L.			+		LC	
Polygonum amphibium L.	+	+	+		LC	LC
Populus alba L.	+		+		LC	LC
Populus nigra L.	+		+		DD	
Populus tremula L.		+			LC	LC
Potamogeton acutifolius Link	+				LC	NT
Potamogeton crispus L.	+	+	+		LC	LC
Potamogeton gramineus L.			+		LC	
Potamogeton lucens L.	+	+			LC	LC
Potamogeton natans L.	+		+			
Potamogeton perfoliatus L.	+				LC	LC
Potentilla palustris (L.) Scop.		+		V	LC	
Prunus avium (L.) L.			+		LC	LC
Pulmonaria officinalis L.			+		LC	LC
Ranunculus lingua L.	+	+			LC	LC
Ranunculus ophioglossifolius Vill.	+					
Rosa gallica L.		+	+			DD
Rumex palustris Sm			+		LC	
Ruscus aculeatus L.	+		+	V	LC	LC
Salix alba L.		+			LC	LC
Salix caprea L.			+		LC	
Salix cinerea L.		+			LC	LC
Salix pentandra L.		+		V	LC	LC
Salix purpurea L.		+	+		LC	LC
Salvinia natans (L.) All.	+		+	V	LC	NT
Sanguisorba officinalis L.		+	+		LC	
Schoenoplectus lacustris (L.) Palla			+		LC	LC
Scilla pratensis Waldst. & Kit., nom. illeg.		+		V		
Scirpus sylvaticus L.			+		LC	LC
Scutellaria galericulata L.	+	+			LC	
Serratula lycopifolia (Vill.) A. Kern.		+			DD	DD
Spirodela polyrhiza (L.) Schleid.	+		+		LC	
Stratiotes aloides L.	+				LC	
Teucrium chamaedrys L.		+				LC
Teucrium scordium L.		+				LC
Thelypteris palustris Schott	+			V	LC	
Trapa natans L.			+	V	LC	
Trifolium hybridum L.		+				LC
Trifolium pratense L.	+	+	+		LC	LC
Trifolium repens L.	+	+	+			LC
Typha latifolia L.	+	+	+		LC	LC
Typha shuttleworthii W. D. J. Koch & Sond.	+	+	+			DD
Ulmus glabra Huds.	+	+			DD	VU
Utricularia vulgaris L.	+	+	+	V	LC	
Vallisneria spiralis L.			+		LC	LC
Veronica anagalloides Guss	+			V	LC	
Veronica serpyllifolia L.		+			LC	
Viburnum opulus L.		+	+			LC
Zannichelia palustris L.	+			V		

From the total number across all three areas, there are 13 plant species represented. Bardača and Livanjsko Polje share 18 common plant species, while Livanjsko Polje and Hutovo Blato share 8, as well as Hutovo Blato and Bardača. According to the preliminary list of endangered species of Bosnia and Herzegovina (PCLBiH), 32 species are vulnerable (V), with the highest number found in Hutovo Blato (20), followed by Bardača (13) and Livanjsko Polje (10). The percentage representation of vulnerable species is 19.88%. These species include *Acanthus spinosus, Acorus calamus, Butomus umbellatus, Caltha palustris, Celtis tournefortii, Convallaria majalis, Cyclamen hederifolium, Cyclamen repandrum, Galanthus nivalis, Gratiola officinalis, Iris sibirica, Marsilea quadrifolia*, and others. According to the IUCN categorization (global IUCN G), 112 species are classified as least concern (LC), 4 species lack sufficient data (DD), 3 species are near threatened (NT), and 2 species are vulnerable (VU). Some of the species listed on the IUCN global list include: *Alisma plantago aquatica, Angelica sylvestris, Baldellia ranunculoides, Cardamine pratensis, Carex nigra, Pinguicula* *vulgaris, Populus nigra, Corrigiola litoralis, Glechoma hederacea, Hordeum murinum, Iris pseudacorus.* The percentage representation of species by categories is: 69.57% LC; 1.86% NT; 1.24% VU; 2.48% DD. According to the IUCN categorization (Europe IUCN E), 82 plant species are least concern (LC), 4 are data deficient (DD), 4 are near threatened (NT), and 1 is vulnerable (VU). These species are: *Anacamptis morio, Anacamptis palustris, Molinia caerulea, Najas minor, Rosa gallica, Salvinia natans, Scripus sylvaticus, Colchicun autumnale, Juncus articulatus, Meliolotus officinalis, Persicaria amphibia,* etc. The percentage representation of species by categories is: 50.93% LC; 2.48% NT; 0.62% VU; 2.48% DD.

Conclusion

The Ramsar sites provide habitat for many endangered plant species, thus offering vital support for biodiversity. Through effective protection measures, such as regulation of human activities, control of invasive species, and promotion of education, we can ensure the long-term sustainability of these ecosystems. Further research and monitoring of plant species populations will be essential for the successful conservation of the Ramsar sites of Bosnia and Herzegovina. To date, three sites in Bosnia and Herzegovina have been designated as Ramsar sites: Bardača in the Republic of Srpska, and Hutovo Blato and Livanjsko Polje in the Federation of Bosnia and Herzegovina. Based on literature data, 161 endangered and protected plant species have been identified in the investigated areas. The analysis determined that the Livanjsko Polje area contains 94 plant species, the Bardača area 69, and Hutovo Blato 59. Of the total number of plant species in the investigated area, 32 species are vulnerable (V) according to the preliminary Red List of endangered plants of Bosnia and Herzegovina. According to the IUCN list (globally IUCN G), 112 species are in the category of least concern (LC), 4 species are data deficient (DD), 3 species are near threatened (NT), and 2 species are vulnerable (VU). In Europe (IUCN E), 82 plant species are least concern (LC), 4 are data deficient (DD), 4 are near threatened (NT), and 1 is vulnerable (VU).

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THE EFFECTS OF NaCl AND KCl ON THE GERMINATION AND SEEDLING GROWTH OF *AEGILOPS CYLINDRICA* HOST SEEDS

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Abstract

This study was conducted to determine the effect of two chloride salts (NaCl and KCl) and their concentrations (0, 50, 100, 150, 200, 250, 300 mM) on the germination and early seedling growth of *Aegilops cylindryca* seeds. The trials were performed in two replicates of 25 seeds for each variant. Germination was carried out between rolls of filter paper with 20 ml of the respective test solutions at a temperature of 20 ± 1°C, for 10 days. Seeds were counted as germinated when the germ has reached at least 2 mm. The following characteristics were calculated: germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day^{-1}), mean germination time (MGT, day) and relative injury rate (RIR). The data for the shoot and root length (cm) (LSh and LR), length of seedling (LS, cm), fresh weigh (mg plant^{-1}) of shoot and root (FWSh and FWR) and dry weight (mg plant^{-1}) of shoot and root (DWSh and DWR) were measured 10 days after germination. The three-way ANOVA analysis showed that the dose was proven to have the greatest effect on the studied parameters. The highest value of the relative injury coefficient above 0.80 was recorded when seeds were treated with NaCl at concentrations of 250-300 mM salt, as well as when they were treated with KCl at concentrations of 200-300 mM. Application of high salinity concentrations of both chloride salts had a greater inhibitory effect on shoot growth compared with root growth.

Keywords: *Aegilops cylindrica* Host, *NaCl, KCl, germination, seedling growth.*

Introduction

Salinity represents one of the most significant abiotic constraints on plant growth and productivity globally. The impact of salinity on plant growth is mediated by its influence on metabolic rates and pathways within the plant. Salinity affects plant growth at all stages of development, and the sensitivity to salinity varies between different growth stages (Yohannes et al., 2020). The presence of excess salts in soils and water can have a detrimental impact on seed germination and plant growth. This can occur either through osmotic effects, whereby the absorption of water is limited due to a low water potential, or ionic toxicity, which can result in cellular injuries and a reduction in metabolic activity due to the presence of excess ions (Dadach et al., 2023; Hadjadj et al., 2023**)**. The presence of soluble salts, such as calcium chloride ($CaCl_2$), potassium chloride (KCl) and sodium chloride (NaCl), has been demonstrated to have a detrimental impact on plant growth. The influx of these ions into the cells can result in the onset of significant physiological disorders. The accumulation of sodium ions (Na^+) has been shown to inhibit the absorption of potassium, an essential element for growth and development. This can lead to a reduction in productivity and, in severe cases, may even result in death (Kanjevac et al., 2021).

Seedlings and young plants are much more sensitive to salt stress than mature plants (Kanjevac et al., 2021). Jasim and Nulit (2019) noted that it is of paramount importance for plants to adapt to salinity during the germination and early seedling stages, as this is a crucial factor in the establishment of the plant stand. The adverse effects of salt stress on germination, seedling growth, and some physiological activities of a number of cultivated plant species have been extensively investigated (Kiran et al., 2021). A number of studies have indicated that salt stress tolerance varies among species and also depends on the stage of plant development, salt type and concentrations (Panuccio et al., 2014; Vishnu Priya et al., 2020). Consequently, the selection of salt-tolerant plant genotypes at both the germination and seedling stages represents an effective approach to addressing the issue of soil salinity (Hadjadj et al., 2023).
The aim of this study was to analyze the effects of NaCl and KCl on germination and seedling growth of *Aegilops cylindrica* Host seeds.

Material and Methods

The study examined the effect of six salinization levels (50, 100, 150, 200, 250, and 300 mM) using two chloride salts (NaCl and KCl) on seed germination and early seedling growth parameters. Deionized water was used as control variant. An experiment was conducted with seeds from five Bulgarian populations of *Aegilops cylindrica* Host, taken from an experiment established at IPGR-Sadovo in 2023, Bulgaria. For each variant of experiment, two replicates of 25 seeds were germinated on rolled filter paper (Grade FT 55) with 20 ml of the respective test solutions. The papers were replaced every 2 days to prevent salt accumulation. The seeds were allowed to germinate in the dark at $20\pm1^{o}C$ for 10 days. Seeds were considered germinated when the radicle had extended at least 2 mm. The number of germinated seeds was recorded daily until a constant count was achieved. From the germination counts several germination characteristics were studied including germination percentage (G, %), coefficient of velocity of germination (CVG), germination rate index (GRI, % day^{-1}), mean germination time (MGT, day) and relative injury rate (RIR). CVG was calculated according to Kader and Jutzi (2004), GRI and MGT according to the formula of Kader (2005), while RIR according to Li (2008).
On the eighth day of the experiment, ten seedlings were randomly selected from each treatment for the measurement of shoot and root length (LSh and LR in cm) and fresh weight of shoot and root (FWSh and FWR in mg/plant). Additionally, data for dry weight of shoot and root (DShW and DRW in mg/plant) were recorded after drying in an oven at 80°C for 24 hours. Seedling height reduction was determined using the method described by Islam and Karim (2010), and salt tolerance index (STI) was estimated according to Mujeeb-ur-Rahman et al. (2008).
A three-way analysis of variance (ANOVA) was employed to assess the influence of genotype, salt type, concentration, and their interaction on germination and seedling characteristics. The Duncan’s Multiple Range Test was conducted at a 0.05 probability to determine the significance of differences between the means of all germination and seedling parameters for each treatment. A simple correlation with the index (Pearson correlation) was employed to ascertain the interrelationships between the values of salt concentrations and germination parameters and seedling metrics. All statistical methods were conducted using JMP 17 statistical software.

Results and Discussion

A three-factor ANOVA analysis (Table 1 and Table2) showed a significant individual effect of genotype, salt type, concentration (dose) and their interaction on the most of studied parameter, except salt type for MGT, ShL and DRW, genotype for DRW, genotype*salt for RL and ShL, genotype*salt*dose for FRW. Dose was shown to have the greatest effect on the all parameters studied.

Table 1. Three-way ANOVA on the effect of genotype, salt type, concentration (dose) and their interaction on the germination characteristic of *Aegilops cylindrica* Host

Source of variation	CVG		GRI		MGT		G	
	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.
Genotype	818.85	0.00	3076.63	0.00	90.61	0.00	26008.33	0.00
Salt	91.00	0.00	856.72	0.00	2.15	0.08	17190.48	0.00
Dose	**22882.64**	**0.00**	**26339.96**	**0.00**	**270.48**	**0.00**	**177107.86**	**0.00**
Genotype * Salt	270.93	0.00	208.57	0.00	54.21	0.00	522.62	0.01
Genotype * Dose	2386.28	0.00	1552.36	0.00	120.29	0.00	8918.33	0.00
Salt * Dose	521.04	0.00	1134.36	0.00	57.51	0.00	15437.86	0.00
Genotype * Salt * Dose	1044.14	0.00	791.72	0.00	110.81	0.00	5424.05	0.00

G-germination percentage, CVG-coefficient of velocity of germination, GRI-germination rate index, MGT-mean germination time

Table 2. Three-way ANOVA on the effect of genotype, salt type, concentration (dose) and their interaction on the seedling characteristic of *Aegilops cylindrica* Host

Source of variation	RL		ShL		FRW		FShW		DRW		DShW	
	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.	Sum of Squares	Sig.
Genotype	11.68	0.00	17.39	0.00	298.72	0.00	881.83	0.00	5.83	0.10	11.72	0.00
Salt	78.02	0.00	1.31	0.15	879.13	0.00	118.20	0.01	0.05	0.80	10.08	0.00
Dose	**2305.49**	**0.00**	**2736.70**	**0.00**	**12136.44**	**0.00**	**66818.05**	**0.00**	**167.09**	**0.00**	**836.67**	**0.00**
Genotype * Salt	2.02	0.46	2.99	0.31	141.15	0.00	236.99	0.01	4.41	0.21	7.63	0.00
Genotype * Dose	40.18	0.00	71.91	0.00	303.18	0.01	2673.20	0.00	61.07	0.00	33.52	0.00
Salt * Dose	27.70	0.00	33.59	0.00	503.86	0.00	619.49	0.00	10.42	0.03	4.15	0.04
Genotype * Salt * Dose	30.73	0.00	55.99	0.00	248.41	0.07	2339.54	0.00	67.13	0.00	20.72	0.00

RL-root length, ShL-shoot length, FRW-fresh weight of root, FShW- fresh weight of shoot, DShW-dry weight of shoot, DRW-dry weight of root

The *Aegilops cylindrica* seeds exhibited differential responses at the studied chloride salt concentrations, as illustrated in Fig. 1. The non-saline-treated seeds (control) exhibited the highest germination rate. The seeds demonstrated a higher germination rate under salinity stress caused by KCl than under stress caused by NaCl. The lowest germination rate was observed in seeds treated with 250 mM NaCl, while at the highest salinity levels of 300 mM NaCl, no seeds germinated in all samples included in the study. Only weak root system development was observed.

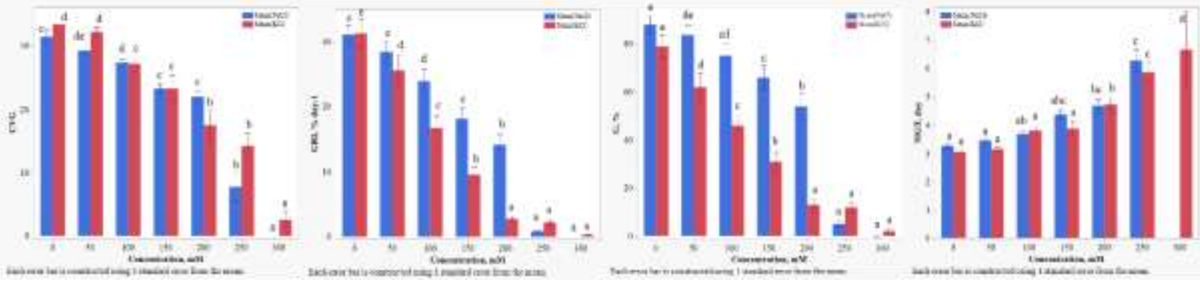

Fig.1 Variation of the germination characteristics of *Aegilops cylindrica* seeds treated with NaCl and KCl. Bars are mean values± standard error. Similar bars with different letters are significantly different from each other (Duncan’s Multiple Range Test, P < 0.05).

The linear regression analysis data presented in Fig. 2 showed a strong negative statistical correlation ($p<0.001$) between G, CVG, GRI values and salt doses with coefficients of determination (R^2) ranging from 0.684 to 0.790 and a strong positive statistical correlation between MGT and salt concentrations with R^2 ranging from 0.552 to 0.571. These results also provide clear indication of the inhibitory effect of NaCl and KCl salts on seed germination of *Aegilops cylindrica* Host.

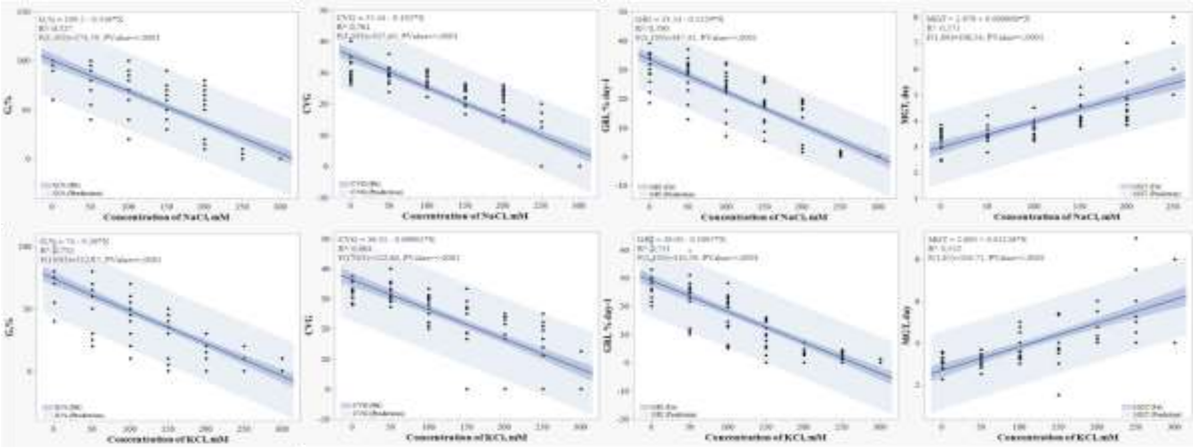

Fig.2 Regression plots for germination parameters (G-germination percentage, CVG-coefficient of velocity of germination, GRI-germination rate index, MGT-mean germination time) of *Aegilops cylindrica* seeds treated with NaCl and KCl

The highest value of the relative injury coefficient above 0.80 was recorded when seeds treated with NaCl at concentrations of 250-300 mM salt, as well as when treated with KCl at concentrations of 200-300 mM. The results also indicated that seeds treated with NaCl exhibited a lower relative injury rate at concentrations of 50-200 mM in comparison to those treated with KCl. However, at the highest salinity concentrations (250-300 mM), NaCl-treated seeds demonstrated the highest value of the relative injury rate (Fig.3).

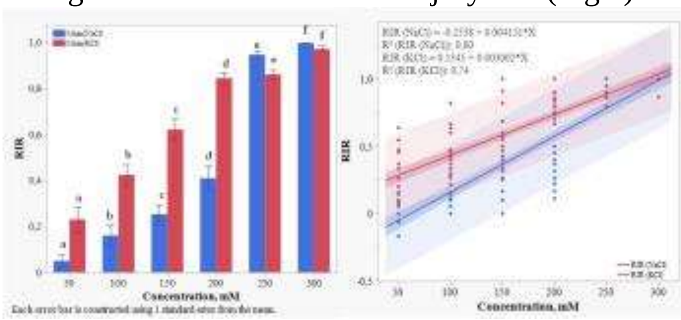

Fig.3. Variation of the relative injury rate (RIR) of *Aegilops cylindrica* seeds treated with NaCl and KCl and regression plot for RIR. Similar bars with different letters are significantly different from each other (Duncan’s Multiple Range Test, $P < 0.05$)

The results of our study showed that both of mean root and shoot lengths were significantly declined with the increase in salt levels. The reduction in root growth ranged between (0.11–0.88) and (0.07-0.91) as compared to those of controls, respectively for NaCl and KCl. For shoot elongation, the reduction rates ranged between (0.16-1) and (0.11–0.98), in this order for NaCl and KCl. Hence the growth of shoot was more sensitive to salt stress than root, particularly at higher salt concentrations (Fig. 4). The significant variations in salt tolerance indices with increasing salinity concentration for both salt types were found. The highest values of the indices were recorded at the lowest concentration, respectively the lowest at the highest concentration (Table 3).

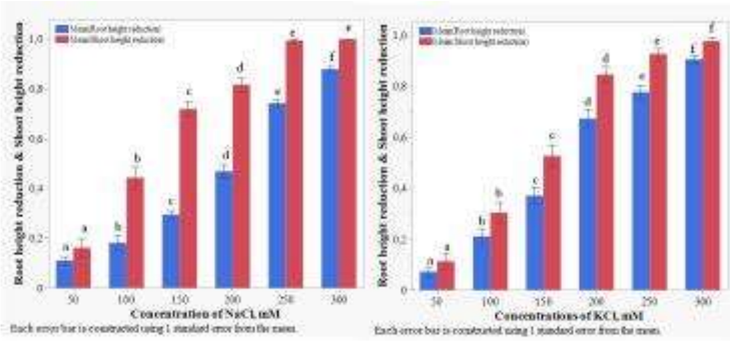

Fig.4. Variation of the root/shoot height reductions of *Aegilops cylindrica* seeds treated with NaCl and KCl. Similar bars with different letters are significantly different from each other (Duncan's Multiple Range Test, $P < 0.05$)

Table 3. Variation in Salt Tolerance Indices of *Aegilops cylindrica* Host with the salt and concentration

Concentra-tion	GSTI, % (NaCl)	GSTI, % (KCl)	RSTI, % (NaCl)	RSTI, % (KCl)	ShSTI, % (NaCl)	ShSTI, % (KCl)
50 mM	96.33f	76.96f	88.91f	92.24f	83.86e	88.71e
100 mM	83.90e	57.56e	81.82e	78.50e	55.65d	69.59d
150 mM	74.64d	37.80d	70.49d	62.56d	28.09c	47.29c
200 mM	58.98c	15.34c	53.06c	32.50c	18.26b	15.35b
250 mM	5.15b	13.73b	25.70b	22.17b	0.55a	7.23ab
300 mM	0.00a	2.66a	12.07a	9.38a	0.00a	2.25a
Average	**53.17**	**34.01**	**55.35**	**49.56**	**31.07**	**38.41**

Germination Salt Tolerance Index (GSTI), Root Salt Tolerance Index (RSTI), Shoot Salt Tolerance Index (ShSTI),
Means within a column that have different superscript letters (a-g) are significant different from each other (Duncan's multiple range test, $p \leq 0.05$)

Aegilops cylindrica seeds showed high to very high tolerance to germination at 50-150 mM NaCl and 50 mM KCl, moderate tolerance at 100 mM KCl and at 200 mM NaCl, low to very low tolerance at 250 mM NaCl and 250-300 mM KCl. At the highest concentration of NaCl, seeds were sensitive to germination. RSTI varied from 9.38% to 92.24%, while ShSTI varied from 0% to 88.71%. At 50-150 mM doses of both salts, Aegilops cylindrica showed very high to high tolerance to root growth, while at the highest concentrations of 250-300, root tolerance varied from low to very low tolerance. In terms of shoot growth, at the highest concentration Aegilops cylindrica was sensitive to salinization with NaCl and very low resistant to salinization with KCl. The calculated average salt tolerance indices indicated that Aegilops cylindrica was more tolerant to NaCl salt in compere to KCl salt.

Conclusion

Genotype, salt type, concentration (dose) and their interactions had significant individual effect on the most of studied germination and seedling characteristics of *Aegilops cylindrica*. Application of increasing concentrations of both type of salts (NaCl and KCl) prolonged mean germination time and suppressed the studied germination and seedling characteristics and had a greater inhibitory effect on shoot growth compared with root growth. The relative injure rate above 0.80 was recorded at 250-300 mM NaCl and 200-300 mM KCl The significant variations in salt tolerance indices with increasing salinity concentration for both salt types were found. The effect of salinity on physiology, molecular and biochemical of properties of *Aegilops cylindrica* should be studied.

Acknowledgments

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SEEDS QUALITY CHARACTERISTICS OF SOME MEDICINAL PLANTS FROM *LAMIACEAE* FAMILY USING FLUORESCENCE SPECTROSCOPY

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Abstract

In resent yeas there is a very special attention of the scientific community towards the investigations connected with the seed quality of the medicinal plants. That is due to the increased demand for these plants for the pharmaceutical industry together with the necessity of creation of rational mechanisms for their cultivation. The aim of the study was to determine the quality of seeds from members of the families. *Lamiaceae* produced in different years and to validate fluorescence spectroscopy in the proposed configuration as a non-invasive method to assess seed moisture, viability and purity. Seeds from 12 accessions from 4 species, belonging to *Lamiaceae* botanical family are included in the study and tested using mobile experimental installation for fluorescence spectroscopy. The water absorption of the seeds shows differences and similarities between species, between accessions as well as between variants. The main similarity is the rapid absorption of water of the seeds in all 12 variants during the first 8 hours, nevertheless that in *Ocimum basilicum* – lemon the process lasts 4 hours. The seeds of all other variants imbibed rapidly, no matter the year of harvest and obtain their maximum weight during the first hours. The average water absorption is similar in all variants (35%). There were no significant differences in % germinated seeds and germination time between Ocimum basilicum-limon and *Ocimum basilicum*. Seed germination was high at 96-100% and completed in 7-9 days except for *Ocimum basilicum*-2012 at 9%. Germination of salvia samples was longer about 20 days and germination percentage were in the range of 63-93% depending on the year of harvest. A mobile fibre-optic installation using fluorescence spectroscopy was successfully set up and tested. In the proposed setup, fluorescence spectroscopy was validated as a non-invasive method to assess seed moisture, viability and purity.,

Key words: *medicinal plants, seeds quality, fluorescence spectroscopy, Lamiaceae family.*

Introduction

Plants are an important source of medicinal substances and play key role for the human health. The increasing population, the insufficient supply with medicinal substances, the extremely high cost of medical treatments, the negative side effects caused by the synthetic drugs and development of resistance to many infection diseases has led to increasing demand of plant materials as a source for medicinal remedies.

Nowadays many medicinal plant species are threatened or nearly extinct. Unfortunately, they are not actively conserved. For example, there is very little material from them in gene banks.

The quality of the seeds is a very important factor for the successful growing of medicinal plants. Nowadays, there is a worldwide recognition of the importance of aromatic and medicinal plants not only on the basis of their medicinal and preventive properties, but also for their use in food, feed, cosmetic and pesticide industries, which is reflected in the growing demand for aromatic and medicinal products in the markets.

Seed germination is a very important stage from the live cycle of the plants ant there is a tendency to be strongly unpredictive in time.

A number of factors as temperature, salinity, light, water supply, nutrient substances and moisture content of the soil strongly influence the seed germination. The moisture content of the seeds as another very important factor for the viability of seeds.

In a well-organized program for production of seeds with high quality, the measurement of seed moisture content also determines the decision-making policies concerning seed harvesting, processing and marketing.

It is therefore important that the moisture content is consistent with the seed storage behavior and desiccation and chilling sensitivity/tolerance of a species,

Water absorption is the first step of seed germination. It starts with the uptake of water by absorption of the dry seeds, followed by expansion of the embryo.

To produce quality seedlings, good germination is necessary, which includes metabolic and morphogenetic mechanisms, summarized as follows: 1) absorption of water by the seeds, 2) activation of metabolism and the process of respiration, protein synthesis and mobilization of embryonic reserve substances, and 3) elongation of the embryo with rupture of the seed coat, through which the exit of the root is observed (Suárez and Melgarejo, 2010, Monroy-Vazquez et al., 2017). Water absorption is the first step of seed germination. It begins with the uptake of water by imbibition of the dry seeds, followed by expansion of the embryo (Suárez and Melgarejo, 2010)

Seed dormancy is one of the important limiting factors for exploitation of economically valuable species.

The chemical composition of seeds that contain essential oils are rich in lipids, in which specific oxidation processes occur during storage. They are believed to be a limiting factor for long-term storage depending on their specific chemical composition (Balešević et al., 2007). Loss of seed viability is associated with an increase in reactive oxygen species (i.e., singlet oxygen, hydrogen peroxide, and hydroxyl radical) causing DNA damage, protein adducts, and lipid peroxidation (Sano et al., 2016, Kurek et al., 2019).Therefore, the storage of such seeds requires special attention, otherwise the oxidation processes can occur too early, which can lead to the loss of seed germination and viability (Genes and Nyomora Agnes, 2018). Dormancy is an important component of seed physiological quality, and plants with a long history of domestication and reproduction generally have lower seed dormancy than wild or recently domesticated species. (Copeland and McDonald, 2001; Khalid et al., 2016).

Over the past decades, imaging and spectroscopy techniques have been developed rapidly with widespread applications in non-destructive agro-food quality determination. Seeds are one of the most fundamental elements of agriculture and forestry. Seed viability is of great significance in seed quality characteristics reflecting potential seed germination, and there is a great need for a quick and effective method to determine the germination condition and viability of seeds prior to cultivate, sale and plant (Xia et al., 2019)

The potential or practical applications in seed industry include the detection of viability, vigour, defect, disease, cleanness and the seed composition determination (Feng et al 2019). Fluorescence analysis of plant powders from medicinal plants is also used as a fingerprint for correct identification of crude drugs when other physical and chemical parameters of crude drugs are not sensitive enough (Beressa et al., 2021). Fluorescence spectroscopy is a high-precision method commonly used in the quantitative analysis of heavy metal elements and is also used to determine nanoscale metal concentrations (Nguyen et al., 2022).

Fluorescence spectroscopy in the analysis of powders can be used to solve the problem of quality control of herbal medicines, as well as in the initial stages when trying to determine their molecular properties and quantify photo components of the herbal products from which they are derived. (Chinese et al., 2020).

The aim of the study was to determine the quality of seeds from members of the families. Lamiaceae produced in different years and to validate fluorescence spectroscopy in the proposed configuration as a non-invasive method to assess seed moisture, viability and purity.

Material and methods

Seeds from 12 accessions from 4 species (table 1), belonging to *Lamiaceae* botanical family are included in the study using mobile experimental installation for fluorescence spectroscopy (Table.1.). They are from the collection of Institute of Plant Genetics Resources (IPGR) - Sadovo.

Table1. Seeds from *Lamiaceae* botanical family are included in the study.

Species	**year**	**Seeds**
Ocimum basillicum, lemon	2022	
Ocimum basillicum, lemon	2020	
Ocimum basillicum, lemon	2017	
Ocimum basillicum	2022	
Ocimum basillicum,	2020	
Ocimum basillicum,	2012	
Salvia sclarea	2020	
Salvia sclarea	2019	
Salvia sclarea	2018	
Salvia officinalis	2022	
Salvia officinalis	2019	
Salvia officinalis	2016	

Seed moisture %;
Determined individually on 50 seeds of each variant. The seeds were dried for 4 days at a constant temperature (70±1 °C) in the dark. Initial and final weights were measured on an analytical balance (Scientech SA120, USA, with an accuracy of ±0.0001 g) and seed moisture was expressed as a percentage of weight loss relative to initial weight (International Seed Testing Association, 2010)
$\% \ Mc = \times 100$

Seed imbibition %
The seeds of the twelve variants were placed in petri dishes on filter papers moistened with distilled water. They are then stored in a controlled environment at a constant temperature (30±1 °C) and in the dark. Three replicates of each variant were evaluated, with 10 seeds each (n=30). Initial weight was recorded every 8 hours for 48 hours, a final recording was made after 72 hours from baseline. Absorption is expressed as the percentage increase in seed weight, by water absorption, relative to the initial weight,

Germination %
Seeds of the twelve variant variants were placed in petri dishes on filter papers moistened with distilled water with a cotton swab. They are stored at a constant temperature (30±1 °C) and in the dark. Four replicates of each variant were evaluated, with 25 seeds each (n=100). Seed germination (%) - ratio of the total number of normally germinated seeds to the total number of seeds at the end of the period. Germination was recorded daily for 20 days or until no germinating seeds were observed for more than five days.

Fluorescence spectroscopy

The mobile fiber-optical spectral installation for the study of fluorescence signals is designed specifically, for the rapid analysis of plant biological samples. The mobile experimental setup used by fluorescence spectroscopy includes the following components:

- Laser diode (LED) with an emission radiation of 245 nm with a supply volt-age in the range of 3V. It is housed in a hermetically sealed TO39 metal housing.
- Rod lens of the achromatic doublet type. It is composed of two bonded lenses with different Schott and Corning dispersion coefficients with an anti-reflective coating. The multimode optical fiber is FG200LEA. It has a core diameter of 200 µm and a step index of refraction.
- Quartz glass area 4 cm2.
- CMOS detector with photosensitive area 1.9968×1.9968 mm.
- Reception of the emission signal at 45°

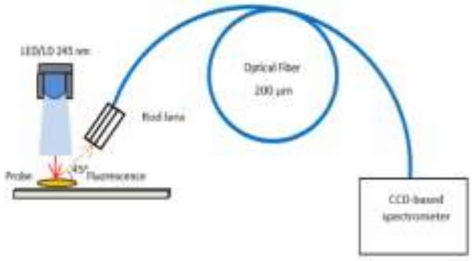

Fig. 1. Mobile experimental installation used by fluorescence spectroscopy

Results and discussion

Seed moisture content showed differences between *Ocimum basillicum*-lemon and *Ocimum basillicum* as well as between *Salvia sclarea* and *Salvia officinalis* samples (Fig.2).

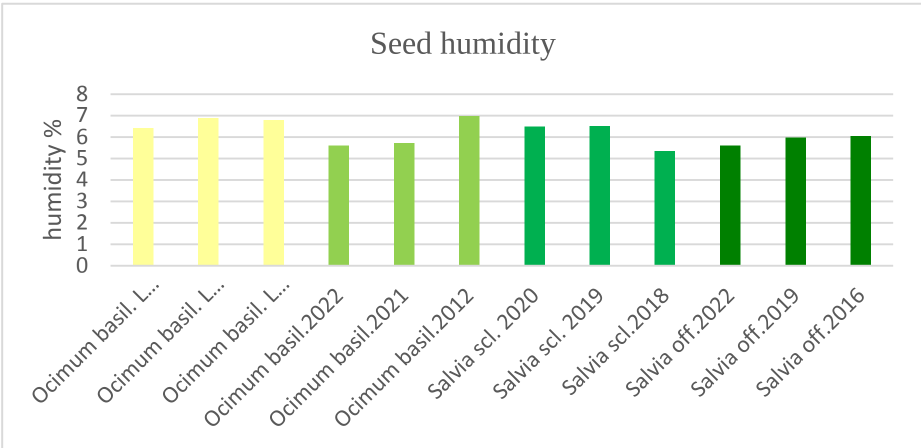

Fig.2. Seed humidity

The process of water absorption of the seeds is very rapid and easy way to be determined whether the seed coat is water transparent or not. The hard seed coat could cause physical latent of seeds (Ranber Chauhan, Sobha and JS Chauhan, 2020).

The water absorption of the seeds shows differences and similarities between species, between accessions as well as between variants (Fig.3). The main similarity is the rapid absorption of water of the seeds in all 12 variants during the first 8 hours, nevertheless that in *Ocimum basilicum* – lemon the process lasts 4 hours. After that rapid phase the average absorption remains constant during the next 24-32 hours. An exception is *Ocimum basilicum* -

2012, whose seeds continue to absorb water rapidly after 32 hours. Similar results are reported for *Opuntia* sp. where water absorption in most intensive during the first 8 hours. In O. megacantha and O. ficus-indica that time is with 4 or 8 h longer. (**Monroy-Vázquez** M.E et al.,2017)

The seeds of all other variants imbibed rapidly, no matter the year of harvest and obtain their maximum weight during the first hours. The average water absorption is similar in all variants (35%), and that coincides with the results, received by Foschi, M.L. et al.,(2022) in Capparis seeds (Capparis spinosa L.).

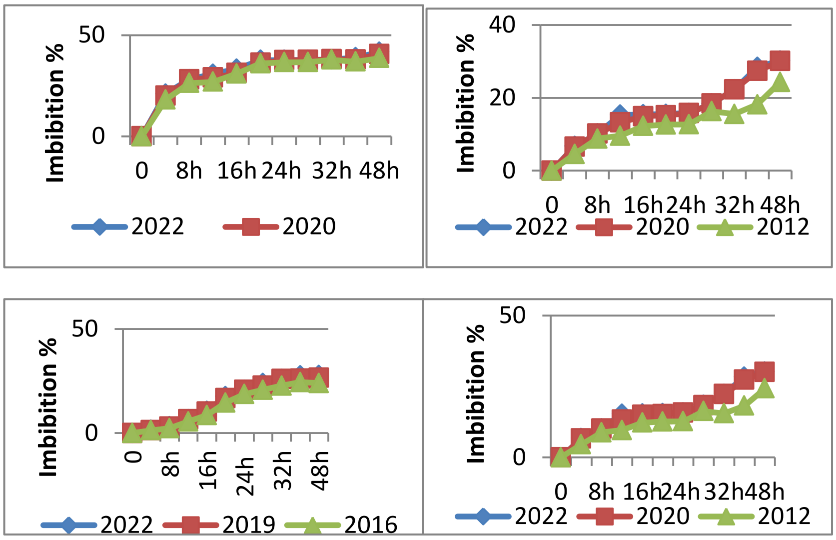

Fig.3. Cumulative water imbibition by seeds of twelve variants of (a) *Ocimum basilicum*-lemon; (b) *Ocimum basilicum*; (c) *Salvia sclarea*; (d) *Salvia officinalis*

The results of the seed germination test are shown in Fig.4. There were no significant differences in % germinated seeds and germination time between *Ocimum basilicum*-limon and *Ocimum basilicum*. Seed germination was high at 96-100% and completed in 7-9 days except for *Ocimum basilicum*-2012 at 9%. Germination of salvia samples was longer about 20 days and germination percentage were in the range of 63-93% depending on the year of harvest.

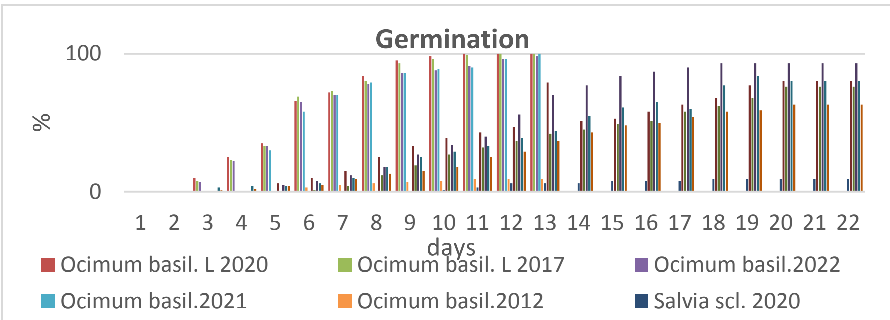

Fig.4. Results of the seed germination test.

The 7 species of medicinal plants, subject to that study (Tiwari and Kuntal, 2014) germinate with different frequency influenced by different storage components - storage materials, effect of storage temperature, Effect of storage duration, different plant species (R. Tiwari and Kuntal Das, 2014).

Results and discussion

Seed purity is directly proportional to the type of spectral distribution of the emission signal. Seed wetness is directly proportional to signal intensity. The viability is directly proportional to the intensity level and the spectral distribution along the ordinate of the signal.
Figure 5 presents the spectral distribution with wavelengths of the reflected emission of *Salvia sclarea* seeds collected in three different periods. Seeds from 2022 have the highest viability, the lowest moisture content and the highest purity, and their emission distribution is close to seeds collected in 2019. Seeds from 2018 have much lower values of these parameters. The close distributions in 2022 and 2019 of *Salvia sclarea* seeds show stability on these parameters for this variety.
Figure 5 presents the spectral distribution with wavelengths of the reflected emission of *Salvia officinalis*. Seeds collected from 2022 have the highest viability, lowest moisture content and highest purity. The close distributions in 2016 and 2019 of *Salvia officinalis* seeds indicate that to have stability in these metrics, seeds should not be stored for a longer period.

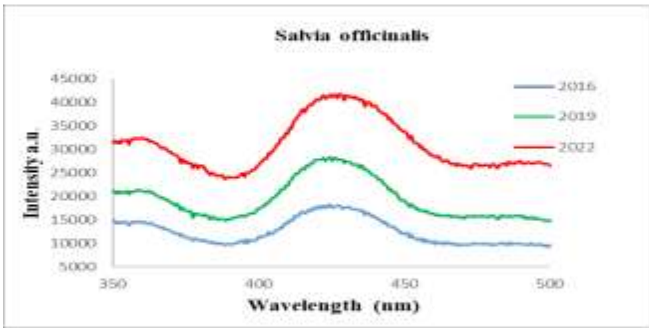

Figure 5. Emission wavelengths of *Salvia Sclarea* seeds harvested in three different periods

Figure 6 shows the spectral distribution with wavelengths of the reflected emission of *Ocimum bacilicum*- lemon. Seeds collected in 2022 had the highest viability, lowest moisture content and highest purity. The emission distributions of seeds collected in 2017 and 2020 were similar. The close distributions in 2017 and 2020 indicate that *Ocimum bacilicum* - lemon seeds are stable over a long period of time.

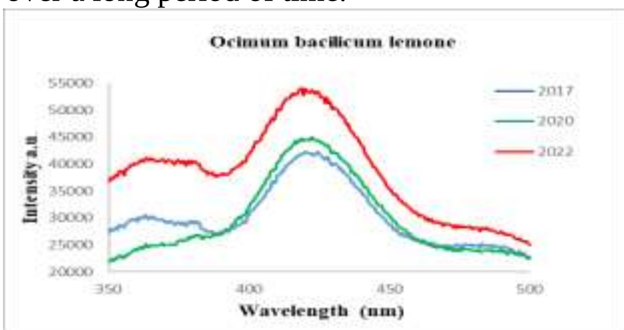

Figure 6. Emission wavelengths of *Ocimum bacilicum* lemone harvested in three different period

Figure 7 shows the spectral distribution with wavelengths of the reflected emission of *Ocimum bacilicum*. Seeds collected in 2012 had the lowest viability, highest moisture content and lowest purity. The emission distributions of seeds collected in 2022 and 2021 are extremely close. The close distributions in 2022 and 2021 of *Ocimum bacilicum* seeds indicate that the seeds are stable in these parameters immediately after harvest or for a short period afterwards.

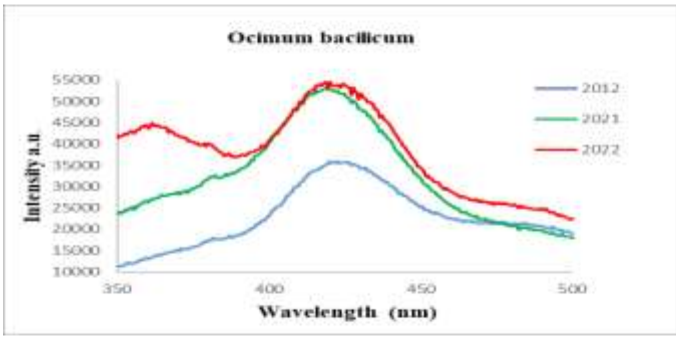

Figure 7. Emission wavelengths of *Ocimum bacilicum* harvested in three different periods

The spectral distributions are unique to the seeds of a particular species, cultivar or breeding line, warranting the use of the plant to identify available seeds of selected members of the *Lamiaceae* family harvested in different years and stored for different periods of time in a non-invasive manner with high accuracy.

Conclusions

The quality of seeds from representatives of the sem. Lamiaceae harvested in different years by standard and non-invasive methods. A mobile fibre-optic installation using fluorescence spectroscopy was successfully set up and tested. In the proposed setup, fluorescence spectroscopy was validated as a non-invasive method to assess seed moisture, viability and purity. Since the proposed method is quick, cheap and non-invasive, and could be easily engineered in order, it is our opinion that it could became an asset in the agro industrial sector helping to determine the quality of seeds in different plants.

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COMBINING ABILITY OF MID-EARLY MUTANT MAIZE LINES FOR THE GRAIN YIELD AND LENGTH OF THE EAR

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Abstract

The general combining ability (GCA) and specific combining ability (SCA) of seven mid-early mutant maize lines for the grain yield and length of the ear were assessed using Savchenko’s mathematical model (1978). The lines were tested in a top-cross scheme with three testers. In 2021 and 2022, their hybrid combinations were tested in field experiments at the Maize Institute – Knezha. High GCA for grain yield was demonstrated by lines XM 2014285, XM 2014298, and XM 2014325, and for length of the ear – by XM 2014285, XM 2014298, and XM 2014284. These lines can be utilized as components in the development of mid-early synthetic populations or as testers when determining GCA in the early stage of the selection. The mutant lines with high SCA for grain yield – XM 2014325, XM 2014298, XM 2014 301, and XM 2014285, and for length of the ear – XM 2014301, XM 2014284, and XM 2014 298, can be incorporated into heterosis selection programs for the purposes of a direct production of high-yielding hybrids with long ears. Lines with high GCA and SCA for both traits can be successfully utilized in both directions of the selection process.

Key words: *General and specific combining ability, Maize inbred lines, Grain yield.*

Introduction

The question of selecting and improving the genetic basis for maize selection is a fundamental aspect in the studies of all selection practitioners. In recent years, the introduction and use of output forms and closely related hybrids in terms of their origin have significantly narrowed the genetic base and created preconditions for vulnerability under stressful conditions. Experimental mutagenesis in maize is one of the possibilities for enriching and expanding its genetic diversity. Its application induces mutations in individual genes or groups of genes, making it possible to obtain a variety of sorts and lines with new traits, without affecting the basic genotype.

Chemical mutagenesis-induced phenotypic changes include important quantitative traits, such as higher grain yield, high protein content, changes in grain type, improved general and specific combining ability and other important traits of agronomic importance (Christov, N., 2004; Kostova, A. et al., 2006; Tomlekova, N., 2010; Gnanamurthy, S. et al. 2011; Eze J. and Dambo A., 2015; Muhammad A., et al., 2018, etc.).

Since 1973, the Maize Institute - Knezha in Bulgaria, has launched a selection program aimed at using experimental mutagenesis and mutational selection to accelerate the selection process by inducing additional genetic variability in elite lines and hybrids (Hristov K. and Hristova P., 1995). As a result, a large number of mutant lines with valuable biological and economic traits have been established at the Institute, and in 1980, for the first time in the world, the first mutant maize hybrids were simultaneously registered in Bulgaria and Ukraine. The combination of hybridization with the methods of experimental mutagenesis is undoubtedly the most effective in enriching the genetic potential of maize. Therefore, an important indicator of the use of a certain selection material in heterosis selection is its combining ability.

The assessment of combining ability allows for its more effective inclusion in well-established selection programs (Petrovska, Genova, 2009; Ilchovska, 2011; Ilchovska, 2013; Valkova, Petrovska, 2015; Valkova, Petrovska, 2016; Petrovska et al., 2019; Valkova et al., 2019, etc.). One of the methods for determining the combining ability of newly stabilized maize lines is the top-cross. In the initial stages of testing, this method allows for a quick and satisfactory assessment of the selection value of these lines (Savchenko, 1978).
The aim of the present study is to analyse the combining ability for the traits grain yield and length of the ear of seven newly developed mid-early mutant maize lines with good productive potential, with a view to their more rational inclusion in appropriate selection programs.

Materials and methods

Studies for the selection and improvement of output material for maize selection through experimental mutagenesis were conducted in the period 2015-2022 at the Maize Institute – Knezha. Heterozygous material treated with a solution of NMU (n-nitroso, n-methyl urea) solution at concentration of 10^{-4}% with an exposure of time of 24 hours was used as the object of mutagenic treatment. The selection of mutagens, exposures, and solution concentrations was applied according to the methodology of Morgun (1983).
After self-pollination and selection for economically valuable traits, the newly-developed mutant lines in the M_5 generation were tested in isolations with three testers: XM 92471, K 4652, and PAU 1617. Their hybrid combinations were tested in field trials using the Latin rectangle method, in three repetitions with a harvest plot size of 10m2, without irrigation, using an agronomic technique adopted for the region of the Institute.
The statistical analysis of the raw data was performed using the method of analysis of variance according to Shanin (1977). To evaluate the general and specific combing ability, the mathematical model of Savchenko (1978) was applied.

Results and Discussion

In the present study, the combining ability of a set of mutant lines was evaluated for the traits grain yield and length of the ear, tested with three testers K 4652, PAU 1617 and XM 92471, belonging to two different heterosis groups – Lancaster and Reid. The testers have a high combining ability and are effective in analysing new source material in the earlier stages of the selection process.
For maize growing, air temperature during the critical for it phenophases, as well as rainfall and its equal distribution during period of vegetation, are agro climatic factors of significant importance. Figure 1 presents data for these factors for the period of the study 2021-2022 compared to a 50-year period (1920-1969). The temperatures for the two years are similar in value and relatively higher than the average for the 50-year period. In 2021, the average daily temperature for the period June - August (flowering, pollination, and fertilization of the maize plant) was 23°C, and the total rainfall amounted to 62.5 l/m². For 2022, the values for the respective indicators were 23.6°C and 54.2 l/m². The data for temperatures and the distribution of rainfall amounts in 2022, especially in July (12.6 l/m²), characterize it as less favourable for maize cultivation, which explains the lower yields and shorter ears of the tested variants (Table 1, Table 2). The tables present averaged data for grain yield and length of the ear of the crosses of the mutant maize lines. The analysis of variance of the field trial data shows significant differences between them (F>Fcrit) for both traits.

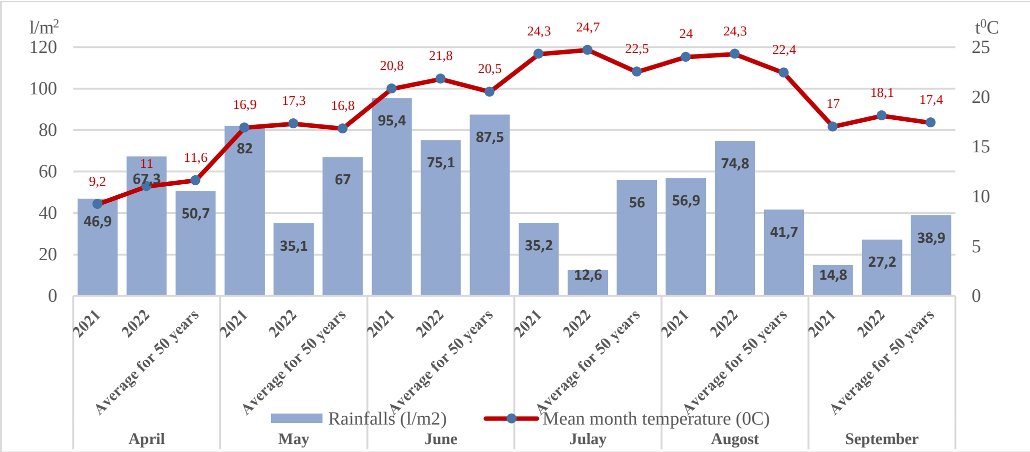

Figure 1. Average month temperature sum of the rainfall through the hybrids vegetation period

The average yield of the studied top-crosses in the first year was 85.460 kg/ha, and in the second year, it was 76.618 kg/ha. The best results for the “grain yield” trait, both by year and on average with the three testers, were shown by the crosses of lines XM 2014285 and XM 2014298 for 2021, and XM 2014325, XM 2014285, and XM 2014344 for 2022. As seen in Table 1 and Table 2, the hybrid combinations involving line K 4652 had higher yields compared to those with the other two testers. Two of them, XM 2014285 x K 4652 and XM 2014344 x K 4652 for 2021, distinguishes themselves with yields over 100.0 kg/ha.

Table 1. Grain yield (kg/ha) of the hybrids with medium-early lines mutant maize. 2021-2022

Tester lines Mutant lines	XM 92471	K 4652	PAU1617	Average for the lines
2021				
XM 2014284	82.560	95.590	74.653	**84.268**
XM 2014285	95.387	**106.170**	**65.753**	**89.103**
XM 2014298	78.170	99.533	86.927	**88.210**
XM 2014301	81.157	96.067	74.747	**83.990**
XM 2014305	98.063	85.393	68.150	**83.869**
XM 2014325	105.147	81.130	66.997	**84.425**
XM 2014344	86.050	100.437	66.567	**84.351**
Average for the testers	**89.505**	**94.903**	**71.971**	**85.460**
$LSD_{5\%}$-55.61				
2022				
XM 2014284	73.477	87.037	65.573	**75.362**
XM 2014285	81.983	79.777	73.677	**78.479**
XM 2014298	76.980	88.800	60.933	**75.571**
XM 2014301	68.493	96.570	64.250	**76.438**
XM 2014305	77.940	85.410	56.600	**73.317**
XM 2014325	83.820	91.893	64.337	**80.013**
XM 2014344	79.360	83.357	68.720	**77.146**
Average for the testers	**77.436**	**87.549**	**64.870**	**76.618**
$LSD_{5\%}$-61.43				

Regarding the trait "length of the ear", the average length of the ear for 2021 was 25.3 cm, and for 2022 it was 19.3 cm. The crosses of lines XM 2014285, XM 2014298, and XM 2014325 for 2021, and XM 2014284 and XM 2014285 for 2022 had long ears both in those years and on average with the three testers. The trend of longer ears in hybrid combinations involving the tester K 4652 persists here as well. Two of the crosses, XM 2014298 x K 4652 (28.6 cm) and XM 2014325 x K 4652 (28.1 cm) for 2022, distinguished themselves with length of the ear over 28 cm. All hybrid combinations with high yields and long ears are of interest for selection, and their testing continues with their inclusion in competitive and ecological varietal trials.

Table 2. Length of the ear (cm) of the hybrids with medium-early mutant lines maize. 2021-2022

Tester lines / Mutant lines	XM 92471	K 4652	PAU1617	Average for the lines
2021				
XM 2014284	23.3	25.8	21.4	**23.5**
XM 2014285	25.8	27.1	25.8	**26.2**
XM 2014298	23.9	28.6	25.7	**26.1**
XM 2014301	25.3	24.8	24.4	**24.8**
XM 2014305	25.1	25.1	25.1	**25.1**
XM 2014325	23.9	28.1	26.1	**26.0**
XM 2014344	22.4	27.3	25.9	**25.2**
Mean for the testers	**24.2**	**26.7**	**24.9**	**25.3**
$LSD_{5\%}$-0.66				
2022				
XM 2014284	20.5	20.3	19.6	**20.1**
XM 2014285	19.8	19.5	19.1	**19.5**
XM 2014298	18.3	19.8	19.7	**19.3**
XM 2014301	18.0	19.8	19.1	**19.0**
XM 2014305	19.3	19.1	19.0	**19.1**
XM 2014325	19.0	19.7	18.7	**19.1**
XM 2014344	19.5	19.5	18.1	**19.0**
Mean for the testers	**19.2**	**19.7**	**19.0**	**19.3**
$LSD_{5\%}$-0.61				

Through analysis of variance of the general combining ability (GCA) and the specific combining ability (SCA) for grain yield and length of the ear, significant differences between the lines participating in the crosses were established (F>Fcrit). This allows for the continuation of the analysis for evaluation of their combining ability. The analysis of variances of the GCA and SCA provides the opportunity to assess the genetic characteristics of these lines, their selection value, and variability in these traits. The results enable their effective use and inclusion in appropriate selection programs, as well as the selection of a suitable selection method (Turbin et al., 1974).

As criteria for this evaluation, the parameters gi, gj for GCA and $\sigma^2 si$, $\sigma^2 sj$ for SCA were used (Table 3, Table 4). The comparison of the effects of GCA with the variances of the effects of SCA for a given line allows for assessing the relative importance of gene action. Lines XM 2014285 and XM 2014298 showed high and positive GCA values for grain yield for 2021, and XM 2014325, XM 2014285 for 2022. These lines are suitable for inclusion in selection programs aimed at obtaining mid-early high-yielding synthetic populations, as they possess more additive genes determining the trait. They can also be used as testers in analysed crosses to determine GCA at an earlier stage of the selection process. The high variances of the SCA effects of line XM 2014325, followed by XM 2014298 for 2021, and lines XM 2014301 and XM 2014284 for 2022, suggest their successful inclusion in combinations to produce hybrids with higher yields. They mainly exhibit dominant and epistatic gene effects.

Table 3. Effects of GCA (gi. gj) and variants of the effects of SCA (σ^2si. σ^2sj) for traits grain yield and length of ear of medium-early mutant maize lines. 2021.

Mutant lines	Grain yield		Length of ear	
	GSA effects (gi. gj)	Variances of SCA effects (σ^2si. σ^2sj)	GSA effects (gi. gj)	Variances of SCA effects (σ^2si. σ^2sj)
XM 2014284	-11.902	2582.804	-1.770	2.184
XM 2014285	36.454	8021.401	0.930	0.309
XM 2014298	27.521	17546.196	0.786	1.318
XM 2014301	-14.679	8530.976	-0.437	2.258
XM 2014305	-15.890	3614.893	-0.181	1.590
XM 2014325	-10.335	22788.860	0.741	0.957
XM 2014344	-11.168	3386.688	0.070	2.306
Standard error	(gi-gj) ±11.24		(gi-gj) ±0.14	
Tester lines				
XM 92471	40.468	11173.36	-1.033	1.68
K 4652	94.406	5682.13	1.405	1.25
PAU 1617	-134.875	5301.78	-0.371	0.72
Standard error	(gi-gj) ±17.17		(gi-gj) ±0.21	

Table 4. Effects of GCA (gi. gj) and variants of the effects of SCA (σ^2si. σ^2sj) for traits grain yield and length of ear of medium-early mutant maize lines. 2022.

Mutant lines	Grain yield		Length of ear	
	GSA effects (gi. gj)	Variances of SCA effects (σ^2si. σ^2sj)	GSA effects (gi. gj)	Variances of SCA effects (σ^2si. σ^2sj)
XM 2014284	-12.562	585.050	0.849	0.177
XM 2014285	18.605	7412.984	0.149	0.144
XM 2014298	-10.473	698.991	-0.040	0.587
XM 2014301	-1.806	8081.939	-0.340	0.562
XM 2014305	-33.017	2025.883	-0.151	0.127
XM 2014325	33.983	1263.295	-0.184	0.036
XM 2014344	5.271	1763.298	-0.284	0.362
Standard error	(gi-gj) ±8.13		(gi-gj) ±0.12	
Линии тестери/ Tester lines				
XM 92471	8.178	1949.98	-0.090	0.37

K 4652	109.306	3463.62	0.357	0.10
PAU 1617	-117.484	1863.55	-0.267	0.20
Standard error	(gi-gj) ±12.42		(gi-gj) ±0.19	

Regarding the trait length of the ear, lines XM 2014285, XM 2014298 for 2021, and XM 2014284 for 2022 show high and positive GCA values. These lines can be included in selection programs to develop synthetics with long ears. The high variances of the effects of the SCA of lines XM 2014301 and XM 2014284 for 2021, and XM 2014298 and XM 2014284 for 2022, allow for their use in selection programs to create hybrids with long ears.
Based on the assessment of the mutant lines, the following most important conclusions are made:

Conclusions

The mutant lines XM 2014285, XM 2014298, and XM 2014325 are suitable for creating synthetics with high yield, and for synthetics with long ears, the suitable lines are XM 2014285, XM 2014298, and XM 2014284. These lines can also be used as testers in the analysis of general combining ability at earlier stages of the selection process.
For obtaining mid-early hybrids with high yields, the mutant lines XM 2014325, XM 2014298, XM 2014301, and XM 2014284, are suitable as parental components, and for those with long ears are suitable hybrids XM 2014301, XM 2014284, and XM 2014298.
The mutant lines XM 2014325 and XM 2014298 for the trait “grain yield” possess relatively high GCA and SCA and can be used in both selection directions.
The mutant lines XM 2014284 and XM 2014298 for the trait “length of the ear” can also be used in both selection directions due to their higher values for both GCA and SCA.

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EVALUATION OF LANDRACE AND FOREIGN MAIZE POPULATIONS FOR BIOLOGICAL AND ECONOMIC PROPERTIES

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Abstract

Two hundred and twenty-two landrace and foreign maize populations were included in a research carried out during the period of 2022 – 2023: 105 of them belonging to the working assortment of Maize Research Institute – Knezha, 15 – to the genebank of IPGR – Sadovo and 102 were foreign, introduced synthetics. The trials were conducted in MRI – Knezha according to block method under non-irrigation conditions and with approval for the regional agricultural technology. Phenological observations (UPOV) were also performed during vegetation. Consequently, the vegetation period and FAO maturity group of the populations were established. Biometric screening of plants and ears (30 of each population) was made. The elements of yield – ear length, number of rows, number of seeds per row, weight of 1000 seeds – were analysed. The laboratory test included the grain chemical content and content of protein, oil and starch. The analysis was conducted with Infraliser (SupNIR – 2700). Forty maize populations, in which the content of protein varied from 10.0 to 14.7 %, that of oil – from 3.5 to 5.3 % and of starch – up to 69 %, were determined as reliable. The aim of the current research is to evaluate the assortment of landrace and foreign synthetic populations, as well as nominate lines for the purposes of hybridisation and creation of new adaptive maize hybrids for various applications (high-oil, high-protein, high-starch).

Keywords: *maize populations, biological and economic properties, chemical content.*

Introduction

The field of agriculture is among the most vulnerable ones to the effects of climate change in any economy. The developing countries that are highly dependent on agriculture and have fewer resources and options to prevent climate change damages are particularly exposed to risk. (Abumhadi et al., 2012). The climate change has been pointed out as one of the main factors leading to a decline in cereal yields in some parts of Europe, despite the continuous development of cultivation technologies. (Brisson et al., 2010). According to Gracen (2008), Hammer and Teklu (2008), Rao and Hodgkin (2002), Brown (2010) the most serious threats to the cultivation of agricultural crops will not be occasional severe droughts or heat waves, but high temperatures during key periods in the life cycle of any crop. Variability and decline in crop yields are expected to increase due to increased frequency of extreme climatic events and the spread of diseases and pests (Martinez, 2016) and the effects of these changes may be diverse in different parts of Europe (Smith, 2005). The preservation of old varieties and populations, landraces and wild relatives of cultivated plants is a priority task worldwide. Many of them have evolved in order to survive in harsh conditions, such as drought, moisture, high temperatures and poor soils. This means they can be a source of new genetic diversity that breeders can use to develop more sustainable food systems, as well as increase food security and ecosystem stability. The knowledge of genetic diversity and its distribution is essential for its conservation and use in specific breeding programs (Valchinkov and Valchinkova, 1993; Bespalova et al., 2017; Esquinas-Alcázar, 1993).

The objective of the current paper is an analysis of the biological and economic qualities of landraces and foreign maize populations in terms of the following indexes: vegetation period, grain moisture at harvesting, mass of 1000 grains, ear length, number of rows in the ear, grain length, protein, oil and starch content. The most valuable ones will be used as source material for hybridisation and creation of a new generation of stress-tolerant, high-yielding maize hybrids in various directions, with an enhanced grain quality.

Materials and Methods

The study was conducted during the period of 2022 – 2023 in the experimental field of Maize Research Institute – Knezha, Bulgaria. Two hundred and twenty-two landraces and foreign maize populations were analysed: 105 of them belonging to the working assortment of Maize Research Institute – Knezha, 15 – to the genebank of IPGR – Sadovo and 102 were foreign, introduced synthetics. The trials were carried out according to block method, in 3 repetitions, sowing density of 5000 plants per ha, harvest plot of 10 m^2, under conditions without irrigation and with approval for the regional agricultural technology. During the vegetation, phenological observations according to UPOV were carried out. As a result, the vegetation period of the populations was reported and their FAO maturity groups were determined. Biometric screening of plants and ears (30 of each population) was made. The structural elements of yield – ear length, number of rows, number of grains in a row, mass of 1000 grains – were also analysed. The laboratory analysis included chemical composition of grain (protein, oil and starch content) and was performed on an average plot sample with an Infraliser (SupNIR – 2700 SERIES). The grain moisture was recorded with an electronic moisture meter.

Results and Discussion

The auspicious crosses from the landraces and the foreign populations, which were pointed out as a consequence of phenotyping a total of 222 samples in relation to some yield elements, are displayed in Table 1. In the observations made during the vegetation period all populations (landraces and foreign ones) were found to be resistant to lodging. No damage from economically important diseases and enemies in the conditions of a natural infectious background was noted. The length of the period from emergence to flowering of the ear varies from 51 to 62 days in different landraces and foreign populations. The flowering periods of ears within each population itself ranged widely and high variability in terms of this parameter was observed among individual plants. The populations Svilengrad and Mestna 1 from the first group and Litkaya – Koreyskaya – visokaya and Synth min spons from the second one silked the earliest.

A slight divergence in the flowering of the ear and the panicle was reported in all populations (from 1 to 3 days), except for the landraces Glogovo and Pishurka, as well as Bst3 Epom 831575 pollan-3-3-1 from the foreign ones, where the desynchronisation in the flowering of the reproductive organs exceeds 5 days. This feature must be taken into account in breeding programs in which the indicated populations are used as parental components for hybridisation. In these cases, it is advisable to apply date sowing.

The selected populations belong to different maturity groups, from 300 to 600 according to FAO. The majority of them are members of the mid-late group and have a period of 55-58 days from germination to flowering of the ear and a standard moisture in the grain (up to 14%). After analysing the obtained results, landrace populations with the longest ear length were selected - Bdintsi, Karamantsi, Bozhentsi, Pishurka, Svilengrad, Spasova mogila, Gnoinitsa, Florosh and Zemen (18-19 cm). Some of them have 14 rows and a longer grain length (9-10 mm.)

Table 1 – Auspicious landraces and foreign maize populations– productivity elements and quality indicators, average values for 2022-2023

No from the assortment	Landrace *Foreign population	Ear length (cm.)	Number of rows	Number of grains per row	Grain length (mm)	Mass of 1000 grains (g)	Days until ear flowering	Moisture %	Protein %	Oil, %	Starch, %	FAO Group
2	Mestna 1	14	12	22	8	213	51	11.9	12.9	4.7	65.1	300-399
3	Mestna 2	17	12	27	8	235	55	12.5	10.9	4.2	65.5	400-499
4	Mestna 3	13	12	25	8	269	55	12.6	12.4	3.8	65.5	400-499
13	Gnoinica	18	14	35	10	259	57	13.2	10.8	4.3	66.6	500-599
20	Vinishte	15	10	27	7	252	53	14.1	12.6	3.9	64.7	400-499
28	Gloginka	17	14	29	9	310	58	13.9	10.9	4.3	66.3	500-599
31	Sadina	17	12	34	9	272	54	13.5	12.1	4.3	66.1	400-499
48	Gyulevo	17	12	30	7	244	55	14.1	11.5	3.5	63.3	400-499
51	Bdintsi	19	12	33	7	285	55	13.6	10.4	3.8	65.9	400-499
56	Sp. Mogila	18	12	36	8	252	53	13.5	11.6	4.2	65.0	400-499
57	Stefanovo	19	12	35	7	269	54	14.0	11.7	4.5	64.9	400-499
60	Markovo	17	12	31	7	213	53	14.0	10.2	4.5	68.1	400-499
75	Karamantsi	19	12	37	8	205	58	11.4	11.2	3.7	65.0	500-599
80	Hlevene	15	14	23	9	259	56	12.8	12.3	4.1	66.3	500-599
81	Glogovo	15	14	28	7	239	60	13.9	10.3	5.2	67.0	500-599
83	Bozhentsi	19	14	34	8	256	55	13.7	11.9	4.4	65.6	400-499
86	Svilengrad	18	14	31	8	267	50	13.7	9.92	4.9	67.7	300-399
91	Florosh	18	12	36	7	236	53	13.7	10.4	4.2	67.3	400-499
92	Pishurka	19	14	29	6	306	59	13.1	9.9	3.7	66.9	500-599
95	Zemen	18	12	33	8	288	53	13.1	11.1	3.7	65.3	400-499
7	*Valentanskaya Knezh	17	14	28	8	279	55	13.5	11.0	4.1	64.3	400-499

9	*SS	14	12	30	6	193	58	14. 0	11. 9	4. 0	65.1	500-599
10	*Yugoslavia - 2	16	16	30	7	215	57	13. 1	10. 0	3.6	65.2	500-599
15	*Amerikanska	17	12	33	7	221	55	13. 3	11. 3	5. 3	65. 5	400-499
16	*Litkaya – Koreyskaya - visokaya	15	16	35	7	169	53	13. 2	11. 6	3. 8	64. 2	400-499
17	*Po Synth	17	12	33	9	242	58	14. 0	11. 6	4. 3	63. 9	500-599
19	*Synth min spons	17	14	33	8	237	54	13. 9	11. 4	4. 3	65. 6	400-499
32	* ICCPT - Fundulla - Hauganse	15	14	27	9	284	58	13. 1	11. 7	3. 6	66. 1	500-599
45	*Population 16 Yugoslavia	18	12	26	7	355	55	13. 7	12. 6	3. 9	63. 8	400-499
53	*No 13 (Argentina)	17	12	29	7	219	59	14. 7	13. 3	4. 0	65. 8	500-599
59	* Synthetic 1-4/86 Romania	17	14	36	7	188	57	13. 4	10. 5	3. 8	66. 2	500-599
64	* High-protein synthetic 9hp CEP – 4	18	16	32	7	242	58	13. 4	10. 7	4. 9	69. 0	500-599
73	* Synthetic - H 99-6-4-1	17	14	31	7	224	59	13. 2	10. 6	3. 8	68. 1	500-599
75	* Bst3 Epom 831575 pollan-21-2	16	14	34	7	224	58	13. 9	9. 4	3. 9	67. 9	500-599
77	* Bst3 Epom 831575 pollan-3-3-1	18	14	35	8	213	62	14. 2	9. 8	4. 0	68. 0	Above 600
82	* Mexican deute pon 831268 - 4 - 2 -1	15	16	28	8	241	55	14. 2	11. 8	4. 8	66. 7	400-499
87	* Synthetic L2 (C_o)	20	12	37	9	328	56	13. 8	11. 7	3. 6	65. 5	500-599
88	* Synthetic (C_2)	18	12	31	9	316	57	13. 4	10. 9	3. 9	66. 1	500-599
95	* Synthetic (C_1)	20	12	31	8	330	56	14. 0	10. 5	4. 0	64. 3	500-599
100	* Synthetic 2/96 "L" (C_o)	21	14	34	8	317	55	12. 9	11. 7	4. 1	65. 9	400-499

The populations Gloginka, Pishurka, Zemen and Bdintsi have the highest mass per 1000 grains. In the landraces listed, the combination of these elements of productivity is favourable and seeds from them have been set aside for testing and determining their combining value.
Synthetic 16 (C_1), Synthetic L2 (C_o), Synthetic 2/96 "L" (C_o) and Synthetic L2 (C_2) belonging to the assortment of foreign populations and in which improvement selection was carried out using the method of recurrent selection posed an interest. The introduced populations have longer ears than the landraces. Two of them are also multi-row ones and can be used as components for synthetics with a long ear or a large number of rows.
The lower absolute weight reported in the populations SS, Litkaya - Koreyskaya - visokaya, Synthetic 1-4/86 Romania can be explained by the participation of teosinte in the genetic foundations of these populations or by a targeted selection of certain quality indicators carried out in them before including them in our breeding programs.
After a chemical analysis of grain quality indicators (Table 1) in the studied populations was done, those with the highest percentage of protein - Mestna 1 (12.9 %), Mestna 3 (12.4 %) and Vinishte (12.6 %) were pointed out. Glogovo (5.2%), Mestna 1 (4.7%) and Svilengrad (4, 9%) have the highest oil content. The landrace populations Markovo (68.1 %), Svilengrad (67.7 %) and Glogovo (67.0 %) have the highest levels of starch content. The populations Mestna 1 and Vinishte have both a high protein content and a relatively high oil content, which makes them a suitable starting material for maize breeding, in the direction of improving the hybrids’ nutritional qualities. The landrace populations Markovo and Svilengrad are most suitable for the food industry and the creation of hybrids with a high starch content. The most balanced in terms of quality indicators is the population Mestna 1, which displays high protein content (12.9 %), oil content (4.7 %), starch content (65.1 %) and low grain moisture at harvest (11.9 %). Then, we placed the landrace Vinishte, for which a high content of protein (12.6 %), oil (3.9 %), starch (64.7 %) and standard moisture of the grain at harvest (14.1 %) were reported. Worthy of interest is the population Mestna 3, which has a balanced profile of quality indicators - protein (12.4%), oil (3.8%), starch (65, 5%) and grain moisture – 12.6%. The landrace Hlevene, which is characterized by high content of protein (12.3%), oil (4.1%), starch (66.3%) and grain moisture – 12.8%, will be included in the breeding programs of Maize Research Institute – Knezha for improving the grain quality indicators. These four populations stand out as the best sources for maize breeding in a direction for enhanced grain quality due to their high protein content, balanced moisture levels and favourable starch and oil content.
The remaining local samples have good indicators too, but due to the higher grain humidity at harvest, they may prove problematic under certain agro-climatic conditions or when stored for a longer period. After analysing the chemical composition of the grain in the foreign synthetics as well, No. 13 (Argentina) proved to be the population with the highest protein (13.3 %), oil (4.0 %) and high starch content (65.8 %). This makes it a suitable source of geneplasm in the selection of high-protein maize hybrids. Population 16 (Yugoslavia) with a protein content of 12.6 % is also suitable in this direction.
In the direction of high-oil hybrids selection, the population Amerikanska can be successfully used as starting material due to its highest percentage of oil – 5.3%, as well as a high content of protein (11.3 %) and starch (65.5 %). The High-protein synthetic 9hp CEP – 4 can also be used successfully for the listed purposes, with oil content of 4.9 %, protein – 10.7 % and highest starch content – 69. 0 %. Synthetic - H 99-6-4-1 can effectively be used in the selection of high-starch corn, since the starch content is 68.1 %.
The listed four synthetic populations of foreign origin can be included in programs to improve the quality of the grain and as donors of geneplasm to increase the content of protein, oil and starch. We evaluated them as having a high potential for creating hybrids or improving selection with a view to creating maize hybrids with a balanced nutritional content.

Conclusions

The study of genetic diversity in landraces and foreign populations of maize is a huge resource for breeding under conditions of stress and unpredictable climatic changes. Phenotyping and evaluation of these starting materials are a reserve for creating adaptively valuable maize hybrids that respond adequately to environmental changes. After a study of 222 samples of local and foreign origin was done, 40 valuable maize populations suitable for different directions of use were selected.

For high-protein and high-oil hybrids, the most suitable landrace material is Mestna 1, which has high protein content (12.9 %), high oil content (4.7 %) and low grain moisture at harvest (11.9 %). The synthetic Argentina from the introduced populations is suitable for high protein hybrids.

For high-starch hybrids, it is recommended to use lines originating from the synthetics Markovo (68.1 %), Svilengrad (67.7 %), Florosh (67.3 %) and Glogovo (67.0 %), and from the foreign ones the High-protein synthetic 9hp CEP – 4 (69.0 %), Synthetic - H 99-6-4-1 (68.1 %) and Bst3 Epom 831575 pollan-3-3-1 (68.0 %) are suitable for this direction.

As a result of the conducted research, the working collection of Maize Research Institute - Knezha was enriched with 222 new samples, which were studied for biological and economic qualities.

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GRAIN YIELD RESPONSE OF MAIZE, WHEAT AND BARLEY TO NITROGEN RATES AND FERTILIZATION SYSTEMS

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Abstract

The grain yield of maize, wheat and barley depending on the nitrogen rate and fertilization system was studied in a long-term field fertilizer experiment for the period 1959-2015. Nitrogen fertilization was N_{80}, N_{160} and N_{240} (for maize); N_{60}, N_{120} and N_{180} (for wheat), and N_{40}, N_{80}, N_{120} (for barley). The fertilization systems were: Control, $N_{300}P_{400}K_{600}$+18 t vetch-oat mixture/ha, $N_{600}P_{400}K_{600}$ (stock fertilization), $N_{900}P_{400}K_{600}$, $N_{600}P_{400}K_{600}$+30 t manure/ha, $N_{600}P_{400}K_{600}$+4 t straw/ha, $N_{600}P_{400}K_{600}$ (annual fertilization) (I-IV crop rotations); Control, $N_{300}P_{300}K_{400}$, $N_{600}P_{300}K_{400}$, $N_{900}P_{300}K_{400}$, $N_{600}P_{300}K_{400}$+40 t manure/ha, $N_{600}P_{0}K_{400}$, $N_{600}P_{300}K_{0}$ (V-VII rotations); Control, $N_{250}P_{300}K_{200}$, $N_{500}P_{300}K_{200}$, $N_{750}P_{300}K_{200}$, $N_{250}P_{150}K_{0}$+60 t manure/ha, $N_{500}P_{0}K_{200}$, $N_{500}P_{300}K_{0}$ (VIII rotation). The obtained grain yields without fertilization were 6127 $kg.ha^{-1}$ for maize, 1453 $kg.ha^{-1}$ for wheat and 1617 $kg.ha^{-1}$ for barley, on average over a period of eight crop rotations. The rates N_{80}, N_{160}, and N_{240} in maize increased grain yield by 38.4%, 57.3%, and 68.8%, respectively, compared to the control. The grain yield of wheat and barley increased by 1.9-3 times an average in the nitrogen-fertilized variants, compared to the yields without fertilization. The regression analysis indicated that without fertilization in the crop rotation, average grain yields of 6164 $kg.ha^{-1}$ was possible to expect from maize; 1547 kg. ha^{-1} from wheat, and 1672 kg. ha^{-1} from barley. The possible additional grain yield for each kilogram of nitrogen input per hectare was 32.7 kg for maize, 43.2 kg for wheat and 40.4 kg for barley.

Keywords: *Maize, Winter Wheat and Barley, Fertilization.*

Introduction

The essence of modern concepts for the development of sustainable agriculture consists in finding the optimal productive level corresponding to each crop, by applying such systems that guarantee obtaining stable or increasing yields for a long period of time and preserving natural resources, including the main means of production – the soil (Alexander et al., 2015; Beckman et al., 2020). The emergence of these concepts is based on the results of long-term stationary experiments carried out in different regions of the world (Merbach and Deubel, 2008). The oldest permanent fertilizer experiment in the world is in in Rothamsted, England (Edmeades, 2003). Cereal crops were studied in it and it continues to this day (Edmeades, 2003). Fertilization is one of the most effective the means to increase productivity of the main cereal crops that require mineral nutrients in large amounts (Traikov et al., 2017). The different rates of fertilization in the agroecosystems and long-term cultivation of crops without fertilization allow establish the regulatory functions of fertilization and its absence on the state of soil fertility and productivity of the studied crops (Kunzova and Hejcman, 2010; Foulkes, 2009). According of Babulicova (2008) the long-term field fertilizer experiments are the main factor for managing the productivity and quality of crops according to the requirements for balanced nutrition and fertilization, for rational use of the land and preservation of soil qualities, and protection of agro-ecosystems as a whole from pollution. The fertilization system affects the physical, agrochemical and biological parameters of the

soil and largely determines the magnitude and stability of yields (Valcheva et al., 2015). Nitrogen fertilization is one of the most effective means of increasing the productivity of the main cereal crops that require mineral nutrients in large quantities (Kismányoky and Tóth, 2010; Lopez-Bellido et al., 2005; Traikov et al., 2017). At the same time this nutrient is easily mobile and its improper use can cause environmental pollution (Fixen et al., 2015). Agroecosystems in which good agricultural practices are followed demonstrate the increased yields, economically optimal nutrition and fertilization, minimal losses of soil nutrients and sustainable soil fertility (Mikkelsen et al., 2012).
The present research aims to study the effect of nitrogen rates and fertilization systems on the grain yields of the main cereal crops in Bulgaria maize, wheat and barley under conditions of long-term fertilizer experiment.

Material and Methods

A long-term fertilizer experiment conducted at the educational and experimental base of the Department of Agrochemistry and Soil Science at the Agricultural University of Plovdiv, Bulgaria, examined the grain productivity of maize, winter wheat, and winter barley over the period from 1959 to 2017. The cereal crops were grown under the conditions of field crop rotation (maize - winter wheat – sunflower - winter barley) with the application of different fertilization systems (Table 1). Maize was irrigated cultivation. Average data of the grain yields of the rotations with different fertilization systems were analyzed. The experimental design included seven variants of system fertilization, which were applied in four replications. The size of the experimental plots was 150 m^2.

Table 1. Fertilization systems by crop rotations

I-IV crop rotations	V-VII crop rotations	VIII-IX crop rotations
1. Control	1. Control	1. Control
2. $N_{300}P_{400}K_{600}$+18 t vetch-oat mixture.ha^{-1}	2. $N_{300}P_{300}K_{400}$	2. $N_{250}P_{300}K_{200}$
3. $N_{600}P_{400}K_{600}$ (stock fertilization)	3. $N_{600}P_{300}K_{400}$	3. $N_{500}P_{300}K_{200}$
4. $N_{900}P_{400}K_{600}$	4. $N_{900}P_{300}K_{400}$	4. $N_{750}P_{300}K_{200}$
5. $N_{600}P_{400}K_{600}$+30 t manure.ha^{-1}	5. $N_{600}P_{300}K_{400}$+40 t manure.ha^{-1}	5. $N_{250}P_{150}K_0$ + 60 t manure.ha^{-1}
6.$N_{600}P_{400}K_{600}$+4 t straw.ha^{-1}	6. $N_{600}P_0K_{400}$	6. $N_{500}P_0K_{200}$
7. $N_{600}P_{400}K_{600}$ (annual fertilization)	7. $N_{600}P_{300}K_0$	7. $N_{500}P_{300}K_0$

Applied nitrogen fertilization during the studied period was in rates N_{80}, N_{160} and N_{240} in the maize; N_{60}, N_{120} and N_{180} in the wheat, and N_{40}, N_{80}, N_{120} in barley. Phosphorus fertilization was done in the form of triple superphosphate and potassium fertilization as potassium chloride, which together with manure were plowed with deep autumn plowing (25-30 cm). The maize was fertilized twice with 1/2 of the nitrogen for pre-sowing fertilization, and the rest of the nitrogen rate for dressing in the 5-6 leaf phase. Nitrogen in wheat and barley was applied in parts: the half of the ammonium nitrate as pre-sowing fertilization, and the rest of nitrogen rate as winter-spring top dressing. The experiment was carried out on alluvial-meadow soil (Mollic Fluvisols) (FAO, 2006), a slightly solontic, a sandy-clay with the depth of groundwater 100 - 200 cm. The content of physical clay in the upper horizons reached 50% (in horizon A was 33%). The soil contained 1.63 – 3.00% calcium carbonate, which determined it favorable physico-chemical and water properties. Horizon A1 was up to 28 cm deep, brown-black, loose, with a rather well-defined powder-granular structure. The average content of humus was 3.72 %. The soil reaction, the content of mineral nitrogen (NH_4+NO_3) and available phosphorus and potassium after VIII crop rotation was presented in Table 2.

Table 2. Soil reaction and content of available forms of nitrogen, phosphorus and potassium in the soil (mg.kg^{-1}) after VIII crop rotation

Fertilizing systems	pH H_2O	Nmineral	P_2O_5	K_2O
1. Control	7.36	26.5	58	337
2. N_1PK	7.58	33.6	89	392
3. N_2PK	7.08	26.9	68	351
4. N_3PK	6.84	27.3	48	337
5. NPK + manure	7.16	43.9	81	415
6. N_2PK with reduced P	7.25	30.1	50	341
7. N_2PK with reduced P	7.19	34.0	93	346

The climate of Plovdiv region is transitional-continental, with frequent invasions of moist air masses from the warm sector of Mediterranean cyclones during the cold half-year. It is influenced by the Mediterranean and is called "Western Thracian", with a relatively low amount of precipitation. The average annual air temperature was 12°C. The area was characterized by high temperatures in the summer months of July and August, a mild winter with not very low average daily temperatures (the lowest average monthly temperature was in January - 0°C) and frequent warming. Spring comes relatively early and has an average monthly temperature of 6.5°C. Summer is hot with the highest temperature in July (23°C). Autumn is usually warmer than spring. The average amount of annual precipitation in the Plovdiv region was less than the average for the country (512 mm) and was unevenly distributed by month. Droughts are very common, starting from the beginning of summer and continuing until the end of October.

The data was analyzed with the ANOVA procedure and Duncan's multiple range test to find significant differences among the average values of grain yields of the crops. A regression analysis was applied to model the dependencies between the result parameter grain yield and the factor characteristic nitrogen fertilization. We used regression models of the type: $y = a + bx + cx^2$, where: y - theoretical levels of the result attribute and x - actual levels of the factor attribute.

Results and Discussion

Low productivity of studied cereals was obtained when the crops grown without fertilization (Table 3). Based on natural soil fertility the average grain yields were 6127 kg.ha^{-1} in maize, 1453 kg.ha^{-1} in wheat, and 1617 kg.ha^{-1} in barley. The lowest yields of corn and barley in the control variant without fertilization were established within I-IV crop rotations, and for wheat in the period of V-VII crop rotations. The lowest yields of maize and barley in the control variant were established within I-IV crop rotations, and for wheat in the period of V-VII crop rotations. The highest maize yield without fertilization was 6670 kg.ha^{-1} (V-VII crop rotations). Within the study the highest grain yield of wheat in control variant was 1610 kg.ha^{-1} (I-IV crop rotations) and the highest barley yield without fertilization was 1920 kg.ha^{-1} (V-VII crop rotations). As a result of nitrogen fertilization, the productivity of maize grain was changed from 7670 kg.ha^{-1} (N_{80} in I-IV crop rotations) to 10550 kg.ha^{-1} (N_{240} in V-VII crop rotations). Low productivity of wheat grain was found at N_{60} rate in VIII crop rotation (3520 kg.ha^{-1}). The application of 180 kg N.ha^{-1} in I-IV crop rotations resulted in a high wheat grain yield of 4880 within the study. Nitrogen fertilization changed barley grain yield from 3000 kg.ha^{-1} (N_{40} applied in V-VII crop rotations) to 4210 kg.ha^{-1} (N_{120} applied in VIII crop rotations). A positive effect of the applied nitrogen on the grain yield of three crops was established over the period of the eight crop rotations. Input of N_{80}, N_{160}, and N_{240} rates to maize increased average grain yield by 38.4%, 57.3%, and 68.8%, respectively, over the control.

Table 3. Grain yield of maize, wheat and barley depending on the nitrogen rates and fertilization systems, kg.ha^{-1}

Fertilization systems	Maize grain	% to N_0	Wheat grain	% to N_0	Barley grain	% to N_0
I-IV crop rotations						
Control	5660	100	1610	100	1270	100
N_1PK	7670	135.5	4550	282.6	3240	255.1
N_2PK	9290	164.1	4670	290.1	3290	259.1
N_3PK	10130	179.0	4880	303.1	3500	275.6
V-VII crop rotations						
Control	6670	100	1190	100	1920	100
N_1PK	8470	127.0	3540	297.5	3000	156.3
N_2PK	9790	146.8	3680	309.2	3190	166.1
N_3PK	10550	158.2	3850	323.5	3380	176.0
VIII crop rotation						
Control	6050	100	1560	100	1660	100
N_1PK	9300	153.7	3520	225.6	3160	190.4
N_2PK	9830	162.5	4340	278.2	3900	234.9
N_3PK	10340	170.9	4540	291.0	4210	253.6
Average I-VIII crop rotation						
Control	6127 c*	100	1453 b	100	1617 b	100
N_1PK	8480 b	138.4	3870 a	266.3	3133 a	193.8
N_2PK	9637 a	157.3	4230 a	291.1	3460 a	214.0
N_3PK	10340 a	168.8	4423 a	304.4	3697 a	228.6

*Values for each crop followed by the same letters are not significantly different at $p<0.05$ according to Duncan’s multiple range test

The average grain yield of wheat and barley raised by 1.9-3 times in nitrogen-fertilized variants, compared to the yields of the control. Higher nitrogen rates (N_{240} in maize, N_{180} in wheat, and N_{120} in barley) increased grain yields, but the difference with the moderate nitrogen fertilization (N_{160} in maize, N_{120} in wheat, and N_{80} in barley) was not significant.

Table 4. Correlations of nitrogen rates with grain yield of maize, wheat and barley

Crop rotation	Maize	Wheat	Barley
I-IV	0.985*	0.827*	0.834*
V-VII	0.984*	0.830*	0.903*
VIII	0.895*	0.926*	0.953*
Average I-VIII	0.965*	0.867*	0.906*

*Correlation is significant at the 0.05 level.

A strong positive correlation ($r > 0.830$) was found between the applied nitrogen rates and grain yields of the cereal crops maize, wheat and barley grown in crop rotations with different fertilization systems. The correlation coefficient of nitrogen fertilization with maize grain yields demonstrated the highest average value ($r = 0.965$*) in the period of I-VIII crop rotations. The obtained correlation coefficient of fertilized nitrogen input with the wheat grain yield was 0.867*. High average value of the correlation coefficient (r = 0.906*) was established and in regard to barley also.

Table 5. Regression dependences of grain yield (y) of maize, wheat and barley on nitrogen fertilization (x) under different fertilization systems

Crop rotation	Regression model	R^2
	Maize	
I-IV	$y = 5640 + 29.7x - 0.045x^2$	0.999
V-VII	$y = 6666 + 25.9x - 0.040x^2$	0.998
VIII	$y = 6185 + 42.4x - 0.045x^2$	0.967
Average I-VIII	$y = 6164 + 32.7x - 0.07x^2$	0.997
	Wheat	
I-IV	$y = 1755 + 50.6x - 0.19x^2$	0.941
V-VII	$y = 1302 + 40.7x - 0.15x^2$	0.947
VIII	$y = 1586 + 38.2x - 0.12x^2$	0.997
Average I-VIII	$y = 1547 + 43.2x - 0.15x^2$	0.968
	Barley	
I-IV	$y = 1374 + 49.8x - 0.28x^2$	0.933
V-VII	$y = 1964 + 28.1x - 0.14x^2$	0.969
VIII	$y = 1675 + 43.2x - 0.19x^2$	0.998
Average I-VIII	$y = 1672 + 40.4x - 0.20x^2$	0.977

The applied regression analysis of the dependences between the result parameter (grain yield) and the factor sign (nitrogen fertilization) in cereal crops indicated that the dependences were not linear (Table 5). The modeled dependencies were represented by quadratic equations. High values of the coefficients of determination ($R^2 > 0.933$) were found for all studied fertilization systems in the crop rotations. The regression model showed that the increase of grain yields was performed with a delay depending on the nitrogen fertilization up to a rate of 30 kg $N.ha^{-1}$ (in maize), up to a rate of 18 kg $N.ha^{-1}$ (in wheat), and up to a norm of 12 kg $N.ha^{-1}$ (in barley). Without fertilization, the expected average grain yields from the crops were as follows: maize 6164 $kg.ha^{-1}$; wheat 1547 $kg.ha^{-1}$, and barley 1672 $kg.ha^{-1}$. According to the standard interpretation of the regression coefficients in a quadratic polynomial, each increase in the amount of nitrogen fertilizer input by one kilogram per hectare will lead to an increase of the additional grain yield by 32.7 kg of maize, 43.2 kg of wheat and 40.4 kg of barley in the period I -VIII rotations.

Conclusions

The analysis of the data from the long term fertilizer trail of Department of Agrochemistry and Soil science of Agricultural University of Plovdiv, Bulgaria demonstrated that obtained grain yields of cereal crops grown without fertilization were 6127 $kg.ha^{-1}$ (maize), 1453 $kg.ha^{-1}$ (wheat) and 1617 $kg.ha^{-1}$ (barley), on average over a period of eight crop rotations. The N_{80}, N_{160}, and N_{240} rates in maize increased grain yield by 38.4%, 57.3%, and 68.8%, respectively, compared to the control. The grain yield of wheat and barley increased by 1.9-3 times an average in the nitrogen-fertilized variants, compared to the yields without fertilization. The regression analysis indicated that without fertilization in the crop rotation, average grain yields of 6164 $kg.ha^{-1}$ was possible to expect from maize; 1547 kg. ha^{-1} from wheat, and 1672 kg. ha^{-1} from barley. The possible additional grain yield for each kilogram of nitrogen input per hectare was 32.7 kg for maize, 43.2 kg for wheat and 40.4 kg for barley.

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MACRONUTRIENTS ACCUMULATION OF BARLEY DEPENDING ON THE SOIL NITROGEN LEVEL

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Abstract

The effect of nitrogen N_0, N_{200} and N_{400} on the uptake of nitrogen, phosphorus, potassium, calcium and magnesium of barley in maturity was studied. A pot experiment was carried out with five Bulgarian varieties of malting barley grown under greenhouse conditions. Nitrogen levels were created by adding NH_4NO_3 dissolved in water. A proven positive effect of the applied nitrogen on the formed dry biomass and the accumulated macronutrients by the plants was found. Fertilization N_{400} increased the average productivity of the varieties by 23.8% for grain, 36.1% for straw and 31.1% for grain+straw, compared to plants grown at N_0. Accumulated nutrients of grain+straw at N_{400} level were higher by 84.9% for nitrogen, 51.8% for phosphorus, 54.4% for potassium, 41.3% for calcium, and 47.3% for magnesium, on average. Grain harvest index decreased from 41.5% at N_0 to 39.3% at N_{400}. The nitrogen level had a slight effect on the percentage distribution of accumulated nutrients to the grain, although a tendency for a lower percentage was observed in regard to nitrogen, phosphorus and magnesium. A strong positive correlation of nitrogen level with grain and straw yield and amount of accumulated nitrogen was established. A negative correlation was observed between applied nitrogen and grain harvest index (r=-0.641*) and nitrogen harvest index (r=-0.479).

Keywords: *Phosphorus, Potassium, Calcium, Magnesium, Malting barley.*

Introduction

The specificity of mineral nutrition is rarely taken into account in the selection process of cereals (Emebiri et al., 2004). Research specifics of mineral nutrition are in two directions: characterizing the response of varieties and hybrids to mineral nutrition and creation of new varieties and hybrids that use nutrients efficiently and economically (Górny, 2009). Efficiency of nutrient uptake is defined as the ability of plants to absorb a particular nutrient at a low level of soil availability or nutrient medium (Gastal and Lemaire, 2002). Usually, however, crop selection is carried out under agrochemical conditions that do not limit their growth and productivity (Abeledo et al., 2008). It is known that barley productivity is mainly related to the assimilation of nitrogen and phosphorus, which are the main cause of donor limitation at grain filling (Popovic er al., 2011; Przulij and Momcilovic, 2001; Rogers et al., 2019). Nitrogen influences the development and maintenance of leaf area and photosynthetic efficiency (Abeledo et al., 2001) and the allocation of dry mass to reproductive organs (Prystupa et al., 2004). Plants take up potassium exclusively from the soil solution, which is in dynamic equilibrium with the exchangeable and, to a lesser extent, the non-exchangeable potassium stock in the soil (Marschner, 1997). The effectiveness of mineral nutrition depends on two integrated groups of plant factors, namely absorption efficiency and utilization efficiency (Barr et al., 2011). Absorption efficiency is the relative uptake of a given nutrient a given provision or supply. Utilization efficiency represents the total or primary production relative to the nutrient uptake. The genotypic specificity of mineral nutrition in agricultural

crops, including barley, has been proven and in a number of countries work is being done to adapt plant diagnostics according to the genotype (Collen, 2006).
The aim of the present research was to determine the effect of the soil nitrogen level on the accumulation and distribution of nitrogen, phosphorus, potassium, calcium and magnesium of barley in maturity.

Material and Methods

A pot experiment was conducted to study the effect of nitrogen levels N_0, N_{200}, and N_{400} on the uptake and distribution of macronutrients in five Bulgarian winter two-row barley varieties. The study was conducted in a cultivation facility in 2022. The nitrogen levels were created by adding NH_4NO_3 dissolved in water. Ten plants of each variety Emon, Obzor, Krami, Kristi and Kaskadior were grown in plastic pots filled with 5 kg Molic fluvy soil under greenhouse conditions. The main soil characteristics were as follows: $pH_{(H2O)}$ 7.3; humus content 3.2%; mineral nitrogen 43.8 mg $N.kg^{-1}$; available phosphorus 209 mg $P_2O_5.kg^{-1}$; and available potassium 450 mg $K_2O.kg^{-1}$. Each experimental treatment was studied in four replicates. The yield of grain, straw and the amount of nitrogen, phosphorus, potassium, calcium and magnesium in them were determined in a physiological maturity. Barley grain and straw were dried at 60°C to a constant weight. The concentration of macronutrients was analyzed using the common methods (Tomov et al., 2009). The content of macronutrients was calculated by multiplying the concentration of each of studied nutrient by the dry weight of plant parts. Grain harvest index was calculated according to the following formula: GHI (%) = Grain yield / (Grain+Straw) yield × 100. Nitrogen harvest index (NHI) was defined as the amount of accumulated nitrogen of grain divided by the amount of accumulated nitrogen of grain plus straw, and multiplied by 100. The harvest indices of the other macronutrients were calculated in the same way. An overall analysis of variance (ANOVA) was performed to evaluate the effect of the experimental treatments on the referred variables, and Duncan's multiple range test ($\alpha = 0.95$) was used in order to establish the difference among the means.

Results and Discussion

The yields of grain, straw and grain+straw proven increased with the higher amount of applied nitrogen (Table 1). The average grain yield of barley varieties without fertilization was 13.8 $g.pot^{-1}$. It rose by 14.5% and by 23.8% at N_{200} and at N_{400} levels, respectively. Grain productivity differences between the unfertilized plants and the plants grown at N_{200} level were not established. No mathematically proven difference was found between the two levels of nitrogen input N_{200} and N_{400} either. The obtained straw yield increased by 20.7% an average at N_{200}, and by 36.1% an average at N_{400}, relative to N_0 level. The grain harvest index changed from 37.2% (Emon variety at N_{400}) to 43.1% (Krami variety at N_0). Higher nitrogen fertilization of 400 mg $N.kg^{-1}$ soil lowered the average GHI of barley varieties from 41.5% in unfertilized plants up to 39.3%.
The accumulated nitrogen, phosphorus and potassium of barley significantly increased in parallel with the applied nitrogen (Table 2). The highest uptake of nitrogen, phosphorus, and potassium was found at N_{400} level. Their average values of grain+straw were 558.4 mg $N.pot^{-1}$, 276.8 mg $P_2O_5.pot^{-1}$, and 551.2 mg $K_2O.pot^{-1}$, respectively. This indicated that the plants accumulated very similar amounts of nitrogen and potassium in maturity. The absorbed phosphorus was almost twice less compared to them. The total nitrogen uptake widely varied from 265.4 mg $N.pot^{-1}$ (Obzor at N_0) to 651.5 mg $N.pot^{-1}$ (Kaskadior at N_{400}). The total phosphorus in maturity changed from 156.3 mg $P_2O_5.pot^{-1}$ (Emon at N_0) to 313.1 mg $P_2O_5.pot^{-1}$ (Krami at N_{400}).

Table 1. Barley productivity (g.pot^{-1}) in dependence of the nitrogen level

Parameter	N level	Min	Max	Average	% to N_0
Grain yield	N_0	12.0	15.3	13.8 b* ± 1.59	100
	N_{200}	14.1	17.6	15.9 ab ± 1.66	114.5
	N_{400}	15.7	18.5	17.2 a ± 1.33	123.8
Straw yield	N_0	17.2	21.2	19.5 c ± 1.54	100
	N_{200}	20.9	25.5	23.6 b ± 1.88	120.7
	N_{400}	24.5	27.6	26.7 a ± 1.24	136.1
Grain+Straw yield	N_0	29.2	36.3	33.4 c ± 3.06	100
	N_{200}	35.2	43.1	39.5 b ± 3.47	118.1
	N_{400}	40.2	46.1	43.7 a ± 2.43	131.0
GHI, %	N_0	39.5	43.1	41.5 a ± 1.24	100
	N_{200}	38.3	41.0	40.3 ab ± 1.11	97.0
	N_{400}	37.2	40.2	39.3 b ± 1.22	94.6

*Values for each parameter followed by the same letters are not significantly different at p<0.05 according to Duncan’s multiple range test

Table 2. Uptake of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) of barley in dependence of the nitrogen level, mg.pot^{-1}

Parameter	N level	Min	Max	Average	% to N_0
N grain	N_0	179.6	240.9	201.4* c ± 25.7	100
	N_{200}	254.9	308.8	274.1 b ± 23.6	136.1
	N_{400}	314.0	422.7	361.0 a ± 47.1	179.2
N grain+straw	N_0	265.4	357.4	302.0 c ± 38.9	100
	N_{200}	374.3	480.4	417.0 b ± 46.2	138.1
	N_{400}	481.4	651.5	558.4 a ± 72.0	184.9
P grain	N_0	82.6	124.1	97.9 b ± 16.3	100
	N_{200}	102.7	153.4	122.1 a ± 19.5	124.8
	N_{400}	124.9	167.3	141.7 a ± 16.2	144.8
P grain+straw	N_0	156.3	219.2	182.4 c ± 25.5	100
	N_{200}	200.1	280.9	234.7 b ± 33.1	128.7
	N_{400}	245.0	313.1	276.8 a ± 27.6	151.8
K grain	N_0	67.8	92.0	83.6 c ± 10.8	100
	N_{200}	91.5	122.5	110.5 b ± 11.7	132.2
	N_{400}	115.4	144.6	131.3 a ± 10.6	157.0
K grain+straw	N_0	289.6	67.4	357.0 c ± 50.1	100
	N_{200}	379.7	529.0	462.3 b ± 63.0	129.5
	N_{400}	461.8	616.0	551.2 a ± 61.1	154.4

*Values for each parameter followed by the same letters are not significantly different at p<0.05 according to Duncan’s multiple range test

The content of potassium of grain+straw ranged from 289.6 mg K_2O.pot^{-1} (Obzor at N_0) to 616.0 mg K_2O.pot^{-1} (Kaskadior at N_{400}). Higher N_{400} fertilization increased absorbed nitrogen by 79.2% an average in terms of grain, by 96.5% of straw, and by 84.9% of grain+straw, compared to N_0 plants. The studied varieties demonstrated a high export of phosphorus and potassium of the aboveground dry mass when grown at N_{400} level. The average content of grain phosphorus and potassium of the fertilized plants exceeded by 24.8% - 44.8%, and 32.2% - 57.0% that of the N_0 plants, respectively.

Table 3. Uptake of calcium (CaO) and magnesium (MgO) of barley in dependence of the nitrogen level, $mg.pot^{-1}$

Parameter	N level	Min	Max	Average	% to N_0
Ca grain	N_0	25.2	38.3	34.4 b ± 5.4	100
	N_{200}	32.8	47.3	42.5 a ± 5.7	123.7
	N_{400}	37.7	54.9	48.5 a ± 6.4	141.1
Ca grain+straw	N_0	160.7	284.6	226.8 b ± 47.7	100
	N_{200}	202.3	323.4	276.5 a ± 51.7	121.9
	N_{400}	245.8	364.3	320.5 a ± 49.1	141.3
Mg grain	N_0	13.2	21.1	16.1 b ± 3.1	100
	N_{200}	17.1	25.9	20.4 a ± 3.4	126.7
	N_{400}	17.3	25.9	21.1 a ± 3.3	130.8
Mg grain+straw	N_0	28.0	38.1	30.5 b ± 4.3	100
	N_{200}	36.0	48.6	40.0 a ± 5.1	131.3
	N_{400}	41.1	56.3	44.9 a ± 6.4	147.3

*Values for each parameter followed by the same letters are not significantly different at $p<0.05$ according to Duncan’s multiple range test

The amount of calcium and magnesium proven increased in the fertilized variants compared to N_0 (Table 3). The average uptake of grain+straw calcium was higher by 21.9 – 41.3%, and that of magnesium by 31.3 – 47.3%. However, no proven difference was found in regard to the calcium and magnesium content of the barley varieties grown at N_{200} and N_{400}. Total calcium uptake varied from 160.7 mg $CaO.pot^{-1}$ (Obzor at N_0) to 364.3 mg $CaO.pot^{-1}$ (Kaskadior at N_{400}), and that of magnesium from 28.0 mg $MgO.pot^{-1}$ (Kristi at N_0) to 56.3 mg $MgO.pot^{-1}$ (Kaskadior at N_{400}). Within the study, variety Kristi accumulated the most grain calcium 54.9 mg $CaO.pot^{-1}$ and variety Kaskadior accumulated the most grain magnesium 25.9 mg $MgO.pot^{-1}$.

Nutrient harvest indices indicate what percentage of the absorbed nutrient in the grain+straw at maturity is allocated to the grain, similar to the grain harvest index. The results of Table 4 showed that the barley plants alocated into the grain the most of accumulated nitrogen in maturity (64.6–66.7%), followed by the share of phosphorus (51.2–53.6%) and that of the magnesium (47.0–52.7%). The studied varieties distributed to the grain the least amount of calcium from its content of grain+staw in maturity, which averaged 15%. Low values of 23.5%-24.1% were also found regarding to the potassium harvest index. The nitrogen studied levels no significantly affect the percentage of nitrogen, phosphorus, potassium, calcium and magnesium distributed into the grain at maturity. There was a tendency to decrease the mean values of NHI, PHI and MgHI in the nitrogen-fertilized variants.

Our results indicated no proven varietal differences in the average yields of grain and straw (Table 5). The assimilated nitrogen, phosphorus and potassium in these parts had similar values. Significant difference was observed only in the uptake of grain phosphorus between Krami variety (average 148.3 mg $P_2O_5.pot^{-1}$) and Emon variety (average 103.4 mg $P_2O_5.pot^{-1}$). The Kristi variety accumulated more grain calcium and the Kaskadior variety more grain magnesium. The Emon variety was distinguished by a lower Grain harvest index of 38.3%, compared to the rest of varieties, where GHI was 40.3-41.3%, on average. The Kaskadior variety allocated less of the absorbed phosphorus (PHI=48.7%) and calcium (CaHI=13.1%) to the grain. Emon variety had a low KHI=21.9%, and the Obzor variety had a low MgHI=43.4.

Table 4. Harvest index of nitrogen, phosphorus, potassium, calcium and magnesium in dependence of the nitrogen level, %

Parameter	N level	Min	Max	Average	% to N_0
NHI	N_0	64.2	69.0	66.7 ns ± 2.1	100
	N_{200}	63.8	68.1	65.9 ± 1.8	98.8
	N_{400}	62.6	65.8	64.6 ± 1.2	96.9
PHI	N_0	49.9	56.6	53.6 ns ± 2.4	100
	N_{200}	48.4	54.6	52.0 ± 2.3	97.0
	N_{400}	47.8	53.4	51.2 ± 2.1	95.5
KHI	N_0	21.3	26.6	23.5 ns ± 2.0	100
	N_{200}	22.1	28.9	24.1 ± 2.7	102.5
	N_{400}	22.3	27.9	24.0 ± 2.2	101.9
CaHI	N_0	12.2	17.9	15.4 ns ± 2.1	100
	N_{200}	13.3	18.0	15.6 ± 1.7	101.1
	N_{400}	13.7	16.5	15.2 ± 1.0	98.7
MgHI	N_0	46.1	58.1	52.7 ns ± 4.6	100
	N_{200}	42.6	54.4	50.9 ± 5.0	96.7
	N_{400}	41.4	53.1	47.0 ± 4.7	89.1

ns* Not significantly different at $p<0.05$

Table 5. Average productivity ($g.pot^{-1}$) and content of nitrogen, phosphorus, potassium, calcium and magnesium ($mg.pot^{-1}$) of barley varieties

Variety	Grain					
	Yield	N	P_2O_5	K_2O	CaO	MgO
Obzor	14.0 ns**	258.5 ns	109.4 ab*	105.2 ns	31.9 b	15.9 b
Emon	14.1	257.9	103.4 b	91.6	42.8 ab	17.4 b
Krami	17.1	299.7	148.3 a	113.8	45.0 ab	19.9 ab
Kristi	16.2	253.9	123.5 ab	112.2	46.8 a	18.5 b
Kaskadior	17.0	324.1	118.3 ab	119.7	42.6 ab	24.3 a
Grain + Straw						
Obzor	34.9 ns	385.0 ns	206.9 ns	377.1 ns	203.0 b	36.9 ns
Emon	36.8	400.9	200.5	416.9	246.8 ab	35.8
Krami	41.5	464.9	271.1	487.3	294.7 a	36.4
Kristi	39.7	381.7	234.5	486.9	304.4 a	35.8
Kaskadior	41.6	496.4	243.5	516.1	324.1 a	47.6
HI						
Obzor	40.3 a	67.4 ns	53.0 ab	27.8 a	15.7 b	43.4 b
Emon	38.3 b	64.5	51.7 b	21.9 c	17.4 a	49.1 ab
Krami	41.3 a	64.5	54.9 a	23.4 b	15.3 b	55.2 a
Kristi	41.0 a	66.9	52.9 ab	23.0 b	15.4 b	51.8 a
Kaskadior	40.8 a	65.5	48.7 c	23.1 b	13.1 c	51.6 a

*Values for each nutrient in the plant parts followed by the same letters are not significantly different at $p<0.05$ according to Duncan’s multiple range test
ns** Not significantly different at $p<0.05$

Correlation analysis indicated that the nitrogen level in the range of N_0 to N_{400} was strongly positively related to straw, grain+straw yields, as well as to the absorbed grain nitrogen (r=0.906**) and straw nitrogen (r =0.894**). Nitrogen fertilization was negatively related to GHI (-0.641*).

Conclusions

Fertilization N_{400} increased the average productivity of the varieties by 23.8% for grain and 31.1% for grain+straw, compared to plants grown at N_0. Nutrients accumulated of grain+straw at N_{400} level were higher by 84.9% for nitrogen, 51.8% for phosphorus, 54.4% for potassium, 41.3% for calcium, and 47.3% for magnesium, on average. Grain harvest index decreased from 41.5% at N_0 to 39.3% at N_{400}. The nitrogen level had a slight effect on the percentage distribution of accumulated nutrients to the grain, although a tendency for a lower percentage was observed in regard to the nitrogen, phosphorus and magnesium. A strong positive correlation of nitrogen level with grain and straw yield and amount of accumulated nitrogen was established. A negative correlation was observed between applied nitrogen and grain harvest index (r=-0.641*) and nitrogen harvest index (r=-0.479).

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KNEJA 652 - A LATE HYBRID FROM PORTFOLIO OF THE MAIZE RESEARCH INSTITUTE - KNEZHA FOR 2024

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Abstract

Kneja 652 is a new high-yielding, stress-tolerant maize hybrid from the FAO Group 600+, selected for grain, silage and biogas production. Suitable for extensive and intensive cultivation. The hybrid is a single cross created by interline hybridisation of inbred lines XM 1617 and XM 9923. The hybrid is for early sowing with a fast initial rate of development. It has a tall, well formed stem, resistant to lodging and has good synchrony in the flowering period of the reproductive organs (ASI = 0-2). The cob is characterised by compact, large cobs 24-25 cm long; 16 rows; 50-52 grains per row, 230 g grain weight per cob; 273.5 g. MNV; hectolitre weight - 75 kg/hl. Kneja 652 has high quality indicators for grain and silage use, as follows: 10.38-10.43 % protein content in grain; 68,01 % starch; 2,07 % fibre. The hybrid is resistant to the economically important maize diseases Helminthosporium turcicum, Ustilago zea and Fusarium ssp. Kneja 652 is recognized by the Expert Commission, after two years of testing, by the Executive Agency for Variety Testing, Approbation and Seed Control and by order RD12-2/15.03.2023 is included in the Official Variety List of the Republic of Bulgaria. Hybrid Kneja 652 has certificate No 11294/25.01.2024 of the Patent Office of the Republic of Bulgaria. The hybrid was developed at the Scientific Research Institute of Maize - Kneja by Assoc. Prof. Valentina Vulkova. The Minister of Agriculture and Food presented an award for Kneja 652 hybrid for scientific activity and development in the innovation competition.

Keywords: *New maize hybrid, Kneja 652, Maize research Institute – Knezha.*

Introduction

As a consequence of the increasing human population worldwide; unfavourable agro-meteorological environmental conditions for plant development caused by the onset of climate change; reduced availability of arable land and soil erosion; and water scarcity, the need for increased agricultural production is growing.

Maize is the most produced crop in the world (FAO Statistical Yearbook, 2023). It is a major forage crop, with the highest feed and energy value of the cereal forage crops. According to Kostova A. (2012), it is a raw material for over 2000 industrial and technical products. Maize breeding in recent years has been very successful both at home and abroad. After the joining of Bulgaria to the European Union, a prerequisite was created for opening the seed market to foreign companies, expanding the varietal list and unhindered dissemination of foreign breeding achievements. The new conditions require radically different approaches to research and higher criteria for our hybrids to survive in the highly competitive environment of the European market.

Along with the requirement for new hybrids to be high yielding, there is an increasing demand for other qualities: resistance to diseases and pests; tolerance to abiotic stress factors; efficient water and nutrient use; grain quality; increased harvest index; rapid initial rate of development; rapid moisture release from the grain and stable yield. At the same time, the production life of maize hybrids is declining at a rapid rate. According to Duvick et al. (1999), hybrids persist in production for an average of seven years. For this reason, maize selection has a permanent character. Each newly recognized hybrid must be tested under different ecological-geographical regions and compared with the best standards of its maturity group to really evaluate its qualities.

In its 100-year history, more than 117 maize hybrids of different maturity groups and uses have been developed at the Maize Research Institute - Knezha, Bulgaria (Genov et al., 2005; Genova et al, 2009; Gacovski et al, 2011; Ilchovska et al, 2014, Petrovska et al, 2018; Valkova et al, 2018; Yordanov, 2019, 2021). Each newly recognized hybrid is better than the previous ones and builds on their qualities. According to a number of scientists (Yugenheimer, 1979; Russel, 1986; Hallauer, 1988; Reid et al, 2014), even the most thorough research and technological developments in breeding would be meaningless if they did not lead to the creation of new higher yielding, quality, sustainable and adapted hybrids and the achievement of real breeding progress.

The objective of this publication is to show the main characteristics, morphological and economic qualities of the new hybrid of the Maize Research Institute, Knezha, Bulgaria - Kneja 652.

Material and methods

Hybrid Kneja 652 is a late maize hybrid intended for grain, silage and biogas production. It was developed in 2017 as a topcross cross to evaluate the combinability of newly stabilized lines with testers that have proven over the years their high general and specific combinability for grain yield. The cross XM 1617 x XM 9923 (Kneja 652) was voted as high yielding in 2018 after testing in a preliminary variety trial. The results were confirmed in competitive and environmental variety trials over the next two years, and in 2021 the hybrid was submitted for evaluation for Distinctness, Homogeneity and Stability (DHS) and Biological and Economic Qualities (BEC) in the official country trial (Executive Agency for Variety Testing, Field Inspection and Seed Control). The trials were conducted under non-irrigated conditions in 2021 and 2022 at 3 sites - Selanovci, Brashlen and Radnevo. High temperatures and minimal rainfall at the latter site led to the scrapping of the trials there. Due to the convincing superiority of the yield of hybrid Kneja 652 over the Executive Agency for Variety Testing, Field Inspection and Seed Control standards, the hybrid was recognised after two years of testing. With Order No 12-2/15.03.2023 The Minister of Agriculture approved the recognition and entry in the Official Variety List of the Republic of Bulgaria of hybrid Kneja 652. The hybrid has certificate No 11294/25.01.2024 of the Patent Office of the Republic of Bulgaria

In 2021 and 2023, the Kneja 652 hybrid was tested under non-irrigated conditions, in randomized block trials in the Republic of Serbia and Romania, where it was compared to their own and foreign standards. The following parameters were studied: grain yield at standard moisture of 14% and moisture at harvest

The parental components of the hybrid are the product of mutational selection. Descriptions of the paternal form - XM 99 23 are available in two previous publications (Valkova V., 2006; Valkova et al, 2018). The maternal line of the hybrid XM 1617 was produced by treatment with chemical mutagens DES (diethyl sulfate) - 0.1% and NMU (nitroso methyl urea) on a heterozygous genotype. It was stabilized in M4 inbred generation in 2016.

Results and discussion

The maize hybrid Kneja 652 is a straightline cross between the inbred lines XM 1617 x XM 9923. According to the FAO classification, the hybrid belongs to the late genotypes - group over 600 with a growing period of 117-124 days. This period is extended by 3-5 days when grown under irrigated conditions. The hybrid is suitable for early sowing (10°C soil) with a rapid initial rate of development. Suitable for extensive and intensive cultivation. Kneja 652 is characterised by a tall stem (260-280 cm), well frondose (13-15 pcs.), resistant to lodging. It has good synchrony in the flowering period of the reproductive organs (ASI = 0-2). The hybrid is monocot. The cob is cylindrical, 24-25 cm long (Fig. 1). There are 16 rows and 50-52 grains per row in the cob. The kernel is orange horse tooth type, 10-12 mm long, and the weight of the kernel per cob is 220 -240 g. The weight of 1000 seeds is 273.5 g and the hectolitre weight is 75 kg/hl. The percentage of grain in the cob measured during the seed cast showed that the Kneja 652 hybrid also has a high percentage of 85-86%. The chemical analysis of the grain showed that the hybrid has an increased protein content in the grain - 10.38-10.43%, and on this indicator it significantly exceeds the standard P9903 with a protein content of 8.5% and the standard Kneja 435 - 9.5%. In the grain of the hybrid the percentage of starch is 68,01 and of fibre – 2.07%. These results give grounds to recommend hybrid Kneja 652 for production of quality forage.

Figure 1. Kneja 652

The results of the first two stages of the hybrid field trial at the Maize Research Institute are shown in Table 1.

Table 1. Results from the testing of Kneja 652 hybrid in condition without irrigation (2018-2019)

Year	Grain yield, kg/ha			% the yields to the standart		Grain moisture at harvest, %		
	Kneja 683A	Kneja M625	Kneja 652	Kneja 683A	Kneja M625	Kneja 683A	Kneja M625	Kneja 652
PVT -2018	1203.1	1116.3	**1289.7**	107.2	115.5	16.5	16.2	15.7
CVT-2019	1083.6	1178.6	**1183.9**	109.3	100.4	15.8	15.0	15.3
CVT-2020	713.3	699.2	**767.2**	107.6	109.7	14.2	15.8	14.0

*PVT - preliminary varietal trails, CVT - competititve varietal trails

The trials were carried out under non-irrigated conditions and following the agronomic technique adopted for the region. The highest yield of the hybrid was obtained in 2018 and its variation from year to year was due to different agro-climatic conditions. The excess of the yield of hybrid Kneja 652 over that of the standards ranged from 0.4% over hybrid Kneja M625 in 2019 to 15.5% for the same hybrid in 2018. The excess of the hybrid's productivity over that of the standards allowed the testing of the hybrid to continue in the ecological network of the country. The trial was conducted again under non-irrigated conditions at three sites - Kneja, Ruse and Pavlikeni. The trials were sown and harvested manually, in three replications, harvest plot size of 10 m^2 and plant density of 55.000 plants/ha (Table 2). It is noticeable that the yields at Pavlikeni are much lower than those at the other two sites. This is due to the late sowing of the experiment (11-12.05.2020), which was hampered by the complicated situation throughout the country, in relation to the spread of Covid 19, and the scarce rainfall in July (6.6 mm/m^2), when flowering and fertilization of reproductive organs took place. For this reason, a high percentage of infertile plants were recorded during the harvesting trials, which inevitably led to yield reductions. In the ecological variety trials the hybrid exceeded the Kneja 683A standard in yield by 23.1% and was 97.1% to that of Kneja M625.

Table 2. Results from the testing of Kneja 652 hybrid in ecological variety trails (2011)

Year	Grain yield, kg/ha			% the yields to the standart		Grain moisture at harvest, %		
EVT	Kneja 683A	Kneja M625	Kneja 652	Kneja 683A	Kneja M625	Kneja 683A	Kneja M625	Kneja 652
Knezha	656.7	838.2	**830.8**	126.5	99.1	15.6	16.4	**17.1**
Rousse	678.9	751.1	**777.1**	114.5	103.5	16.0	17.0	**17.1**
Pavlikeni	346.2	501.1	**443.8**	128.2	88.6	15.2	15.8	**16.3**

In order to continue testing the productive potential of the hybrid under study, it was included in the country's official variety trial. Testing was carried out under non-irrigated conditions in three locations in the country - Selanovtsi, Brashlen and Radnevo (Table 3). In 2021, Kneja 652 realized a grain yield of 7645 kg/ha, 112.4% of the official standards. The yield in 2022 was 5650 kg/ha - 99.5% towards the standards, where the differences with the average standard are mathematically unreliable. The average yield from the six locations over the two years is 6647.5 kg/ha, with an average exceedance of 106% over the Kneja 683A and Kneja M625 standards. Yields in the official trial were quite low, on one hand because of the climatic conditions at the time of pollination, fertilisation and grain filling and on the other hand because of the inclusion of the Radnevo site, where conditions for maize cultivation are not favourable and low yields are obtained. According to reports from the IACAC, Kneja 652 is resistant to the economically important maize diseases *Helminthosporium turcicum*, *Ustilago zea* and *Fusarium ssr*. The hybrid is recognized after two years of testing by the Executive Agency for Variety Testing, Field Inspection and Seed Control and holds certificate No 11294/25.01.2024 of the Patent Office of the Republic of Bulgaria.

Table 3. Results from official testing of Kneja 652 hybrid in EAVTFISC

Hybrid	Grain yield, kg/h	% average Standart (Kneja M625 and Kneja 683A)	number of days from germination to harvest	Grain moisture at harvest, %
2021				
Kneja 652	764.5	112.4	121	14.9
Kneja M625 and Kneja 683A	684.9	100	121	14.8
2022				
Kneja 652	565	99.5	117	12.8
Kneja M625 and Kneja 683A	567	100	117	13.9

*Executive Agency of Variety Testing, Field Inspection and Seed Control (EAVTFISC)

In the years 2021 and 2023, the hybrid was included under international cooperation for bilateral hybrid testing by the Maize Research Institute - Knezha, Bulgaria with related institutes in Zemun Polje Serbia and Fundula, Romania. In the first year of the trial, the hybrid performed better in Romania (7925 kg/ha) and in Zemun Pole the yield was 7285 kg/ha. In 2023, due to unfavourable conditions (lack of rainfall and high temperatures), the trial in Romania was scrapped. In Serbia, higher yields were obtained in Tovarishevo - 8663 kg/ha and in Zemun Pole - 7792 kg/ha.

The technology of seed production of hybrid Kneja 652 requires simultaneous sowing of the parental components. The sowing scheme can be 6:2, because the panicle of the parent component is very rich and retains its fertilisation capacity 8-10 days. The recommended and optimum sowing densities are: under non-irrigated conditions 55 000 pl/ha and under irrigated conditions 65 000 pl/ha. Seed production of the hybrid is so far on a fertile basis with complete detasseling of the mother rows. A sterile analogue of the maternal form is in the process of being created to switch seed production to the fertility restoration method. This is possible because the paternal component, the XM 9923 line, is a universal restorer, and this in turn will lower the cost of hybrid seed produced. The breeding coefficient of the maternal form - line XM 1617 reaches 3900-4200 kg/ha of standard seed. The hybrid is suitable for inclusion in System Plus with all late hybrids to increase grain yield per unit area.

At the International Agra 2024 exhibition, the Kneja 652 hybrid was awarded a prize for research and development in the innovation competition.

Conclusions

Hybrid Kneja 652 has high productive potential and very good resistance to economically important diseases in maize *Helminthosporium turcicum*, *Ustilago zea* and *Fusarium ssr*.

Once a sterile analogue of line XM 1617 is developed, seed production of the hybrid will be carried out using the fertility restoration method, which will eliminate manual detasseling and increase profit per unit area.

The grain of the hybrid has very good quality indicators: protein content 10.38-10.43%, starch 68.01% and fiber 2.07%.

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STUDY OF THE INFLUENCE OF DIFFERENT PERCENTAGE STERILE PLANTS ON THE MAIZE HYBRIDS YIELD

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Abstract

The maize hybrids Kn-442, Kn-509, Kn-511, Kn-M625 and P35F38 of different maturity groups were studied for a period of four years (2014-2015 and 2017-2018). Each hybrid has three variants - 100% fertile; 50% fertile and 50% sterile; 100% sterile plants. Sterility was achieved by means of castration (*detasseling*) before anthesis. The trials were carried out in the field of the Maize Research Institute - Knezha, by the randomized block design, on a 10 m^2 harvest plot, in 3 replications, without irrigated conditions. The results of the three-factor Analysis of Variance (ANOVA) points out significant variances for hybrids, years (environmental conditions) and the interaction between them. The remaining variances, including: variants of the hybrids with percentage of sterility; first-order interactions – hybrids x variants; years x variants, as well as the triple interaction – hybrid x years x variants, are not significant. Significant differences in mean yields between the tested variants (100% fertile, 50:50% and 100% sterile) are observed in some cases at P=5%. The effect of variants with 50% castrated tassels tended to be higher yielding by 0.4 to 12.1% in 2014. With 100% sterile plants, there is a relative excess of yield compared to 100% fertile ones in 4 out of 5 hyrids with a range of 1.3-5.0%. For the period of the study, the highest yield was performed by hybrid Kn-442, followed by P35F38.

Key words: *maize hybrids, percent of detasseling, yield and stability*.

Introduction

According to FAO-data from 2023 worldwide maize production amounts to more than 1.2 *bn/t*, witch mades it the most produce cereal crop on the planet. Maize has good ecological stability and is grown on all five continents from 50°N to 35°S latitude. Mainly used for grain and silage, food for people in developing countries, it is a raw material for the production of over 2,000 products for the food, pharmaceutical and chemical industries (Ranum et al., 2014). Due to its major economic importance, it is the object of constantly increasing scientific and practical interest (FAO Statistical Yearbook, 2023). In recent years, in relation with the global climate changes, the yields of all cultural crops are threatened by yields reduction, including those of maize too. In the Third National Action Plan on Climate Change (Ministry of Environment and Water, Republic of Bulgaria, 2012) it is stated that spring agricultural crops will be increasingly vulnerable to these changes in the future. Increasing the resistance of maize to drought is an extremely important problem not only for Bulgaria, but also for other countries where this crop is grown, due to a significant reduction in yields.

The hybrid is the most important, effective and intensive factor in maize production. The yield obtained and the added value per unit of area largely depends on the correct choice of hybrid, and on the other hand, the choice of correct agricultural techniques. In the last 20-25 years, to deal with this problem, as well as to increase the yield from a unit of area without additional costs for the producers, the so-called "Plus System" has been implemented. It was proposed by Weingartner et al. (2002) and is a mixed crop of two or more maize hybrids of

the same vegetation group, some of them obtained by the mixing method. The expected higher yield results from the combined effect of the higher yielding sterile plants and the higher seed mass obtained from non-exogenous pollination. The combined effect of this system affects two components of the yield – the cytoplasmic male sterility (CMS) leads mainly to an increased number of grains, and the xenic effect increases the 1000 seeds weight (Weider et al., 2010; Vulchinkov et al., 2014). A large part of the newly recognized hybrids of the Maize Research Institute - Knezha have sterile analogues of the maternal component. The use of maternally sterile counterparts in hybrid maize seed production results in greater production efficiency and purity of the resulting hybrid seeds by eliminating hand or machine detasseling. The data on the impact of CMS on yield are contradictory. According to a number of authors (Duvick, 1958; Beckett, 1971; Ilchovska, 1991), the yield of sterile forms is higher than that of their fertile parent lines, due to the weakened competition for assimilates between the ear and the tassel. On the contrary opinion are Valkova (2012) and Vulchinkov et al., (2014), according to which the yield of sterile forms is influenced by the genotype, the year of the study, as well as the genotype-environment interaction. The same thesis has been confirmed by a resent studies by Costa et al., (2019), Ghețe et al., (2020) and Prodanović et al. (2022), as well as the significant importance of the method of detasseling – by hand or mechanized. In some hybrids with sterile counterparts of the maternal form, the paternal component lacks fertility restorer genes. This necessitates the use of a “compromise” mixing scheme (Valkova, 2012). With it, 1/3 of the plants in the mass hybrid are fertile (flowering), which in practice is also a "Plus" system. If at least two hybrids produced according to such a scheme are sown, their sterile plants will cross-pollinate, resulting in a xenia effect.
The aim of the study is to determine the influence of different levels of detasseling on the grain yield of the exanimated hybrids from different vegetation groups over a four-year period.

Materials and Methods

The experiment is conducted in the experimental field of the Maize Research Institute - Knezha during the periods 2014-2015 and 2017-2018. The trails are set in a randomized complete block design with 10,0 m^2 experimental plot size in three replications for each genotype tested; under non-irrigated conditions. Hybrids of different maturity groups were studied - Kneja 442, Kneja 509, Kneja 511, Kneja M 625 and P35F38. Each hybrid is tested in three variants – 100% fertile (1), 50% fertile and 50% sterile (2) and 100% sterile plants (3). Sterility is achieved by hand detasseling the plants immediately after tassel emerging and before anthesis. The grain yield is measured in kg/dka with 14% moisture content at harvest. A three-factor *Analysis of Variance* (ANOVA) on the data is performed. The data processing is done by Microsoft Excel and SPSS 25 Statistical program.

Results and discussion

Of the climatic conditions, rainfall, temperature, and relative humidity are of greatest importance for the growth and development of maize. According to Vulchinkov S., (personal correspondence), a year with a sum of precipitation during the growing season (April-September) higher than 400 ml is defined as favorable for the maize development, and one with less than 300 ml of precipitation can be considered as dry. According to these, two of the years of the study period (2014 and 2018) are considered as favorable with rainfall totals of 627.2 ml and 447.5 ml, respectively. With a more even distribution of rainfall and lower temperatures during pollination, 2014 was the most favorable year during the study period (Fig. 1).

Figure 1. Average mount values of temperatures and precipitation for April-October for the study period (2014-2015, 2017-2018)

The results of the multifactorial Analysis of Variance (ANOVA) show significant differences both between the years of study and between the hybrids and the interaction of environmental conditions and studied genotypes (Table 1). The other factors: the variants of the hybrids with percentage of sterility; hybrid by variants; years by variants, as well as the triple interaction – hybrid, year, variant do not have significant variances.

Table 1. Thee-factor ANOVA analys

Source	df	SS	MS	F-count	F-table 5%	F-table 1%	P-value
S	3	123,917.2073	41,305.7358				
L	2	3,859.0143	1,929.5072				
S*L	6	46,950.5710	7,825.0952				
REP*S*L	24	354,103.3613	14,754.3067				
G	4	679,965.8231	169,991.4558	4.91**	2,93	4,59	0,0076
G*S	12	360,115.1738	30,009.5978	3.91**	1,85	2,38	0,0001
G*L	8	49,337.7096	6,167.2137	0.80ns	2,04	2,7	0,634
G*S*L	24	184,251.2229	7,677.1343	0.95ns	1,63	1,99	0,5335
Error	96	773,622.2587	8,058.5652				
Corrected Total	179	2,576,122.3420					

CV = 11.19 %; S – Years; L – Variants; G - Genotypes (hybrids); Rep – repetitions

Table 2. Average grain yields of five hybrids from different vegetation group with in different levels of detasseling for four years period

Hybrid	Variants	Grain yield by years 2014	2015	2017	2018	Average	Min	Max	STDEV	CV. %
Kn 442	*(1)*	898.3	894.1	851.8	986.5	907.7	851.8	986.5	56.6	6.2
	(2)	926.9	849.6	900.2	893.9	892.7	849.6	926.9	32.1	3.6
	(3)	942.8	851.3	1190.3	932.1	979.1	851.3	1190.3	146.6	15.0
Kn 509	*(1)*	730.3	654.8	704.5	895.9	746.4	654.8	895.9	104.5	14.0
	(2)	785.7	673.6	653.7	650.6	690.9	650.6	785.7	64.0	9.3
	(3)	740.1	691.8	731.9	815.9	744.9	691.8	815.9	51.8	7.0

Kn 511	*(1)*	786.8	783.1	770.7	806.5	786.8	770.7	806.5	14.8	1.9
	(2)	789.9	797.7	745.7	935.8	817.3	745.7	935.8	82.3	10.1
	(3)	824.4	768.6	734.6	826.9	788.6	734.6	826.9	45.0	5.7
Kn M625	*(1)*	775.0	782.9	772.8	620.4	737.8	620.4	782.9	78.4	10.6
	(2)	869.1	777.8	724.9	642.0	753.5	642.0	869.1	95.2	12.6
	(3)	796.1	745.5	878.2	623.7	760.9	623.7	878.2	106.6	14.0
PR 35F38	*(1)*	950.0	811.2	895.1	807.9	866.1	807.9	950.0	69.0	8.0
	(2)	983.1	841.5	783.4	738.3	836.6	738.3	983.1	106.4	12.7
	(3)	894.7	776.6	762.2	816.1	812.4	762.2	894.7	59.4	7.3

(1) 100 % fertile plants; (2) 50 % fertile / 50 % sterile; (3) 100 % sterile plants.
LSD at P=5 % is 102,6 kg

Averaged over the years of the study (Table 2), the highest yield is obtained from hybrid Kn 442 (979.1 kg/ha) under complete detasseling, followed by PR 35F38 (866.1 kg/ha) at 100% flowering plants, Kn 511 (817.3 kg/ha) when 50% of the tassels are removed, Kn M625 (760.9 kg/ha) with 100 % castration and Kn 509 (746.4 kg/ha) in fully flowering plants.
Of the calculated least significant differences at a probability level of 5% for the entire trial is *LSD*=102.6 kg. This means that in hybrid Kn 442 in 2017, the variant with complete detasseling (1190.3 kg/dka) proved to be different from those with partial and no detasseling. This is also the highest yield of all hybrids for the entire research period. Hybrid Kn 509 in 2018, the variant with 50/50 sterility clearly differs from the one with fully fertile plants. A proven difference was also reported in 2018 hybrid Kn 511 for dust 50/ castration, compared to the other variants in the experiment. Differences in the levels of detasseling levels were also observed for Kn M 625 and PR 35F38 in 2017.
Quantitatively, the yield picture is as follows. The sterile variant in hybrid Kn 442 had a higher yield than that of fertile plants by 7.9% on average over the test period and varied from (39.7% in 2017 to 5% in 2014). In the other two years, the yield of the sterile variants is lower than that of the fertile plants by an average of 5%. In the hybrids Kn M625 and Kn 509, higher yields is obtained than the sterile variants in three of the years and in two of those with 50% participation of sterile plants. The excess in yields of sterile variants over that of 100% fertile plants in hybrid Kn M625 varies from 13.6% (2017) to 0.5% (2018), and in Kn 509 from 5.6 (2015) to 1.3 (2014). In hybrid Kn 511, higher yields than the mixed variant ape reported in three of the studied years and in two of 100 % sterile plants. The influence of the number of sterile plants in the crop with the foreign hybrid PR 35F38 is insignificant. Excess in the grain yield from the variants with 50% fertile and 50% sterile plants over that with fertile plants was in 60% of the tested cases. The largest excess is reported for the hybrid Kn 511 with 16% (2018) to 0.4% (2014) for the same hybrid.
The results show that there is relation between the yields of the individual variants and the years of testing in some cases. There is no tendency for a higher yield from the sterile plants in drier conditions, on the contrary, in 2014, the most favorable year, of all the Bulgarian hybrids from the variants with the participation of sterile plants, higher yields are obtained than the variant with 100% fertile plants. These results are in coherency with the results obtained earlier by Valkova. (2012) and contrary to what was demonstrated by Kannenberg (1974) and Hoekstra et al. (1985).

Conclusions

The results of the three-factor Analysis of Variance (ANOVA) points out significant variances for hybrids, years (environmental conditions) and the interaction between them. The remaining variances, including: variants of the hybrids with percentage of sterility; first-order interactions – hybrids x variants; years x variants, as well as the triple interaction – hybrid x years x variants, are not significant. Least significant differences in mean yields between the tested variants (100% fertile, 50:50% and 100% sterile) are observed in some cases at P=5%. The effect of variants with 50% castrated tassels tended to be higher yielding by 0.4 to 12.1% in 2014. With 100% sterile plants, there is a relative excess of yield compared to 100% fertile ones in 4 out of 5 hyrids with a range of 1.3-5.0%. For the period of the study, the highest yield was performed by hybrid Kn-442, followed by P35F38.

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USING OF HETEROSIS SELECTION IN SESAME (SESAMUM INDICUM L.)

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Abstract

The phenomenon of heterosis has opened wide perspectives in a number of crops and continues to play a crucial role in the genetic improvement of plants. The heterosis effect is most expressed in cross-pollinated plants, however, they are also the most sensitive to inbreeding depression. Heterosis is less expressed in self-pollinating species, but inbreeding depression is lesser. The crosses in sesame were made in an incomplete diallel pattern. Biometric measurements of major yield elements were made on the parental forms from the working collection of IPGR - Sadovo and the hybrid progeny. Seed mass per plant, number of the branches, total number of boxes per plant were recorded. The mean total parent height (MPH) and heterosis (BPH) were determined. The variation in MPH and BPH among different hybrids was measured. The level of genotypic variance (σ2G) was determined by its components, the levels of additive variance (σ2A), dominance variance (σ2D) and epistasis variance (σ2E). The inheritance coefficients were determined in the broad (H2) and narrow (h2) sense, as a possibility to compare them. Selection success (R) was accounted for by the selection differential (S) and heritability in the broad sense.

Key words: *sesame, heterosis, genotypic and phenotypic variance*.

Introduction

Sesame (*Sesamum indicum* L.) is an oilseed crop that has been cultivated and used by humans since ancient times. It originates from Africa, although India is considered to be the first place of appearance of cultivars. The high nutritional value and health benefits of sesame consumption are undisputed and the interest is growing rapidly (Noureldin S. and Butovchenko A. 2019; Wu 2007). Sesame seed oil is among the most expensive and desirable edible oils in the world. It is one of the healthiest, containing 38.84% oleic acid and 46.26% linoleic acid, which are high in unsaturated fatty acidy. They call it the "Queen" of oils because it reduces the LDL-cholesterol complex, regulates blood sugar, and in addition the antioxidant properties of sesamin, sesamol and sesamolin not only contribute to its high level of resistance against oxidation, but also make it a unique and very high-quality functional food (Oboulbiga et al., 2023)

The production of sesame seed, according to FAO estimates, is 7 706 642 tons in 2020. This is 3.89% more than the previous year and 57.37% more than 10 years ago. In 2019, the 12.8 million hectares used for sesame production resulted in a total of only 6.5 million tons of sesame produced, which must be considered a low yield for such a huge area (FAO statistics database: production, trade, supply. 2019).

Selection for new sesame varieties has focused on several morphological traits and issues related to overcoming biotic and abiotic stress (Teklu et al., 2022). Heterosis in sesame has been reported in a number of works (Disowja et al., 2021; Mishra & Sikarvar, 2021; Chaudhary et al., 2017; Aladji et al., 2014).

The aim of the present study was to determine the heterosis in the inheritance of key morphological traits related to yield, including seed mass per plant, number of branches, and total number of pods.

Material and method

Crosses in sesame were conducted using an incomplete diallel pattern. Biometric measurements of major yield components were taken on both the parental forms and the hybrid progeny, recording seed mass per plant, number of branches, and total number of pods. In the first cross Milena x Sadovo 3959 105 progeny were processed in the F_1 population, in the second Sadovo 3959 x Milena - 83, in the third Sadovo 1 x Sadovo 3959 - 82 and in the fourth Sadovo 3959 x Sadovo 1- 70 progeny. The mean (x), mean error (Sx %), mean square deviation (S %) were determined. Biological characteristics as heterosis - hypothetical and true (%) and heterosis effect (BPH) were analyzed. The type of inheritance of traits in F_1 hybrids was determined by the ratio of the dominant to the additive parameter, d/a (Mather & Jinks, 1971). The heritability coefficients in broad (H^2) and narrow (h^2) sense. The additive variance (σ2A), dominance variance (σ2D) and epistasis variance (σ2E) were calculated. The selection differential (S) was determined - S = Sx defenses in F_2 - Sx in F_1. The (R) was calculated by the formula: $R = S \times H^2$ .The optimal genetic distance (GD) at which the highest heterosis effect is obtained was determined using the quadratic deviation between parents (Nei, 1979).

Results and discussion

The heterosis behaviors of progeny in different hybrid combinations of seed mass per plant are presented in Fig. 1. The average seed mass per plant of F1 progeny in the combination Milena x Sadovo 3959 was 21.50 g. It was 11.70 g in Milena and 7.20 g in Sadovo 3959. Heterozygosity is highly expressed (82.90%) and is manifested by overdominance of the higher yielding parent. Heterosis for seed yield has been reported in a number of studies by Vekaria and Dhadak (2018), Chauhan et al. (2019) and Dela and Sharma (2019). In the reciprocal cross (Sadovo 3959 x Milena), seed mass of a plant is inherited through incomplete dominance of the higher yielding parent, but here heterosis is absent. In a cross with the parents Sadovo 1 x Sadovo 3959, the seed mass per plant for Sadovo 1 is 18.50 g and for Sadovo 3959 is 2.40 g. The resulting average plant mass of the hybrid population was 25.30 g. The observed heterosis effect of 36.60% was due to overdominance of the maternal parent. Daba C., (2019) also found that seed yield, number of capsules and number of tillers are under the influence of dominant gene effects and provide opportunity for heterosis selection in sesame. In the reciprocal cross the seed mass obtained from one plant for Sadovo 3959 was 12.40 g and for Sadovo 1- 45.10 g, and the progeny plants formed a seed mass from one plant of 25.50 g. The indicator d/a has a negative value less than one. Inheritance occurs by incomplete dominance of the lower-yielding parent.

In the combination Milena x Sadovo 3959 variants of additive genes, has a minimum value of 5.1, while dominant and epistatic gene effects have values of 147.7 and 127.1, respectively.

The selection differential (Table 1) has a negative sign, indicating the high degree of depression in F_2 of this trait. In the inheritance of the trait in the reciprocal cross, the variance of additive interactions matters the most.

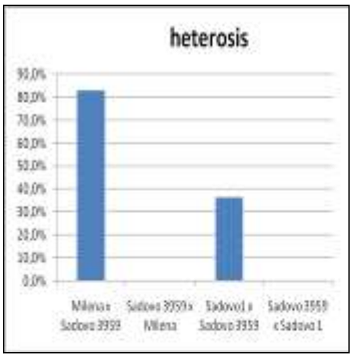

Fig. 1. Heterosis effect by seed yield

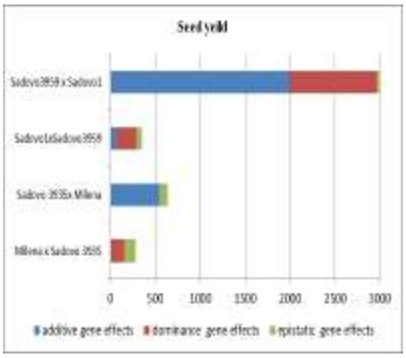

Fig. 2. Gene effects in inheritance by seed yield

This, together with the equalized broad and narrow - sense inheritance coefficients (Table 1), indicate independent genetic control of seed mass per plant in the hybrid population as opposed to epistatic control in the straight cross. Again, the depression in the F_2 generation is strong and the selection differential is negative (-7.78 g). Because of this, selection by phenotype is appropriate and higher yielding progeny can be selected. In the combination Sadovo 1 x Sadovo 3959 the variance of additive and epistatic interaction between genes is almost equal 64.80 - 65.10 with the highest value being the dominant interaction 219.40. This and the higher coefficient of inheritance in a broad sense over that in a narrow sense, make the progeny under consideration suitable for heterosis selection for the trait seed mass per plant. The selection differential was of negative sign (-3.8 g), which was due to inbreeding depression in the F_2 progeny. The epistatic interaction of genes in the reciprocal cross in this trait has the highest score. The selection differential is negative sign (-2.5g) due to inbreeding depression in F_2 progeny. Plant height and number of branches per plant are the bearing surface of capsules and are considered important attributes of biological yield. The heterosis manifestations of progeny in different hybrid combinations are presented in Fig. 3. The number of branches in the hybrid combination Sadovo 1 x Sadovo 3959 was 3.00 for Sadovo 1 and 2.50- Sadovo 3959. In the progeny the average number is 4.00. The dominance (d/a) was 5.00. Inheritance was overdominant, to the parent with the higher number of branches. In this combination, there is a heterosis effect in terms of the number of branches, 33.30%. On this trait, high value heterosis has been reported by Rao (2011), Monpara B.A. and Pawar A.K., (2016). In the reciprocal cross, inheritance of the trait was incompletely dominant, to the parent with fewer branches, the d/a index has a negative value less than one, and heterosis is absent. The values of additive, dominant and epistatic gene interaction in the combination Milena x Sadovo 3959 showed that the highest proportion of epistasis. Inheritance in the broad sense has a higher value than inheritance in the narrow sense, and allelic and non-allelic epistatic interactions were involved in the formation of the number of branches. The selection differential is low and the success of selection by phenotype is unfortunate.

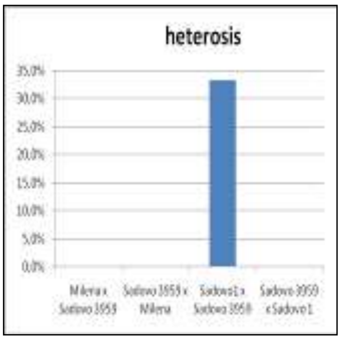

Fig. 3. Heterosis effect by number of branches

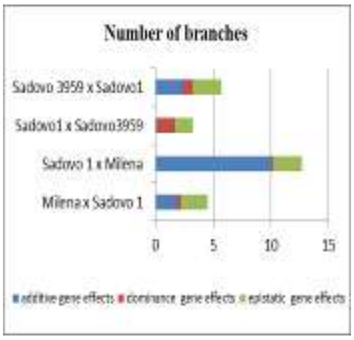

Fig.4. Gene effects in inheritance by number of branches

Additive interactions have the strongest influence on the number of branches in the reciprocal cross. The coefficient of inheritance in the narrow sense, greatly exceeds that in the broad sense. Selection by phenotype for the trait number of branches was successful.
In the combination Sadovo 1 x Sadovo 3959 the highest value were dominant genes effects. The coefficient of inheritance in the broad sense significantly exceeds that in the narrow sense, indicating the interaction of non-allelic and allelic genes. Forms with fewer branches are suitable for mechanized harvesting, the selection differential is negative (-0.27 counts), and the team success is low 5.80. The high value of the inheritance coefficient in a broad sense gives us a reason for successful selection by phenotype. The selection differential is 0.20, and the success of the team has a high value.

In the cross Milena x Sadovo 3959, cultivar Milena formed 92 number of boxes in plant, Sadovo 3959 - 181, and the progeny of F1 population 163.90. The inheritance of the trait was incompletely dominant to the parent with higher number of boxes, and heterosis was absent. In the reciprocal cross, heterosis is again absent, and inheritance of the trait follows the same pattern (fig 5). In the reciprocal cross, the d/a index has a negative value lower than unity inheritance was incomplete to the parent with the lower number of boxes and heterosis was absent. In a study by Rani S., et al. (2015), thirty-three hybrids showed positive heterosis for the number of capsules, while five hybrids showed negative heterosis to the mean parent, supporting our findings.

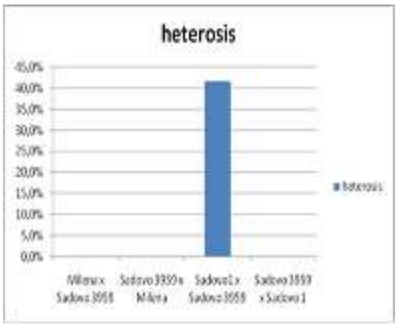

Fig. 5. Heterosis effect by number of boxes

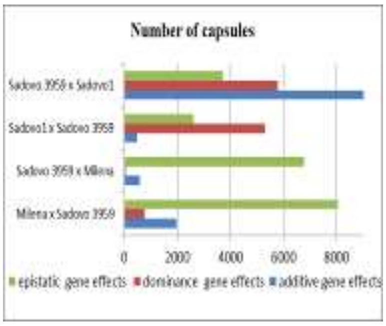

Fig.6. Gene effects in inheritance by number of boxes

Additive gene interactions have the highest proportion in inheritance of this trait in reciprocal cross. This fact combined with the fact that the narrow sense inheritance coefficient has a higher value than the broad sense makes the team in the early generations unsuccessful.

Table. 1 Inheritance coefficients (H^2, h^2) selection differential (S) and team success R

Hybrid combinations	Seeds per plant, g	Nimber of branches	Total number of boxes
♀ *Milena x ♂ Sadovo 3959*			
H^2	23,3	30,0	12,8
h^2	1,5	27,0	10,7
S	-3,4	-0,4	-23,873
R	-80,3	-12,0	-304,8
♀*Sadovo 3959 x♂ Milena*			
H^2	75,2	17,8	4,3
h^2	75,1	1,3	4,1
S	-7,8	-1,1	-46,0
R	-585,2	-191,1	-195,7
♀ Sadovo 1 x ♂ Sadovo 3959			
H^2	57,3	21,5	37,5
h^2	21,3	1,6	5,8
S	-3,8	-0,27	-11,3
R	-217,7	-5,8	-422,7
♀ Sadovo 3959 x ♂ Sadovo 1			
H^2	89,0	35,0	71,9
h^2	74,8	3,1	84,1
S	-2.5	0,2	-0,1
R	-134.2	141,9	-1,2

Genetic distance and heterosis.
In the inheritance of all yield elements in sesame, the interaction between allelic and non-allelic genes were too complex, and in this case no optimal genetic distance can be established in the parents used to justify obtaining the maximum heterosis effect. The nature of the resulting curve has no direction.

Conclusion

In the incomplete diallel scheme used, the manifestation of heterosis and gene effects depend on the direction of crossing. In combinations with high heritability coefficients, where the influence of gene factors in the overall phenotypic manifestation of traits was greater, effective selection in earlier hybrid generations (F_2 - F_3) is expected. This analysis model confirms the hypotheses of heterosis manifestation (dominance, overdominance or epistasis) and can be used with success in any specific hybrid combination included in breeding programs and in other crops.

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STABILITY OF BULGARIAN MAIZE HYBRIDS TESTED IN SERBIA

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Abstract

Twenty Bulgarian maize hybrids (FAO 400-600), including one check – Zp560, are tested in three locations – Zemun Polje, Pancevo and Becej in 2018 and 2019. ANOVA results pointed out a significance of all variances – locations, hybrids and GxE with the first ones’ predomination (51.42%). PC_1 and PC_2 scores occupied 61.6% and 18.2% of the interactions variability. Grain yield stability of hybrids was determined by regression, variation and AMMI analyses. The first three hybrids ranking by grain yield are: Zp-560, Kn-564 and Kn-648. Three hybrids with lowest AMMI stability values (ASV) are Kn-466, Kn-571 and Kn-320, well adapted to all environments. Kn-466 is located almost in the theoretical center of the AMMI-2 biplot – zero point of PC_1 and PC_2. That hybrid also took first rank by ASV. According AMMI-2 analysis five hybrids are unstable – Kn-573, Kn-649, Kn-648, Kn-321 and Kn-572. They had also the biggest values of σ_i^2 – first parameter of Shukla. Ten hybrids (50% from the trial) are located in the more productive zone of AMMI-1 biplot – above grand mean. Five of them are with negative scores of PC_1 including check Zp-560, and the rest five have positive PC_1. A distribution of all hybrids, according yield general mean and mean value (b_i=1.0) of the first stability parameter from Eberhart and Russel model is very similar to AMMi-1 biplot, that confirmed a previous study of ours. Conditions of the three locations in 2018 are better for hybrids performance than in 2019, but Pancevo had biggest PC_1 and PC_2 scores for both years. Vectors of Zp-2018 and Zp-2019 are closest to the center of AMMI-2 biplot.

Key words: *maize hybrids, stability parameters, AMMI, ASV.*

Introduction

The multienvironments testing of maize hybrids is one of the final stages of the breeding process. The main purpose of that testing is the genotypes stability assessment as a result of the genotype-environment (GxE) interaction and determining of the best locations for their zoning as well. A significant GxE interaction has very important consequences for the breeding programs. For such an important quantitative trait as the grain yield, this interaction can seriously limit the efforts in the selection of new genotypes that are important for the final stage of the selection process. The agronomical (phenotype) stability can be defined by means of parametrical methods – most often the regression or variation analysis, or by nonparametrical ones – the rang and cluster analysis (Delic, Stankovic, & Konstantinov, 2009; Branković-Radojčić et al., 2022). Contemporary methods like the AMMI analysis – the Additive Main Effects and Multiplicative Interaction effects model (Zobel et al., 1988) and its modification – the GGE biplot analysis (Yan and Tinker, 2006; Gauch, 2006; Gauch, Piepho and Annicchiarico, 2008) are also applied for determining of the genotype stability and suitable cultivation areas. As hybrid models they use the Analysis of Variance (ANOVA) and the Principle Component Analysis (PCA) with coordinates placement of genotypes and environment in the biplot for a final data interpretation. Purchase et al. (2000) developed the

AMMI Stability Value (ASV) indicator, based on the AMMI-model, in which the ANOVA and the Principal Component Analyses are used simultaneously as a quantitative parameter, in order to the genotypes to be ranked according to the stability of their yields. According to Mitrović et al. (2012) there is not a significant difference between the AMMI and the GGE-biplot analysis and they could be used with equal success for maize hybrids stability evaluation at testing in different environments (locations). Brancovic et al. (2018) make a comparison between linear regression models with the AMMI-analysis in relation to maize hybrids stability. A similarity is observed, but regression models evaluate this stability by one axis – the regression line, while the AMMI-analysis makes rates by two ones – PC_1 and PC_2 (Shojaei et al., 2021). At the prediction of genotype performance and their yield stability in different locations some unpredictable factors may occur – the so-called “noise” (Crossa, 1990; Crossa et al. 1990).
The aim of this study is to evaluate the stability of Bulgarian maize hybrids tested in multi-location field trails in Serbia.

Materials and Methods

Based on a cooperation agreement twenty Bulgarian maize hybrids (FAO 300-600), including one check – Zp560, are evaluated in multi-location field trails in Serbia for a two years’ period (2018-2019). The locations are Zemun Polje (Zp), Pancevo (Pan) and Becej (Be). The climatic conditions of test locations are shown in Figure 1. Combination of years (2018 and 2019) and 3 locations (Zp, Pan and Be) are treated as 6 environments. The trails are set in a randomized complete block design with 7,5 m^2 experimental plot size in two replications for each genotype tested; under non-irrigated conditions. The standard agro-technical practices according to local agro-ecological conditions are applied. The grain yield is measured in kg/ha with 14% moisture at harvest. A two-factor *Analysis of Variance* (ANOVA) on the data is performed. The parameters of stability of genotypes are determined according to the models of *Eberhart* & *Russell* (1966) and first parameter of *Shukla* (1972). *Additive Main Effects and Multiplicative Interaction* effects (AMMI) – according to Zobel at al., (1988) and Gauch (2006); *AMMI Stability Value* – ASV (*Purchase* J. L. et al, 2000) and *Principle Component Analysis* (PCA) with coordinates placement of genotypes and environment in biplot figures for a final data interpretation are applied. The data processing and analyses are done by Microsoft Excel and SPSS 25 Statistical program.

Results and Discussion

The average monthly values of the sum of precipitations and temperatures for a ten-year period (2010-2019), which also includes those of the study (2018-2019), are shown in Figure 1. According to these values, the precipitation in 2019 appears to be average for the period, while 2018 has less rainfall. Rainfall amounts in 2019 at all three locations are over 400 mm (April – October), as follows: ZP (426.6 mm); Pan (460.3) and Be (438.6). In 2018, two of the locations (ZP and Pan) had a lower total rainfall of 313.4 and 344.4 mm, respectively, while Be had 443.3 mm. The results of the ANOVA and AMMI analyses of the trials are shown in Table 1. The variances are significant for all sources of variation – locations, hybrids and their interaction. Locations have the largest variance, followed by genotype (that of hybrids). The genotype-environment interaction has a lesser appearance. In principal components analysis, PC1 accounted for 61.6% of this interaction and PC2 accounted for 18.2%. These results confirm the research of several authors (summarized by Zobel et al., (1988) and Gauch, 2006) on the dominant role of variation in environmental conditions and on PC1 of the genotype-environment interaction.

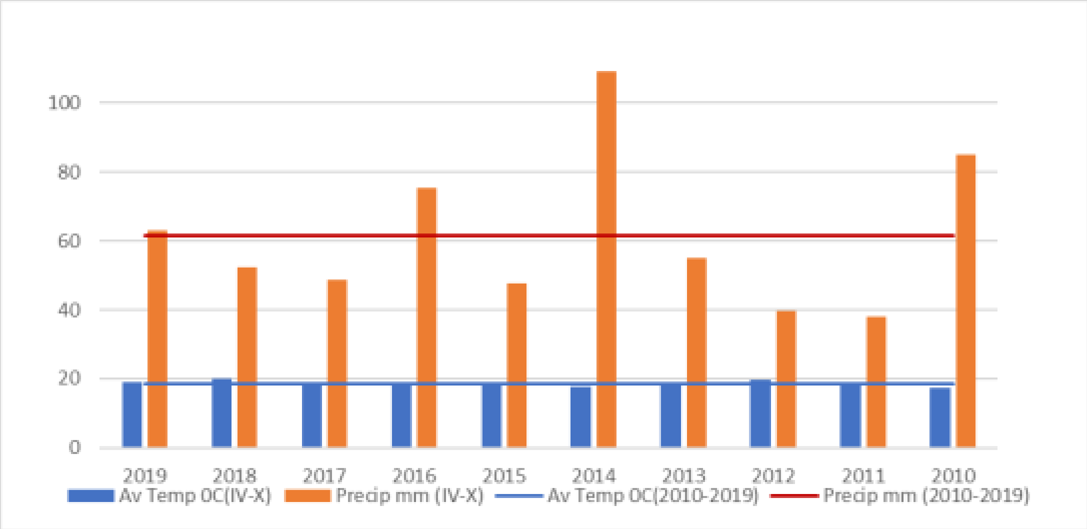

Figure 1. Average mount values of temperatures and precipitation for April-October for the three locations for ten years' period (2010-2019)

Table 1. ANOVA results for grain yield of 20 maize hybrids tested at 6 locations (2018-2019), and AMMI (PC scores)

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Locations	5989430.718	5	1197886.144	75.13263	0.00	2.289851
Reps in loc.	459705.3105	6	76617.55176	4.805531	0.00	4.38075
GEN (hybrids)	1947356.983	19	102492.4728	6.428432	0.00	1.673879
Interaction LxG	3711844.509	95	39072.04747	2.450638	0.00	1.373607
Error	1817572.637	114	15943.61962			
Total	13925910.16	239				

	SS	PORCENT	PORCENAC	DF	MS	F	PROBF
Locations	5989430.72	51.42	51.42	5	1197886.14	63.12	0.00
GEN (hybrids)	1947356.98	16.72	68.13	19	102492.47	5.40	0.00
Interaction LxG	3711844.51	31.87	100.00	95	39072.05	2.06	0.00
PC_1	2288783.80	61.66	61.66	23	99512.34	5.85	0.00
PC_2	675442.21	18.20	79.86	21	32163.91	1.89	0.02
Residuals	2277277.95	0	0	120	18977.31623	NA	NA

Table 2 presents the results of the test for grain yield from 6 environmental conditions - two years and three locations. Yields ranged from 8552.1 kg/ha for Kn-M-625 to 12021.8 kg/ha for the ZP-560 standard. A wide range of hybrids by vegetation groups were included in the trial. In the early FAO group (300-400) there are two hybrids; the mid-early FAO group (400-500) – five hybrids; mid-late FAO (500-600) – ten hybrids (including the standard); and in the late FAO (over 600) are three hybrids. This spectrum of hybrids roughly reflects the distribution of Bulgarian hybrids by FAO groups in the official variety list of the Republic of Bulgaria, where the mid-late group are most represented, although not all hybrids from the experiment are included in it. The arrangement by grain yield puts the standard ZP-560 in first place, but as can be seen from the LSD value at P=5%, a total of 8 hybrids do not differ from it significantly. The yield of the ninth ranked Kn-320 is 88.5% of the standard. These nine hybrids represent 45% of the experiment, as from the early group is Kn-320; the middle early group – Kn-466 and Kn-472; the mid-late group – Kn-564, Kn-568 and Kn-572; from the late group – Kn-648 and Kn-649. As a sample, the distribution by vegetation groups is approximately the same as their distribution in the entire experiment. The lack of significant differences between hybrids from different vegetation groups partially confirms the larger study of Videnović et al. (2013) on this matter.

Table 2. Ranking of hybrids by means of the grain yield (kg/ha) and ranking by ASV and PC scores by hybrids and locations

Hybrids	Name	Yield, kg/ha	Rank	PC_1	PC_2	ASV*	Rank
1	Kn-320	10635.1	9	-0.28	0.11	0.954	3
2	Kn-321	10470.8	10	-0.75	0.5	2.595	18
3	Kn-466	10829.9	8	0.05	0.02	0.165	1
4	Kn-467	9367.4	18	-0.46	0.43	1.632	9
5	Kn-471	9294.6	19	0.43	-0.5	1.548	7
6	Kn-472	11100.8	5	-0.35	0.07	1.19	4
7	Kn-473	9943.0	13	-0.43	-0.25	1.493	6
8	Kn-509	9664.4	17	0.49	-0.15	1.655	10
9	Kn-564	11927.4	2	0.35	-0.33	1.248	5
10	Kn-565	9924.2	14	-0.49	0.43	1.713	12
11	Kn-568	11533.1	4	0.46	0.55	1.655	11
12	Kn-569	10140.7	12	-0.51	-0.23	1.738	14
13	Kn-570A	10191.1	11	-0.47	-0.31	1.629	8
14	Kn-571	9895.9	15	0.07	-0.18	0.304	2
15	Kn-572	10837.1	7	-0.7	-0.43	2.421	16
16	Kn-573	9822.7	16	0.81	1	2.926	19
17	Kn-648	11583.5	3	0.73	-0.56	2.523	17
18	Kn-649	10946.4	6	0.92	-0.27	3.142	20
19	M-625	8552.1	20	0.64	0.2	2.188	15
20	ZP 560	12021.8	1	-0.51	-0.08	1.715	13
	LSD 5% LSD 1% LSD 0.1 %	1450.7 1917.2 2464.0					

*AMMI Stability Value (Purchase J. L. at al, 2000)

Locations**		Yiled, kg/ha	PC_1	PC_2
Zp 2018	1	10277.1	0.19	0.18
Pan 2018	2	12218.4	0.50	-0.47
Be 2018	3	12596.4	0.47	-0.21
Zp 2019	4	8548.4	0.06	0.39
Pan 2019	5	8554.5	-1.00	-0.38
Be 2019	6	10409.9	-0.22	0.50

** Zp – Zemun Polje; Pan – Pancevo; Be - Becej

ASV appears as a parametric evaluation of the stability of genotypes, resulting from AMMI-analysis (Purchase et al., 2000). Lower ASV values characterize hybrids as more stable under less favorable conditions. Table 2 shows three groups of this parameter: with values of

ASV<1.0 there are three hybrids; ASV=1.0 to 2.0 are eleven hybrids (the most common group); ASV>2.0 are six hybrids, the last ones can be defined as unstable. The 3 hybrids with ASV<1.0 are Kn-466, Kn-571, Kn-320, which are from three different vegetation groups, and Kn-571 is significantly lower yielding than the standard. There is no overlap between the yield ranking and ASV in the studied hybrids. Hybrids Kn-565 and Kn-569 have a relatively close ranking, the latter is not significantly different from the yield standard.
In the following Table 3, besides the average yield, the variation by hybrids, the stability parameters from the model of Eberhart & Russell (1966) and the first parameter from the model of Shukla (1972) are presented. We commented on the scope of the yield in the previous table, and the variation for the experiment is 8.85%, which defines it as weak. Variation by hybrids ranges from 10.17 to 32.24%, with ten accessions averaging between 10-20% and the other 10 genotypes is over 20 %, which defines it as strong. The first parameter bi according to Eberhard & Russell model has values <1.0 in ten hybrids, which can characterize them as stable under unfavorable conditions. In six hybrids, S2di had negative values that were determined to be zero (according to Russell 1988, personal communication).

Table 3. Means of the grain yield (kg/ha), variation and stability parameters (Eberhart & Russel, 1966) and first stability parameter of Shukla, (1972).

Gen.	Hybrids	Yiled, kg/ha	CV(%)	Eberhart & Russell			Shukla
				b_i	S^2d_i	R^2	σi^2
1.	Kn-320	10635.1	16.80	0.61	18081.14	0.35	27171.32
2.	Kn-321	10470.8	10.48	0.22	5225.90	0.12	30746.70
3.	Kn-466	10829.9	17.30	1.00	-1522.73*	0.85	4647.18
4.	Kn-467	9367.4	14.95	0.58	3972.08	0.51	15418.35
5.	Kn-471	9294.6	29.08	1.46	3665.84	0.87	16264.15
6.	Kn-472	11100.8	10.24	0.61	-5767.31*	0.86	5929.25
7.	Kn-473	9943.0	17.08	0.78	5226.53*	0.63	12236.58
8.	Kn-509	9664.4	28.66	1.58	-5706.01*	0.98	12178.28
9.	Kn-564	11927.5	22.93	1.56	-6103.51*	0.98	11168.16
10.	Kn-565	9924.2	10.17	0.41	-1470.45*	0.49	16339.57
11.	Kn-568	11533.1	21.12	1.24	9086.55	0.77	15918.80
12.	Kn-569	10140.7	11.51	0.45	1496.95	0.44	17421.85
13.	Kn-570A	10191.1	14.32	0.62	4368.71	0.54	14741.85
14.	Kn-571	9895.9	19.94	1.13	-7150.08*	0.98	214.50
15.	Kn-572	10837.1	14.16	0.48	12792.06	0.29	26319.49
16.	Kn-573	9822.7	31.63	1.32	47440.14	0.54	51587.67
17.	Kn-648	11583.5	27.78	1.75	6443.43	0.89	30583.59
18.	Kn-649	10946.4	32.19	1.93	7956.39	0.90	41781.72
19.	M-625	8552.1	32.24	1.42	11507.71	0.80	22121.22
20.	ZP 560	12021.8	16.20	0.85	12549.97	0.57	17930.26
	Average	10434.1					
	Min	8552.1					
	Max	12021.8					
	STDEV	924.2					
	CV, %	8.85					

*S^2d_i=0

Hybrid Kn-466 has parameters b_i=1.0 and S^2d_i=0, which exactly correspond to the theoretical model. Ten of the hybrids studied had b_i values, including Kn-466, of 1 and above 1, and the rest are below this value. The coefficient of determination R^2, which is taken as an indicator of genetic stability, in four hybrids (Kn-321; Kn-565; Kn-569 and Kn-572) has a value lower than 0.5. The first parameter (σi^2) of Shukla characterizes the stability of genotypes, as a result of their linear interactions with the environmental conditions. Good convergence with the S^2d_i variance from the Eberhart & Russell model is observed. The six hybrids with zero values of this parameter also have low values of σi^2. The results are in good coherency with the study performed by Pavlov et al. (2023) on different stability evaluations in late maize hybrids.

The following **Figure 2** describes the distribution of the studied hybrids by the general mean for grain yield of the trials and the first stability parameter b_i from the model of Eberhart & Russell (1966). In previous research (Vulchinkov., et al., 2021); this distribution was shown to be more correct in characterizing the genotypes in terms of their yield and stability, than the well-known model of Francis & Kannenberg (1978) using mean yield and coefficient of variation. In the figure, half of the hybrids are in the high-yield zone above the general mean of the trials, and also half of them are of the stable type (b_i<1.0). In the most interesting quadrant – higher than average yield and lower value of b_i<1.0, there are five hybrids – Kn-321, Kn-572, Kn-570, Kn-320, Kn-472 plus the standard ZP-560, and Kn-466 with a value of b_i=1.0 can also be added to them. Four hybrids – Kn-568, Kn-564, Kn-648 and Kn-649, which are in the highly productive zone, but of the “responsive” type (b_i >1.0), are also of interest for the selection. The hybrids with low ASV values (see Table 2), four accessions (Kn-320; Kn-466; Kn-472 and Kn-473) falls into the stable zone in Figure 2.

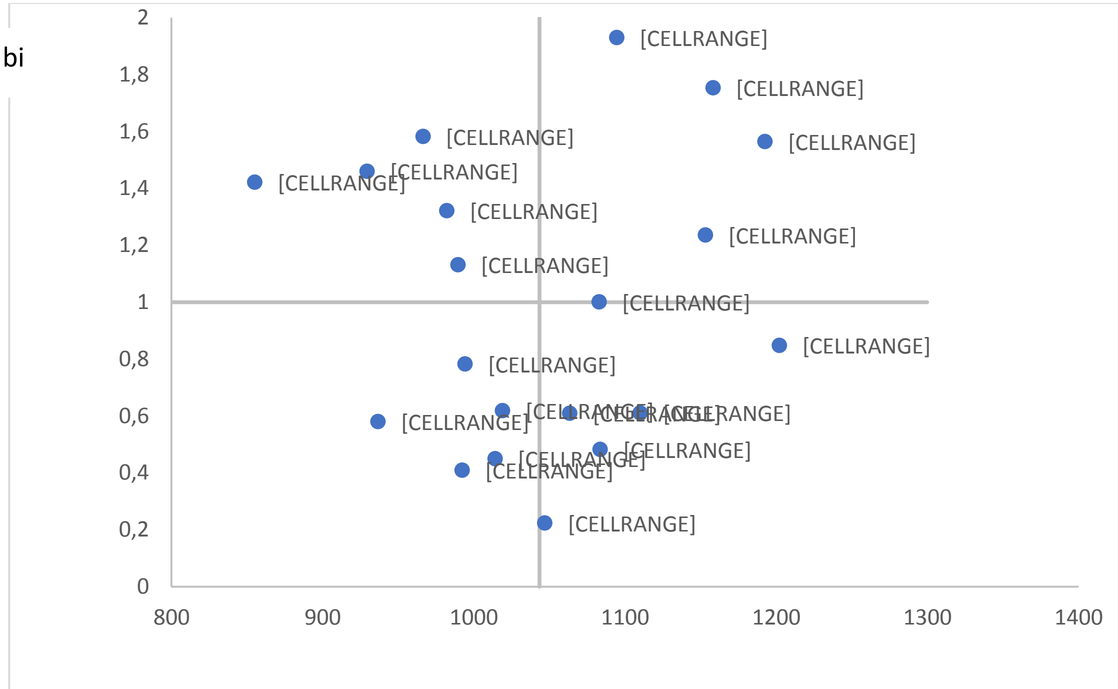

Figure 2. Distribution of 20 hybrids according general means of the grain yield (kg/ha) and first stability parameter (bi) – Eberhart & Russel (1966), tested in 6 locations, 2018-2019.

Figure 3 presents the biplot distribution of AMMI-1 for the grain yield of 20 maize hybrids tested at 6 locations. AMMI-1 operates on the average yield of the hybrids and on the first component of the PCA, that has the largest share of variation, which in this particular case is 61.66%. A high- and low-productivity zone is also observed as in Figure 2. The distribution in the high-productivity zone in terms of the coordinates of the hybrids, relative to their PC1 values, is very similar to the distribution in Figure 2.

The highly productive zone with negative interactions, which we can take as an analogue of the first quadrant (higher than the average yield and bi<1.0) of the Eberhat & Russell model (Fig. 2), practically covers the same hybrids, as only the Kn-466 hybrid (g3) falls in the second quadrant (the zone of positive interactions), in which the remaining four hybrids from

Figure 2 are also distributed. It can be tentatively concluded that good convergence between the yield and stability assessments between the two figures is observed.

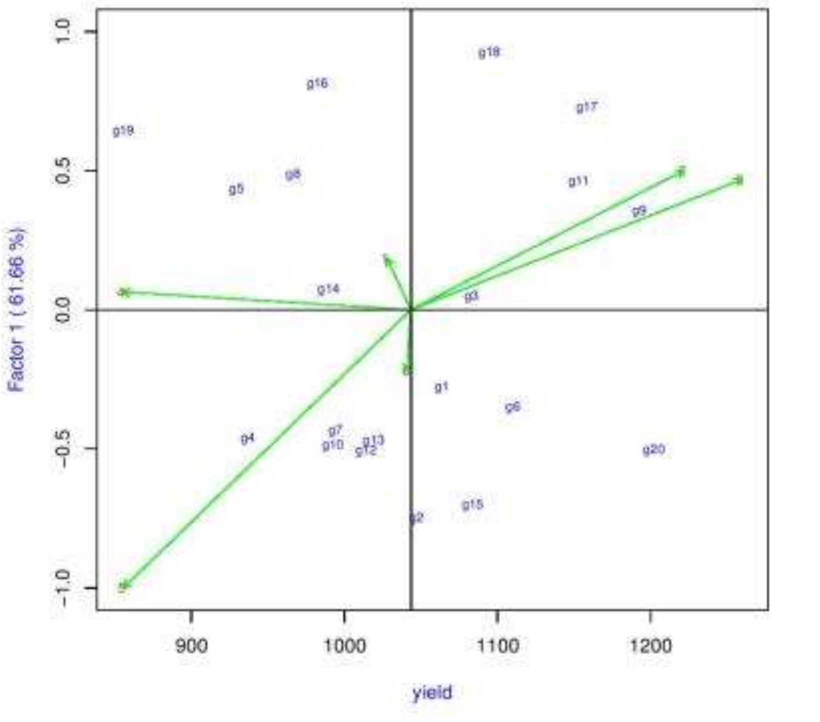

Figure 3. AMMI-1 biplot of grain yield (kg/ha) of 20 maize hybrids, tested in 6 locations, 2018-2019

AMMI-1 also provides additional information on test locations. The Be-2018 (point 2) and Pan-2018 (point 3) of Fig.3 are in the highly productive zone with positive interaction. The remaining locations are in the low-productivity zone, with Pan-2019 being the furthest from the center of AMMI-1.

Figure 4 presents the AMMI-2 biplot, on which the coordinates of the tested hybrids and their locations are plotted, with the corresponding values of their two components (PC1 and PC2). The points at the vertices of the outlined pentagon are the genotypes that are at the greatest distance from the center of the biplot, i.e. with the lowest stability. Clockwise, they are as follows: g2; g16; g18; g 17 and g15 or the hybrids Kn-321; Kn-573; Kn-649; Kn-648 and Kn-572 from Table 2, respectively. These genotypes are from three FAO groups, and their ranking according to ASV values from Table 2 places them in the last five places (from sixteenth to twentieth). Good convergence is observed in the ASV parameter evaluations and the biplot distribution from the AMMI-2 model.

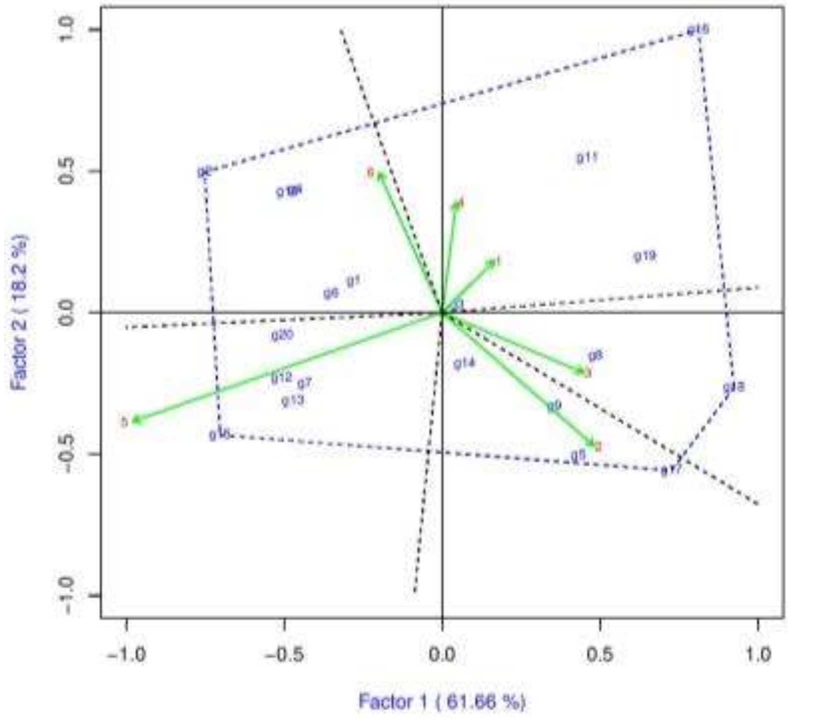

Figure 4. AMMI-2 biplot of grain yield (kg/da) of maize 20 hybrids, tested in 6 locations, 2018-2019

Of the six hybrids (Table 2) with ASV>2.0 values, only Kn-M-625 is not in the periphery of the biplot, but in the quadrant with positive interactions. At the center of the AMMI-2 is a Kn-466 (g3) hybrid, confirming its best stability, also indicated in the other models.
The PC_1 and PC_2 projections of the locations can also be seen on the AMMI-2 biplot. Pancevo has the highest principal component values for both test years. The vectors of Zp-2018 and ZP-2019 are located closest to the center of the biplot, i.e. at this location hybrids with more stable yields may also be presented.

Conclusions

The conducted evaluation of the stability of the studied maize hybrids gives us grounds for the following conclusions:

1. The average productivity of the tested hybrids is over 10 000 kilograms per hectare, and half of them are above it. There are no significant differences between hybrids from different vegetation groups.
2. Half of the studied hybrids are stable under unfavorable conditions (bi<1.0), with 5 varieties in the above-average productivity zone, including standard ZP-560.
3. Three hybrids – Kn-466, Kn-571 and Kn320, are with low ASV values, well adapted to all conditions. The stability of Kn-466 is confirmed by all stability assessment methods in the study applied.
4. A very good convergence between the distribution of the hybrids against the mean values of b_i and grain yield and the AMMI-1 biplot is observed, which is of a confirmatory nature.
5. Test conditions are better in 2018, with principal components at locations Zp-2018 and Zp-2019 being closest to the center of the AMMI-2 biplot.

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TRADITIONS OF USING GEORGIAN WHEAT IN GEORGIA

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Abstract

Georgia is an ancient country of agriculture and agrarian civilization. The ancient tribes who lived here 8000 years ago, along with the creation of the first dwellings, began to domesticate and cultivate wheat and grape cultures . Georgian vine and wheat still attract great interest: Georgian vine and wine, wheat and bread are the treasures of the country and carriers of national values. Owing to the importance of wheat, people around the world have worked on the improvement of this crop for thousands of years. As a result, up to 40,000 varieties of 22 types of wheat have been created. Today, 14 of the 27 wheat species distributed in the world are grown in Georgia, of which 5 endemic species are widespread in different ethnographic parts of Georgia (Zanduri - *T. timopheevii Zhuk*, Kolkhuri Asli - *T.Karamyschevii Nevski*, Dika - *T. persicum Vav.*,Hexaploid Zanduri - *T. zhukovskyi Men. et Eriz*, Macha-*T. macha Dekapr. et Men.*.). Prominent triticologists confirm the origin and evolution of wheat in Georgia: N. Vavilov, M.Mackay, P. Zhukovsky, M. Yakubtsiner, V. Dorofeev, L. Decaprelevich, V. Menabde, P. Naskidashvili. The center of wheat culture origin is Western Georgia, namely the Kolkhida Plain, Racha-Lechkhumi. History has confirmed that wheat culture played an important role in Georgia's independence. The Georgian people created forms adapted to local conditions, which guaranteed the steady production of crops. That is why the population of Georgia has created such wheat biodiversity, which is unique around the world. Georgian endemic species are characterized by unique properties, therefore they are considered the best genetic source in world breeding. The attention of triticologists of the world is especially attracted by: Chelta Zanduri, Dika and Hexaploid Zanduri (Zhukovsky), characterized by phenomenal complex immunity to fungal diseases; Chelta Zanduri is characterized by high protein content in the grain and high degree of baking ability; the endemic species Kolkhuri Asli is resistant to various types of rust; Macha wheat is characterized by an abundance of leaf mass on the plant and the strength of the stem, well tolerates excess moisture. The native varieties of soft wheat of Georgia are very diverse, which are represented in ecological groups and are characterized by a relatively low, but guaranteed solid yield. They adapt well to dramatically changing soil-climate conditions. Their germplasm carries the necessary characteristics for modern varieties of the intensive type. Wheat traditions in Georgia have survived from the Neolithic period to the present day. The historical experience of the Georgian people in the production of wheat is one of the oldest in the world. At the end of the 19th century, Georgia still produced wheat for export.

Keywords: *wheat, traditions, endemic species, history, evolution.*

Introduction

The attitude of the Georgian people to the wheat culture is exceptional. Historically, Georgia was in a constant war with neighboring countries. From ancient times, the Georgian people were considered permanently residing people and who did not leave their home even in war times. None of the conquerors - Macedonians, Mongols, Iranians, Turks and Russians - were able to destroy Georgia. The high level of agriculture, the love of wheat and viticulture played a large role in the survival of Georgia. The writer Xenophon the First gives us information

that "the Hellenic army, passing through the land of the Muscovites of the Kartvel tribe, robbed houses where they came across a large supply of bread, which, according to the Muscovites, were distributed last year by the will of their ancestors. The freshly cut wheat was bound in sheaves. Especially a lot was "Asli" (Berdzenishvili, 1956; Mikeladze, 1967)
According to ethnographic studies, wheat cultivation in Georgia has been at high level since the ancient times. Endemic elements of material culture have survived to this day, such as "shnakvi" a simple tool for collecting keeled wheat, and a threshing tool "kevri".

Historical data on wheat in ancient times

In the works of ancient Greek historians Herodotus and Xenophon, we come across the information about the distribution of many types of wheat in ancient Georgia. The first monuments of the wheat civilization belong to the Mesolithic period, which is confirmed by archaeological excavations. Several types of wheat have been found in Neolithic settlements - Arukhlo, Khramis Gora, Shulaveris Gora. Among them are hard and soft wheat, whose age dates from 6,000 years BC (Rusishvili et al., 2019). As a result of similar archaeological excavations in a settlement in the village of Digomi, carbonized grains of Zanduri of the late Bronze age were discovered (Maisaia et al., 2005). Archaeological research in the Mtkvari-Araksi Valley revealed the remains of Tavtukhi, dating from the 4th and 3rd millennium BC (Dzidziguri, 2000). According to these materials, the primary wheat species existing in Georgia revealed phylogenesis of the wheat genus and confirmed the diversity of wheat species. Georgian farmers in ancient times gave corresponding names to all this great variety of wheat species. Georgian names for wheat by biological group were "dzveltesli" or autumn, and "akhaltesli" or spring. By species and varieties - Zanduri, Asli, Dika, Tavtukhi, Ipkli, Doli Puri, Khulugo, Khozo, Khotora. By place of origin - Chveneburi, Rachuli, Akhaltsikhe, Kolkhuri, Corbouli. These names give a peculiar folk classification of a wide variety of Georgian wheat varieties. Ecotope is a mesophyte. The distribution area - Racha, Lechkhumi, Chiatura, Sachkhere, Kvemo Svaneti. Varieties: Khulugo, Khotora, Khozo. Later Korboulis Dolis Puri and Ipkli. According to morphological characteristics - Shavtavtava, Shavtavela, Shavpkha, Tsiteli doli, Shavi dika, Tetri dika. These names are a kind of folk classification of a wide variety of of Georgian wheat varieties. According to Ivane Javakhishvili, this is not only the result of observations of farmers, in ancient Georgia there were such writers - agronomists and naturalists as Katon, Varon, Columbella and Plinus. The first information about the biodiversity of Georgian wheat belongs to Sulkhan-Saba Orbeliani (XVII century) and Vakhushti Batonishvili (XVIII century). Foreign naturalists also traveled to Georgia - Guldstadt, Georg and Klaport (18th-19th centuries). In Georgia, even today, it is possible to detect sub-contoured forms resulting from the natural interbreeding of wild and cultural forms. Such forms were discovered in the village Eredvi of Kartli area. Of all the richness of varieties that have been created in Georgia for centuries, autumn varieties of soft wheat with red grains called "Dolis Puri" are especially valuable. These varieties had the most important properties, such as high nutritional value, baking ability, and long-term storage of the baked bread. Based on its meaning, the people called it mother's bread. Thanks to these qualities, wheat played the role of a mother in the history of the Georgian people. Over the centuries, the wheat variety developed following the conditions of historical development. Georgian warriors could take bread with them to the war, which did not got staled or change its taste for two weeks. Thanks to the love of wheat culture, in the highlands of Georgia (Racha-Lechkhumi, Meskhet-Javakheti, Mtatusheti) keeled wheat is still grown today and use old Georgian terms: "Jejili" is a newly sprouted field, "Namja is what is left after harvesting, "Kalo" is a place for threshing ears, "Kevri" is a board for threshing ears, "ulo" means strong binding of wheat stems, "shnakvi" is a tool for collecting wheat.

Traditions of use of wheat in Georgia

Wheat has many uses in Georgia. By the church, it is considered as a holy culture. In the Georgian Orthodox Church from ancient times, flatbreads were made from wheat flour in honor of the holy fathers. In Tusheti, small loaves were baked, which were called “kveri”. In every corner of Georgia, church holidays are celebrated with food made of wheat grains or flour. During the holiday Barbaloba, it is customary to bake “lobiani” (a pie with beans). On Christmas in Guria they bake pies with cheese and boiled eggs. In Kartli-Kakheti, Ertso-Tianeti and Gudamakari is prepared “korkoti” (boiled wheat grains). Church rituals are celebrated in each corner in different way.

Wheat was widely used in folk medicine: pus was squeezed out of a suppurated place with the help of "khavitsi." Stomatitis was treated with roasted wheat grain, into the broth of which alum was added and was prepared infusion for rinsing. Wheat starch is used to treat cough and gastrointestinal diseases. Various ointments are made from wheat flour. Wheat flour porridge is used for gastrointestinal diseases and to eliminate hemorrhoids (wheat grains should be soaked for two days, when it germinates, dried, ground, boiled and mixed with butter and salt). Wheat malt is also used as a laxative for animals. Zanduri is used in Samegrelo to stimulate sweating in patients with fever, and patientswithneurosis are given a boiledgrain broth with honey. Macha seed broth is used to improve vision. In folk medicine, the boiled wheat grains brothis used as a refreshing drink.

Modern achievements of Georgian wheat

The multi-species biodiversity of Georgian wheat has not gone unnoticed by world scientists. Scientists in Japan, Russia, Germany, England, the United States and France are intensively working on Georgian wheat.

It is important that on the basis of endemic species of Georgia, 8 new varieties of wheat were obtained: *Tr. milinae* $2n = 28$, a naked analogue of *Tr. Timopheevii*;*Tr. Miguschovii* $2n = 42$, an immune analogue of hexaploid wheat; *Tr. kiharae* $2n = 42$, wheat spelt homolog; *Tr. Timonovum* $2n = 28$ obtained by Zanduri autopolyploidy; *Tr. fungicidum* 2n=56, obtained by Zhukovsky by crossing Dika and Zanduri;

With the participation of Chelta Zanduri in the USA, Australia, Kenya, Japan, England, many new forms and varieties of wheat were obtained: Melanopus 5, Melanopus 6, Melanopus 7, Steinwedel, Timstein, Mengavi, Lepard, SRPC 67;

The future of the Georgian wheat

The diversity of wheat created by the Georgian people over 8,000 years of activity will allow geneticists and breeders of the world to create new varieties of intensive type of wheat using modern methods. The immunity of Georgian species to fungal diseases is especially important for the future. Chelta Zanduri also has cytoplasmic sterility genes that allow the creation of hybrid wheat.Also a gene, found in Georgian soft wheat Dolis Puri 35-4 allows the restoraton of fertility. The positive characteristics of Georgian wheatspecies and soft wheat varieties are the best source for creating promising varieties and forms using gene modification and nanotechnology. This is confirmed by data from the Institute of Molecular Genetics in Liverpool. According to German researchers, in the future, humanity should consume Zanduri.

Conclusion

The Georgian people are the ancient nation, thanks to their traditions and development, have created a huge biodiversity of the wheat culture over the centuries. These traditions continue today and create new opportunities to provide the world's population with new promising source material for wheat.

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BIOSTIMULANTS ADVERSELY AFFECTED BALSAM ESSENTIAL OIL DURING ITS FIRST YEAR OF ESTABLISHMENT

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Abstract

The perennial plant Hypericum perforatum, commonly referred to as balsam, is renowned for its therapeutic properties, including potent anti-inflammatory and antidepressant effects. This study aimed to evaluate the impact of various biostimulants on both the essential oil content (%) and essential oil yield (ml/ha) of balsam during its initial year of establishment. To achieve this, a field experiment was conducted at the experimental farm of the Agrotechnology Department at the University of Thessaly, located in the Larissa region. The tested factors were three different biostimulant treatments (T1: Control, T2: seaweeds, auxin 1.0 mg/lt and cytokinin 0.031 mg/lt from *Ecklonia maxima*, T3: special formulation containing amino acids 10% w/v, pure protein 11.3% w/v, sucrose 22% w/v, UV filter 10% w/v) in six replicates, for the topaz variety. Harvest took place in late July by hand and samples were dried to constant weight. Distillation was then performed using a lab-modified Clevenger apparatus. It was observed that, in the initial year of establishment, the percentage of essential oil content did not exhibit a statistically significant difference. However, due to the higher dry drogue yield of the control, there was a statistically significant difference in essential oil production compared to the other treatments. Consequently, it appears that the application of biostimulants during the first year of establishment may have an adverse effect. Nonetheless, the results from the second and third years, when the perennial plant will be at the peak of its reproductive phase, are highly anticipated.

Keywords: *Balsam, biostimulants, essential oil, Aromatic-Medicinal plants.*

Introduction

Worldwide, there are more than 480 species of tiny trees, shrubs, and herbs in the family Hypericaceae, which includes the genus Hypericum (Napoli et al., 2018; Becker et al., 2016). It originated in Madeira and is currently found all over the world (Xu et al., 2019; Nürk et al., 2013). It is native to Europe, North Africa, West Asia, India, China, and the Azores. As per Crockett and Robson (2011), several species are well-known for their ability to generate an extensive array of advantageous secondary metabolites, which are often utilized in traditional medicine.

Hypericum perforatum L. is widely utilized in both traditional and modern medicine because of its biological qualities (Ersoy et al., 2020; Sarikurkcu et al., 2020; Butterweck 2003). Nevertheless, the plant's geographic range is restricted to Europe and Asia, even with its extensive therapeutic use (Molins et al., 2014; Morshedloo et al., 2015).

As more is known about the pharmacological activity of St. John's Wort plants and the chemicals that make plant extracts unique, such as hypericin and hyperforin-like substances, interest in these plants is growing. Since then, St. John's Wort has grown to be one of the most sought-after therapeutic herbs over the past 20 years (Russo et al., 2014; Wölfle et al., 2014).

Furthermore, the quality of St. John's Wort can differ according to cultivars or subspecies, the region's geography, and harvest season (Verotta, 2003). St. John's Wort plant chemical composition is also influenced by genotype, environment, or genotypes x environment interactions (Buter et al., 1998). The various populations of St. John's Wort may differ significantly in terms of genotype and phenotype (Walker et al., 2001). Such genetic diversities are defined by morphological and biochemical characterizations (Rahnavrd, 2017).
There is little information available regarding the cultivation and production of Hypericum, even if the medical benefits of its aerial parts are widely documented (Caraci et al., 2011; Saddiqe et al., 2010; Birt et al., 2009).
The perennial shrub St. John's wort (Hypericum perforatum) is 1-3 feet tall and can reproduce both vegetatively and sexually. Important physical characteristics of H. perforatum include its taproot system, woody stems, rhizomes, runners, leaves with pellucid glands everywhere on the lamina, and cyme of yellow flowers that turn into dehiscent capsules that store seeds (Velingkar et al., 2017).
Moreover, as previously noted, St. John's wort is primarily used as a food additive and for medicinal purposes. Therefore, it is important to explore the potential for large-scale production using organic cultivation methods. To this end, biostimulants—biologically derived molecules or microbial products—should be employed. These substances, when applied to plants, enhance their natural processes and growth. (Yakhin et al., 2017).
Seaweed extracts have drawn more attention as a biostimulant category in recent years, partly as a result of studies conducted (du Jardin et al., 2015; Khan et al., 2009). According to Ali et al. (2019), these seaweed-based biostimulants improve crop productivity, root system growth, blooming, stress tolerance, and nitrogen absorption.
The objective of this study was to determine the impact of various biostimulants on the essential oil yield of Hypericum perforatum (Topaz variety) during the initial year of cultivation establishment.

Materials and Methods

On May 18, 2022, a field experiment was initiated at the experimental farm of the Agrotechnology Department at the University of Thessaly, located in the Larissa region. The aim of the experiment was to assess the essential oil yield of balsam plants. Three treatments with six replications were used: T1 was the control, T2 consisted of seaweeds, auxin 1.0 mg/lt and cytokinin 0.031 mg/lt from *Ecklonia maxima*, and T3 was a specific formulation including 10% w/v of amino acids, 11.3% w/v of pure protein, 22% w/v of sucrose, and 10% w/v of UV filter. Harvest took place by hand towards the end of July. The samples were then air-dried to achieve a consistent weight.
All measured and derived variables' collected data were subjected to an analysis of variance (ANOVA) using the statistical program GenStat (7^{th} Edition), where the $LSD_{.05}$ test criterion was used to evaluate the differences between means of the main and/or interaction effects (Steel and Torrie, 1982).

Results and Discussion

The study's findings, presented in Table 1, indicate that treatments 2 and 3 (T2 & T3) and the control treatment (T1) yielded significantly different amounts of essential oil per hectare. Specifically, the control group produced more essential oil—1194 ml per hectare—than treatments 2 and 3, which displayed lower essential oil yields of 699 and 737 ml per hectare, respectively. It is interesting to note that treatment 2, which applied algae together with auxins (11.0 mg/lt) and cytokinins (0.031 mg/lt), had the lowest yield—699 ml per ha.

This outcome contrasts with the literature (Giannoulis et al., 2020; Eisa, 2016; Elansary et al., 2016; Gehan, 2015), which suggests that the application of seaweeds generally enhances the biomass yield of various aromatic and medicinal plants. However, there is limited information available on their effects on balsam yield specifically.

Table 1. Essential Oil Content and Essential Oil Yield of Balsams' inflorescence.

	ES. OIL CONTENT (%)	ES. OIL YIELD (ml/ha)
T1	0,218	1194
T2	0,162	699
T3	0,137	737
$LSD_{.05}$	**ns**	**363,2**
CV (%)	**34,8**	**32,2**

Furthermore, no statistically significant differences were observed among the evaluated components. However, treatments T1 and T2 exhibited a numerical advantage over T3 with respect to the essential oil concentration in the flowers. Thus, it was found that none of the biostimulants increased the concentration of essential oils, contrary to findings commonly reported in the literature (Rasouli et al., 2023; Nassar et al., 2020; Fokom et al., 2019; Gehan, 2015). Consequently, as balsam is a perennial and will promote the growth of its root system in the first year of installation, the effects of the biostimulants on essential oil yield and concentration are anticipated to become more evident in the subsequent year.
Based on existing research, the use of biostimulants in field crops is increasingly prevalent. Seaweed extracts (SWE) represent a novel class of organic fertilizers that compete with growth stimulants and conventional agrochemicals (Elansary et al., 2016; Sharma et al., 2014). Studies suggest that SWE could potentially replace some fertilizers due to their content of both major and minor nutrients (Nassar et al., 2020; Zodape et al., 2010; Hong et al., 2007). The results of this study, which diverge significantly from the literature, suggest that during its establishment year, balsam, being a perennial crop, prioritizes the development of a robust and healthy root system. Therefore, repeating the experiment in the second and third growing years is essential to further investigate these discrepancies and gain a clearer understanding of the effects.

Conclusions

It appears that alternative balsam cultivation methods have yielded substantial amounts of essential oils from the initial year of establishment. However, the use of biostimulants seems to have negatively impacted the outcomes of the first year, with algae extracts demonstrating the lowest performance.
Research is ongoing, as all treatments have been repeated for the second year. Data from this year is currently being processed and analyzed.

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BIOSTIMULANTS EFFECT ON LAVENDER YIELD IN ITS SECOND GROWING YEAR

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Abstract

Lavender (*Lavandula angustifolia*), a common medicinal and aromatic species in the Mediterranean, belongs to the Lamiaceae family. It is one of the most common medicinal and aromatic species cultivated in the Mediterranean Region, including Greece, where the climate is characterized by cool, wet winters and hot, dry summers and its cultivation is preferred to produce dried lavender but also its essential oil. The objective of this study was to determine the lavender flower yield for the 2022-2023 growing season, where lavender was in the second year of its biological cycle, and to evaluate farmer’s profit as well. The experiment was set up on the farm of the University of Thessaly in Greece where the effect of biostimulants on lavender yield and their economic viability was examined. In a field experiment with three replications and six levels of biostimulant application (C: control, A: Ascophyllum nodosum algae extract, S: plant amino acids, A+S: combination of previous two, K: Ecklonia maxima algae extract, PLUS: biostimulant with absorbable calcium), all necessary procedures were conducted for two harvests in June (without irrigation) and September (with irrigation) to assess flower yield. It was observed that biostimulants K and A significantly enhanced productivity, with K exhibiting the highest increase (2599 kg/ha). Formulations A and K resulted in the highest yield. In terms of lavender cultivation economics, K emerged as the most efficient biostimulant (16.5% increase). Algae-based formulations were found to maximize agricultural profitability and flower productivity, which provides long-term promising prospects for lavender cultivation using such biostimulant formulations.

Keywords: *Lavender, biostimulants, yield, dried flowers, profit*.

Introduction

Lamiaceae family includes lavender (Lavandula angustifolia Miller), according to the ITIS (Integrated Taxonomic Information System). The subfamily Nepetoideae, which comprises the lavender species, is one of the seven subfamilies that make up the family Lamiaceae. It is characterized by its square-sectioned stems and opposing and dehiscent leaves (Héral et al., 2021).

Originally from southern Europe and the Mediterranean region, lavender is a plant that is now widely grown throughout the world, with France and Bulgaria being the two primary nations where it is grown the most (Zheljazkov et al., 2012).

The Latin verb lavare, which means "to wash, to clean," is the source of the English name "lavender." This plant, and more especially its flowers, were used in Roman baths to sanitize the body and spirit as well as to give the waters a distinctive scent (Gul et al., 2015).

Lavandula angustifolia Miller is the most widely used species of lavender due to its widespread use, high level of study, and numerous applications for its essential oil. Lavandula angustifolia Mill. is also known by the common names "true lavender," "English lavender,"

and "common lavender" (Pokajewicz et al., 2023). The term "stenofyllis" is synonymous with the plant "angustifolia" (Charles D., 2012).
L. angustifolia is a small, strongly aromatic, erect, herbaceous, perennial shrub that can grow up to 60 cm in height (Pokajewicz et al., 2023). Its leaves are sessile, opposite, linear-lanceolate, narrow, and have spirally curved edges (Fakhriddinova et al., 2020; Héral et al., 2021; Pokajewicz et al., 2023). The root is woody and branched at the top. The structure of the inflorescence is organized in apical dichotomous flower clusters, which means that it consists of a main axis with at least two secondary axes (Héral et al., 2021). The inflorescence spikes of L. angustifolia extend above the leaves and can have a variety of colors. The flowers are densely distributed at the tops of the spikelets and are either purple, blue or purple-blue (Wells et al., 2020).
An important step in the production of lavender is irrigation, which guarantees that the right amount of water is absorbed and that the water movement provides the nutrients the lavender plants need for a healthy growth and development. Furthermore, irrigation helps preserve the distinct, powerful scent associated with lavender farming (Jigău et al., 2022). The significance of irrigation for L. angustifolia yield and quality features has been highlighted by numerous research carried out in a variety of soil and climate conditions (Karamzadeh, 2003).
Although as mentioned above, lavender is drought tolerant, it requires supplemental irrigation for excellent establishment and maximum production (Kimbrough & Swift, 2009). In general, both the yield and the essential oil content of various aromatic plants, including lavender, are affected by the amount of water applied (Aqeel et al., 2023).
Since lavender is a plant with modest requirements for high soil fertility and nutrient concentration, it can grow and perform admirably without fertilizers (Salehi et al., 2018). However, it is well recognized that potassium (K), phosphorus (P), and nitrogen (N) play a major role in the development and production of essential oils in aromatic plants (Chrysargyris et al., 2016). But too much nitrogen can lower lavender's oil yield, making the plant unhealthful and weedy (Salehi et al., 2018).
In order to feed the world's growing population, agriculture heavily relies on the usage of pesticides and fertilizers. High concentrations of chemicals found in the environment, however, pose a major risk to human health, non-target animals, and the ecosystem as a whole because of their harmful effects (Mandal et al., 2023). Because of this, biostimulants are crucial to sustainable agriculture because their application helps to activate a number of physiological processes that improve nutrient use efficiency, promote plant growth, and permit a decrease in fertilizer use (Kunicki et al., 2010). A successful strategy to meet the increasing demand for non-traditional products that have minimal ecological impact, negligible adverse effects on non-target organisms, and biological activity must include biostimulants (Kocira et al., 2020; Lau et al., 2022; Pahalvi et al., 2021).
Any substance or combination of naturally occurring substances or microorganisms that modifies a culture in a beneficial way while having the least detrimental effect on it can be considered a biostimulant (du Jardin, 2015). It is important to note that biostimulants and biofertilizers are not the same thing. Rather than giving crops nutrients directly, the former help them absorb them via altering the rhizosphere and plant metabolism.
This improves crop quality, increases tolerance to abiotic stress, and improves the utilization of nutrients (Drobek et al., 2019). Ultimately, it should be noted that superior agricultural products are not invariably associated with elevated nutrient availability (Bulgari et al., 2015). According to Zamljen et al. (2023), biostimulants have applications in sustainable agriculture and can be utilized to lessen plant stressors.
Greece and the wider region of the Mediterranean basin are distinguished by the extremely beneficial soil and climatic conditions for the growth of lavender, which has led to its widespread popularity. Lavender production can be increased when the right cultivation

methods are used in conjunction with the required formulations. Therefore, the goal of this study is to determine the lavender crop's flower yield in its second year following the application of different biostimulants. Additional goals of this study were to calculate the producer's ultimate profit and evaluate economically the used treatments.

Materials and Methods

Experimental Field

The experiment was conducted University of Thessaly farm in Velestino, Magnesia, at coordinates 39^{o}2' N, 22^{o}45' E. The experimental field's soil is classified as *calcixerollic xerochrept* (USDA, 1975) and its fertility is characterized as sufficient and satisfactory, with the soil being clay-sandy (54% sand, 30% clay and 16% silt), with an alkaline pH (7.82) and the organic matter being ranges from low to moderate levels (2.51%) for Greek data. The experiment took place in the period 2022-2023.

A completely randomized experimental design with one factor and three blocks (replicates) was used. The tested factor was the application of different biostimulants. The biostimulants were applied at 6 levels (C: control, B1: *Ascophyllum nodosum* algae extract, B2: plant amino acids (predominantly glutamic and aspartic acid), B1+B2: combination of the previous two, B3: containing easily digestible calcium, and B4: *Ecklonia maxima* algae extract). For the experiment, lavender cultivation, of the species *Lavandula angustifolia*, of the second year was used. Each trial plot had a 3.15 m^2 area, measuring 2.25 m in width and 1.4 m in length.

Irrigation of the crop started after the first harvest, on 06/21/2023. Until the first cut, the crop was not irrigated. Irrigation was done through drip irrigation system with self-adjusting drippers with a constant supply of 4L/h per plant.

Two harvests took place in total during the experiment: on June 21, 2023, for the non-irrigated crop, and on September 22, 2023, for the irrigated crop.

At harvest, lavender antennae (flowers) were collected manually with a sickle from the inner row of the plot (ground coverage area 0.525m^2). The fresh weight was measured at the farm site, and then, the lavenders from each treatment were placed in paper bags and transported to the Laboratory of Agriculture and Applied Plant Physiology for drying in a hot air dryer at a temperature of 40°C, till constant weight.

The Department of Agriculture, Plant Production and Rural Environment's meteorological station, situated on the University of Thessaly farm in Velestino, provides the meteorological data.

Lastly, an analysis of variance (ANOVA) for all measured and derived variables was carried out on the collected data within the sample durations using the statistical program GenStat (7th Edition). The disparities in the means of the main and/or interaction effects were assessed using the $LSD_{0.05}$ test criteria (Steel and Torrie, 1982). The data could be thoroughly analyzed using statistics, and any differences between the variables under investigation that were discovered would be statistically significant rather than merely accidental.

Finaly, for the financial data of this work were obtained from the supply invoices of the required materials-equipment, and from the average selling price of the dried lavender flowers in the local market.

Results and Discussion

Climatic Data

The meteorological information gathered from the farm's meteorological station in Velestino was recorded and related to the experiment's requirements for the March 2023–September 2023 timeframe. The temperatures (^{o}C) and rainfall (mm) recorded every ten days for the months of March through September of 2023 are displayed in Figure 1. Specifically, the highest recorded temperatures were during the second and third 10 days of July, reaching

38.2°C and 37.8°C, respectively. The maximum temperature rises sharply from the second decade of June to the second decade of July before stabilizing until the third decade of July. Furthermore, the maximum temperature drops sharply. As predicted given the intense weather events that affected the larger region during this time, there is also a noticeable drop in the maximum temperature from the third decade of August to the first decade of September.

Figure 1. Temperature and precipitation (10-days mean values) occurring in studied site during the growing period of lavender in 2023.

In terms of precipitation, Fig. 1 indicates that the first and third 10 days of September get the greatest rainfall. More precisely, because of the flooding phenomenon in the Thessaly region, 416 mm of rain were recorded in the first 10 days of September and 224 mm in the third ten days of September. A week after the second harvest, there was a second wave of terrible weather, with the first wave occurring roughly two weeks before to the second harvest. Furthermore, rain fell during the first and second 10 days of April, with totals of 43.62 mm and 45.17 mm, respectively. This led to the quick growth of weeds in the crop. As the temperature rises, no notable rainfall is seen during the second ten days of May, which also happen to be the times when the biostimulants were applied. Eventually, there was some rainfall with a total height of 23.35mm during the second ten days of June, which is just before the first harvest.

Lavender Flower Dry Yield

Table 1's data shows that there are just numerical variations, not statistically significant differences, between the treatments for the dry weight of the flowers and the first and second cut.

Specifically, treatments B1 and B4 outperform the other treatments quantitatively in the case of the first harvest (Table 1). Upon the second cutting, the B1+B2 and B4 treatments exhibit greater values in comparison to the remaining treatments. As compared to the first and second cuts, the values of the C, B2, and B1+B2 treatments rise; the B1+B2 treatment increases by the greatest amount. By comparison, from the first to the second cutting, the costs of treatments B1, B3, and B4 drop; treatment B1 experiences the greatest value decline.

Table 1. Flower dry yield (kg ha^{-1}).

	1st Harvest	2nd Harvest
C	1081	1145
B1	1439	1055
B2	1177	1196
B1+B2	1032	1331
B3	1212	916
B4	1312	1287
LSD$_{05}$	ns	ns
CV (%)	16,7	20,9

The dry weight of the *L. angustifolia* species ranged from 17.8 to 18.8 g/plant under various phosphorus fertilization conditions, according to a study by Peçanha et al. (2021). As can be observed, the current experiment's conclusions contradict the findings of the previous study. Specifically, it was discovered that the dry weight of flowers varied across treatments in the first cutting, ranging from 25.8 to 36 g/plant, and between 53.2 and 65 g/plant overall.

While the flower dry weight yield in the current work, as indicated in Table 1, ranges from 212.8 to 259.9 g m^{-2}, in another study conducted by Seidler-Lozykowska et al. (2014) in four different lavender organic cultivation locations, the flower dry weight yield in that study was found to be between 7.8 and 38 g m^{-2}. Kucharski (2010) discovered a dry lavender flower yield of 300 to 500 kg ha^{-1} in another study, which is significantly less than what we found. Furthermore, Biesiada et al. (2008) report that during the second year of the experiment, the dry weight yield of lavender flowers under various nitrogen fertilization doses ranged from 1.34 to 1.67 kg m^{-2}. Out of all the studies described above, this one is unique in that the performance in terms of flower dry weight surpasses the results of the current research on flower dry weight across all applied treatments. This could be the outcome of the crop receiving nitrogen treatments in the Biesiada et al. (2008) study.

Finally, Sönmez et al. (2018) reported that the range of the second year's floral dry weight output was 2.90 to 113.30 g/plant. As previously noted, the yield in dry weight of flowers during the first harvest was calculated from 25.8 to 36 g/plant, and in the total number of cuts from 53.2 to 65 g/plant. These results are completely consistent with the findings of the current investigation.

Economic data

The financial data of this work were obtained from the invoices for the supply of the required materials-equipment, from the price databases of the required equipment made available by the University of Thessaly, from the payments of the works and from the calculation-estimation of the cost of personal work for the preparation of the specific experiment. These data are presented in Table 2.

Table 2. Total costs of lavender cultivation.

Establishment Costs	€ ha^{-1}	Annual Costs	€ ha^{-1}
Field preparation	250	Working Cost	600
Basic fertilization	200	Fertilization	150
Weed destruction	100	Irrigation	150
Plants cost	2000	Harvest	300
Establishment Total	**2550**	**Annual Total**	**1200**
Total Costs divided with 7 years of lavenders' life cycle			**1564**

The production in flowers for each of the various biostimulant treatment (C, B1, B2, B1+B2, B3, and B4) is displayed in Table 3. Together with the treatment costs, the gross margin is displayed when the unit sales price was set at 4.14 € kg^{-1}. Gross margin is subtracted from total cost to determine profit per hectare. The final column displays the percentage (%) impact of each treatment in relation to the gain from the control's treatment (C).

Table 3. Biostimulants’ financial

Biostimulants	**Flower yield (kg ha^{-1})**	**Gross Margin (€ ha^{-1})**	**Biostimulant quantity (L ha^{-1})**	**Biostimulant Cost (€ L^{-1})**	**Total Cost (€ ha^{-1})**	**Profit (€ ha^{-1})**	**Value Earnings (%)**
C	2226	9215,6	0	0	0	7651,6	(C-C):C 0
B1	2494	10325,2	1,5	14,5	21,75	8739,4	(A-C):C 11,8
B2	2374	9828,4	1,5	10	15	8249,4	(S-C):C 6,5
B1+B2	2363	9782,8	1,5	24,5	36,75	8182,1	(A+S-C):C 5,8
B3	2128	8809,9	1,0	50	50	7195,9	(P-C):C -4,9
B4	2599	10759,9	1,5	15	22,5	9173,4	(K-C):C 16,5

Table 3 shows that biostimulant B4 (16.5%) has the best return on value, followed by biostimulant B1 (11.8%). It is important to remember that biostimulant B3 has negative value. This finding leads one to the conclusion that it actually decreases medication production rather than increasing it or yielding a higher profit. Applying B3 biostimulant to lavender growing for flowers (dry drogue) may therefore be deemed unnecessary.

In conclusion, B4, or the treatment using the extract of *Ecklonia maxima* species algae, is the biostimulant that produces the best results when lavender is grown for flowers (dry drogue) while simultaneously increasing profit. The formulations containing algae (either the *Ecklonia maxima* species in the case of the B4 biostimulant or the *Ascophyllum nodosum* species in the case of the B1 biostimulant) appear to be the most economically advantageous of all the biostimulant formulations applied to the second-year lavender crop, as they yield the highest production and, consequently, the highest profit in cultivation.

Conclusions

The findings of this study indicate that the most profitable biostimulant formulations for the second-year lavender crop are those that contain algae (either *Ecklonia maxima* species in the case of B4 biostimulant or *Ascophyllum nodosum* species in the case of B1 biostimulant). This is because the algae-containing formulations yield the highest production, which translates into the highest profit in cultivation.

In lavender cultivation, biostimulant B1 is less effective (final product increase of 11.8%) than biostimulant B4 (final product increase of 16.5%). The biostimulants B2 (from plant amino acids, dominantly glutamic and aspartic acid), B1+B2, and B3 (containing readily digestible calcium) follow in descending order of return on value.

Using the previously mentioned biostimulant formulations containing algae appears to have a positive impact on the lavender's yield per hectare. Additionally, it maximizes the lavender crop's overall profit, making it a profitable crop with long-term potential for the Greek economy.

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INTERSPECIFIC HYBRIDIZATION BETWEEN *VICIA FABA* L. AND *VICIA NARBONENSIS* L.: PRESENT STATUS AND FUTURE PROSPECTS

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Abstract

Faba bean is an important annual crop and by far the most economically important species of the genus *Vicia.* Faba beans in developing countries are mainly used for human consumption, because of its high feeding value. Faba beans are of high interest to our country as well, mainly for animal feed. However, there are some problems, concerning the disease resistance, the abiotic stress tolerance, seed quality etc., that should be solved, before faba beans can be cultivated in large areas.The interspecific hybrid between faba beans (*Vicia faba*L.) and one of the closest relatives *V. narbonensis* could improve faba beans breeding, because it is an efficient way to introduce desirable traits to faba beans. Efforts to obtain the interspecific hybrid were attempted by many researchers but they did not result in the successful cross.The double fertilization in both reciprocal crosses was held, but the interspecific embryo was aborted at a very young stage because of post-zygotic barriers. In this case crossing the most compatible genotypes of the two species and the development of an embryo rescue technique for the young embryos of both species was the only way to get this hybrid.Itwas concluded that crossing the F_1 intraspecific *V. narbonensis* hybrids with *V. faba*genotypes or the *V. narbonensis* genotypes with F_1 intraspecific*V. faba* hybrids, and then culturing the pods of a 4-day-old hybrid *V. narbonensis*x *V. faba* in large numbers promised a successful hybrid in the future.

Key words: *fababean, human consumption, stress resistance, hybridization.*

Introduction

Faba beans is an important annual crop and by far the most economically important species of the genus *Vicia.* Faba beans in developing countries are mainly used for human consumption, because of its high feeding value, contributing to food protein. Faba beans (*Vicia faba*L.) is of high interest to our country as well, mainly for animal feed. However, there are some problems that should be solved before faba beans can be cultivated in large areas. Thus, the main targets in faba bean breeding except yield, are disease resistance, abiotic stress tolerance, seed quality and other agronomic traits (Gnanasambandam et al. 2012). *V. narbonensis* is a wild legume, and is one of the closest wild relatives of *Vicia faba* (Cubero, 1982), despite their differences in nuclear DNA content (Kew Science's data and resources, 2017). Furthermore, *V. narbonensis* carries many useful agronomic characteristics, such as disease resistance, winter hardiness, resistance to black aphids (*Aphis fabae*) and chocolate spot disease caused by *Botrytis fabae* (Maalouf et al, 2018), drought resistance and generally resistance to adverse environmental conditions (Lazaridou and Roupakias 1993). In addition, a gene that makes proteins rich in certain amino acids has been transferred to *V. narbonensis* (Pickardt et al., 1995*).* For all these reasons, the interspecific hybrid between the two species could help faba bean breeding (Roupakias, 1986). Efforts to obtain the interspecific hybrid were attempted by many researchers who did not succeed to cross the two species (Caracuta et al., 2016) as it was the case of other legume crops, and tried to identify the causes that did not allow the successful cross (Ramsay et al. 1984).The double fertilization in both reciprocal

crosses was held, but the interspecific embryo was aborted, at a very young stage (Roupakias and Tai 1986). In this case the development of an embryo rescue technique for the young embryos of both species is the only way in order to take this hybrid (Lazaridou and Roupakias 1993).
In this review the various efforts to obtain the interspecific hybrid between *Vicia faba* and *Vicia narbonensis* as well as the future prospects are underlined.

Interspecific hybrids between *Vicia faba* (L.) and *Vicia narbonensis* (L.)

Attempts to cross the two species began in 1974 when Van Cruchten observed that although the pollen of one species was germinated on the stigma of the other in the reciprocal crosses, it could not reach the embryosac. Pickersgillet al. (1983) reported that the fertilization occurred in a very low numbers, especially when *Vicia faba* was used as female parent. Crosses between the two species made by Roupakias and Tai (1986) in a controlled environment resulted in double fertilization in both reciprocal crosses, but the interspecific hybrid embryo sac was aborted few days later, at a very young stage. Thus, it would be useful to identify the causes that did not allow the successful cross between the above mentioned species. In addition, it was reported by Roupakias (1986) that the endosperm development of *Vicia narbonensis* was faster than the respective of *Vicia faba*. The genotypes of the two species used in the reciprocal crosses were Polycarpi (*V. faba*) and cv. A-201 (*V. narbonensis*). It was known from previous reports that there was interspecific variability in the endosperm cell cycle time of the two species *(V. faba* and *V. narbonensis*). In order to obtain the hybrid, someone must find out first if there are more compatible genotypes in either species regarding the growth rate of the endosperm. Since cultivars Polycarpi (*V. faba*) and A-201 (*V. narbonensis*) were used in the reciprocal crosses, a study of the endosperm development of *V. narbonensis* was attempted in order to indentify genotypes of *V. narbonensis* with a slower growth rate than the population A-201. To achieve this goal, seven populations of *V. narbonensis* (A-174, A-194, A-201, A-202, A-203, A-205, and A-206) were studied, in a controlled environment with day-night temperature regime 23^{o}C / 16^{o}C respectively. It was found in this work that there was intraspecific variability in endosperm cell cycle time. Additionally it was found that the populations A-174 and A-202 compared to population A-201 were more compatible with *V. faba* cultivar Polycarpi (Lazaridou and Roupakias, 1993).
In order to obtain the interspecific hybrid between *V. faba* and *V. narbonensis*, Lazaridou and Roupakias (1991) studied the embryo sac development of two varieties of *V. faba* (A-108 and A-151).The plants were grown in a controlled environment with day-night temperature regime of 23^{o}C and 16^{o}C respectively. The results revealed that both species (*V. faba* and *V. narbonensis*) followed the same model of endosperm growth but they were different in the growth rate, which is higher in *V. narbonensis*. The identification of the aforementioned populations of *Vicia narbonensis* (A-202 and A-174), possessing a lower growth rate than the respective of A-201compelled us to look for *V. faba* genotypes with an endosperm growth rate higher than that of Polycarpi. Thus, the growth rate of the embryo sac was studied in seven *V. faba* varieties (A-90, A-58, A151, Polycarpi, Tanagra, A-108, A-107) grown in a controlled environment and differences in endosperm growth rate were revealed between the genotypes studied (Lazaridou and Roupakias, 1993). It was also found that cultivar Tanagra had an endosperm growth rate higher than that of Polycarpi. This indicated that Tanagra could be more compatible with the population of *Vicia narbonensis* compared to Polycarpi. All the aforementioned data give evidence that the most compatible genotypes in terms of endosperm growth rate are A-202 and Tanagra. For this, five different crosses of *Vicia faba* x *V. narbonensis* and the reciprocal ones were done, using genotypes with smaller and larger

differences in endosperm cell cycle time (Lazaridou and Roupakias, 1993).The success of V. *faba* x *V. narbonensis* crosses ranged from 9 to 59% while the reciprocal ones ranged from 12 to 30%. The results showed that the crosses between genotypes of the two species with small differences in endosperm cell cycle time did not result in either higher fertilization rates or larger hybrid embryos. Therefore, in order to obtain the interspecific hybrid a new embryo rescue technique, applicable to young embryos of both species should be developed and this was attempted by Lazaridou et al. 1993.This attempt resulted in the rescue of 4-day-old *V. narbonensis* embryos and 11-day-old *V. faba* embryos. Results support the view that cultivation of pods of the 4-day-old hybrid *V. narbonensis* x *V. faba* in large numbers and the simultaneous isolation of more compatible genotypes in both species promises a successful hybrid production in the future (Lazaridou et al. 1993). It was found that the genotypes of *Vicia narbonensis* A-205, A-211, A-194 and A-175 responded better to embryo culture (Lazaridou et al. 1993) but did not produce higher percentages of successful interspecific crosses with *V. faba*. On the contrary, the A-201 population crossed successfully with *V. faba* but had little response to embryo culture. It was found that the intraspecific hybrids A-201 x A-205, A-201 x A-211, A-201 x A-194 and A-201 x A-175 exhibited a better response to embryo culture compared to their common parent (A-201) and a similar response to the second parent (A-175, A-194, A-205, A- 211; Lazaridou and Roupakias 1995). However, when one of the male parents was used as the maternal plant, the embryo culture response of the hybrid was higher than the one of the best parent. If these hybrids revealed good compatibility with *V. faba*, then they could be successfully used in interspecific crosses with *V. faba.* Subsequently interspecific crosses between *Vicia faba* and *Vicia narbonensis* and interspecific crosses between F_1 *V. faba* and *V. narbonensis* intraspecific hybrids were carried out (Zamani unpublished data, 1997). Four interspecific embryos were obtained, three of them from the cross (A-201 x A-194) x (A-107 x A-90) and one from the cross (A-201 x A194) x Polycarpi. However, only one of them was rescued. Besides, in faba bean, techniques such as *in vitro* culture and genetic manipulation were still very difficult to be applied compared to the same effort in many other crops (Pickardt et al. 2004).

Other researchers tried to obtain the hybrid using protoplast fusion. For this to be done Pickardt et al. (1989) tried to regenerate plants from calluses of *Vicia narbonensis* using *in vitro* culture techniques. Somatic embryos derived from calluses but the produced plants that obtained from this could not reach the maturity. A few years later, Tegeder et al. (1995) studied the regeneration capacity of ten *V. faba* cultivars. Calluses derived from protoplast fusion did not manage to induce embryogenesis but the addition of thidiazuron in the culture medium resulted in shoot development of one *Vicia faba* cultivar (Mythos). An increased content of thidiazuron with the presence of naphthalene acetic acid in culture medium affected positively the regeneration frequency of the species (Tegeder et al. 1995). Regarding the regeneration capacity of *Vicia narbonensis,* Tegeder et al. (1996) reported the positive effect of thidiazuron and the production of mature plants from somatic embryos derived from *Vicia narbonensis* protoplasts. Zenkteler et al. (1998) studied the crossability between *Vicia faba* and *Vicia narbonensis*. Several genotypes were used but only a very small percentage (78/5320) of *Vicia narbonensis* flowers pollinated *in vivo* and *in vitro* produced globular hybrid embryos. In addition only a small proportion (124/3860) of *Vicia faba* flowers, pollinated *in vivo* and *in vitro*, produced globular hybrid embryos. One *Vicia faba* line was more compatible with some *Vicia narbonensis* lines. However, the attempt to obtain the interspecific hybrid between the two species had been unsuccessful, due to disturbances in the cells of embryos and endosperm, noticed at earlier stages (Zenkteler et al. 1998). In order this procedure to become successful, more efforts are required and larger number of plants and crosses have to be involved. It should be mentioned that since 1998, due to many unsuccessful attempts, the breeders have stopped dealing with these interspesific hybrids and turned their interest to other breeding methods. However, later results encourage scientists to devote further research to this plant species known to be very recalcitrant to manipulate *in vitro* (Pickardt et al. 2004).

Conclusion and perspectives

The interspecific hybrid between *Vicia faba* and *Vicia narbonensis* could assist faba beans breeding, because it is an efficient way to introgress desirable traits to faba beans. However post-zygotic barriers may prevent the development of interspecific embryos. The various published results indicate that using more compatible genotypes of the two species in the crosses may give larger embryos that could be cultured *in vitro*. More precisely, the existing results indicate that crossing the F_1 intraspecific *V. narbonensis* hybrids with *V. faba* genotypes or by crossing *V. narbonensis* genotypes with F_1 intraspecific *V. faba* hybrids, and then culturing the pods of 4-day-old hybrids of *V. narbonensis* x *V. faba* in large numbers, could contribute to the production of a successful hybrid in the future.

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INVESTIGATION OF THE EFFECTS OF DOMESTIC WATER BUFFALO, HUNGARIAN RACKA SHEEP AND HUNGARIAN GREY CATTLE GRAZING ON DIFFERENT WOOD-PASTURES IN HUNGARY

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Abstract

Nowadays, the role of nature conservation and animal husbandry has increased drastically due to the effects of climate change. Formerly, wood-pastures were a characteristic farming type in the Pannonian region. In the current work, we studied wood-pastures in the North Hungarian Mountain Range and in the Transdanubian Central Mountain of Hungary. Our purpose was to find out the nature conservation values and grassland management values of wood-pastures grazed by different animals. Among the sample areas of North Hungarian Mountain Range, the sample area of Cserépfalu was grazed by Hungarian Grey Cattle, while the Szurdokpüspöki was grazed by domestic water buffalo. The sample areas of Transdanubian Central Mountain were grazed by water buffalo and Hungarian Racka Sheep. Coenological surveys were between 1994 and 2024 in the main vegetation period according to the method of Braun-Blanquet. We applied the diversity and grassland management value to evaluate the state of the vegetation. Grazing of the studied livestock produced different results among the areas. Based on the results, cattle grazing resulted in a variable, mosaic-like, shrubby area with high cover values, sheep grazing resulted in vegetation with higher grassland management values and buffalo grazing resulted in a variable and nutrient rich area with optimal diversity and grassland management values. Hence, grazing by cattle provides adequate solution to create and conserve wood-pasture habitats. However, grazing by sheep forms valuable grassland with better grassland management values. Nevertheless, grazing by buffalo is probably proper for nature conservation and grassland management values as well.

Keywords: *grazing, nature conservation, Pignatti life form, ruminant.*

Introduction

Wood pastures are one of the oldest land-use types in Europe, where livestock graze in mosaic habitats characterised by grasslands with different tree and shrub species. Over the centuries, wood pastures have been important traditional elements in Carpathian-basin as one of the dominant farming types of the country (Moreno *et al*., 2018; Burgess and Rosati, 2018) (Figure 1). The importance of wood-pasture habitats has increased significantly because of the current global climate change issues, as shrinking grasslands lead to potential for animal husbandry in areas that were formerly not considered of high management relevance.

Öllerer *et al.* (2019) provided a complex review of the effects of domestic livestock grazing on temperate forest vegetation. They concluded that successful wood-pasture conservation depends on the choice of grazing animal species and that the lack of grazing can negatively affect biodiversity and forest management. It needs to be taken into account when treating wood-pastures as semi-natural habitats (Bernes *et al.*, 2018; Burrascano *et al.*, 2013).

Figure 1. Location of Hungary (Carpathian-basin) in the map of Europe

Large herbivores have essential role in the formation of forests, shrublands and grasslands (Mitchell, 2005). Therefore, grazing livestock can contribute to the current forest-grassland mosaic (Varga *et al.*, 2020). Different native and introduced livestock such as cattle, sheep and horses can provide a substitute for wildlife activity (Póti *et al.*, 2007). The grazing type influences the vegetation structure and the yield of grassland (Naveh, and Whittaker, 1980, Török *et al.*, 2018).
There were also some investigations on the effects of grazing animal choice in Hungary. Most of the research confirms the traditional nature conservation management that cattle grazing is suitable for habitat conservation (Török *et al.*, 2014; Turcsányi-Járdi *et al.*, 2022; Penksza *et al.*, 2022). Penksza *et al.* (2024) confirmed that grey cattle grazing is a suitable practice for wood-pasture conservation. However, in this study, it was also reported that sheep also have similar grazing characteristics in mountain wood-pasture conditions.
In addition, Fűrész *et al.* (2023) studied a domestic water buffalo grazed shrubland in a mountain with a similar feature to wood-pasture. In this study found that the domestic water buffalo could be a more effective animal for habitat formation and conservation than the grey cattle because it has better digestibility (Mihailou and Massaro, 2021; Escarcha *et al.*, 2018; Warriach *et al.*, 2015).
In the current work, our aim was to study a wood-pasture from Transdanubian Central Mountain (Balatoncsicsó), which is grazed by sheep and domestic water buffalo.

Materials and methods

Data collection and surveyed areas

In the present study, authors took into account two previous studies conducted in the North Hungarian Mountain Range, which focused on the effects of domestic water buffalo, grey cattle and sheep grazing (Fűrész *et al.*, 2023; Penksza *et al.*, 2024). The current work was carried out in Balatoncsicsó (Figure 2) which belongs to the Transdanubian Central Mountain of Hungary where grazing is by domestic water buffalo and sheep. Authors conducted ten

Figure 2. Wood-pasture sample area in Balatoncsicsó

coenological surveys of the woody sample areas, ten in the grassland sample areas in 1994, 2009 and 2024 and ten in the control, untreated grassland sample area in 2024 in the vegetation period (May and June) based on the Braun-Blanquet method (1964) in 2 × 2 m quadrats. The coverage was estimated by percentage for each present species. The name of the species was recorded based on the nomenclature of Király (2009).

Statistical analysis

To evaluate data, the non-parametric statistical method was used to analyze the cover values of species of different sample areas, as these variables were not normally distributed according to the Shapiro–Wilk test ($p < 0.05$). Accordingly, the non-parametric Kruskal–Wallis test (= 0.05) was used, and the non-parametric Dunn’s test with Bonferroni correction was used for multiple pairwise comparisons (Addinsoft XSTAT, 2016).

Results and discussion

Based on the results (Figure 3), it can be seen that the wood-pasture sample areas in Balatoncsicsó have different grazing impacts in terms of changes in cover values.
Compared to the cover results of the control, untreated area, no significant results were found for several sample areas. The results of the control area were significantly different only from the data of the grassland coverage of the survey in 2009. The results of woody areas clearly show that there has been no significant change over 30 years, although the records from 1994 show less similarity with the other records. The results of grassland areas unequivocally show that there has been no significant change over 30 years, however, the records from 2009 show more similarity with the other data. The results of the woody sample area survey from 1994 were statistically significantly different from the mean cover values of grassland. Whereas the mean cover value from 2009 grassland area was significantly different from the cover values of the control area and the woody sample area. The mean cover of the control area was most similar to the data from under the trees of 2009 and 2024.

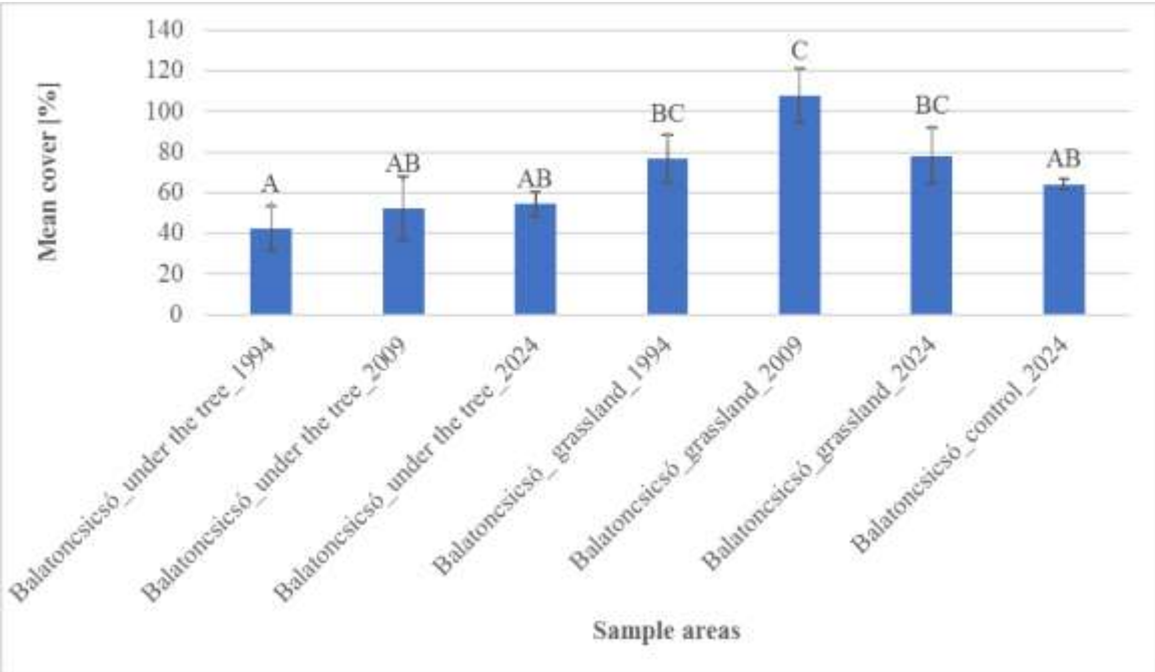

Figure 3. Cover (mean, standard deviation) in the sample areas. Based on the Kruskal–Wallis and Dunn's post hoc tests with Bonferroni correction. Bonferroni-corrected significance level: 0.005. Stand-alone letters indicate homogeneous groups (A or B or C) that significantly differ from each other. When heterogeneous groups have at least two different letters, they are not significantly different from groups with a common letter.

Conclusion

The literature review highlights the outstanding nature conservation and farming values of wood-pastures. To sustain wood-pasture, grazing is essential, but it requires the adequate choice of animal. Preliminary studies have confirmed that cattle are suitable for wood-pasture habitat conservation and biodiversity maintenance, while sheep grazing produces similar vegetation with more beneficial grassland management value. In addition, domestic water buffalo grazing, which has not been practiced in dry grasslands before, may be a more appropriate method to conserve habitat, maintain biodiversity and increase the proportion of plants with high grassland management value.

Consequently, based on the literature reviewed and the results described, it can be concluded clearly that cattle, sheep and domestic water buffalo are capable of sustaining and conserving wood-pasture biodiversity.

Nevertheless, the present work has only investigated the mean cover values, and in the future, it would be useful to carry out a Pignatti life-form data processing and the grassland management values of this sample area to obtain a more complex overview of the impact of grazing.

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BIOLOGICAL DIVERSITY OF *SPINACIA OLERACEA* L. ACCESSIONS COLLECTED IN IRAN USING SOME MORPHOLOGICAL TRAITS

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Abstract

The spinach *(Spinacia oleracea* L.) is an important crop in most regions of the world which is native to Iran. For evaluation of its biological diversity, sixteen landraces collected from different geographical regions of Iran and some quantitative traits remeasured as leaf length (LL), leaf width (LW), petiole length (PL), petiole diameter (PD), leaf area (LA), leaf numbers in flowering (LN), days to flowering (DF), female plants percent (FP), fresh yield (FY) and dry yield (DY). The landraces showed high variability in the morphological characteristics according to analysis of variance. Accession Arak had the high amounts of dry yield, fresh yield, leaf area and leaf numbers and regarding the importance of yield and low properties of petiole length and diameter, accession Arak followed by accessions Karaj and Birjand were identified Dendrogram analysis of a cluster constructed from genotypic distance matrices showed that sixteen landraces could be grouped into three clusters. The number of clusters was confirmed by multivariate analysis using the Wilks lambda statistic test of variance. Accessions Arak, Karaj, Rahnan, Birjand and Sirjan were grouped in cluster-I, accessions Ardestan, Zabol, Bojnord, Esfahan and Shiraz were grouped in cluster-II, accessions Brojerd, Drood, Langrood, Maragheh, Salmas and Tabriz were grouped in cluster-III. The accessions the cluster-I were useful for yield performance while the accessions the cluster-III were useful for improving the quality of spinach.

Keywords: *biological diversity, morphological variation, multivariate analysis.*

Introduction

The spinach *(Spinacia oleracea* L.) is an edible, annual vegetable plant that grows quickly and can survive mild winters. It is native to Southwest Asia and is generally thought to have originated in Iran (Swiader and Ware 2002) and was first mentioned by the Chinese as a Persian herb. It was first cultivated in North Africa, reached Northern Europe through Spain, was documented in Germany, and then was a common garden plant in England and France before 1500 (Decoteau 2000). It is versatile, used as a salad, as a cooked vegetable or as part of many other cooked meat and vegetable dishes which is rich in beta-carotene and folate and is also a good source of vitamin C, calcium, iron phosphorus, sodium and potassium. Spinach, as a dioecious species of male and female plants, is an herbaceous leafy vegetable of the Amaranthaceae family and its leaves are alternate, simple, oval to lanceolate, with larger leaves at the base of the plant and smaller leaves higher on the flowering stem.

Since Iran is the center of genetic diversity of many crops such as wheat, alfalfa, spinach, etc., it is important to conserve these important natural resources. Most *Spinacia oleracea* L. species are cultivars that are highly adapted to specific environmental conditions and are useful sources of genetic variation. However, exploiting the genetic potential of different germplasms requires detailed knowledge of their gene collections, including characterization, evaluation and classification (Lopez-Velasco et al. 2013). Multivariate procedures are useful for characterizing, evaluating, and classifying germplasm collections when a large number of accessions must be evaluated for multiple traits. The usefulness of multivariate methods in dealing with morphological variability in plant genetic resources has been demonstrated for many plants. The aim of this study was to determine the distribution structure of morphological variation for some traits in a collection of 16 original Iranian *Spinacia oleracea* L. germplasm taken from a wide geographical area of Iran and to identify groups with similar traits.

Table 1. Geographical properties of the 16 locations which spinach landraces are collected

No.	Name	Longitude	Latitude	Altitude (meter)	No.	Name	Longitude	Latitude	Altitude (meter)
1	Arak	49° 41′ E	34° 05′ N	1755	9	Langrood	50° 14 ′ E	37° 19′ N	-25
2	Ardestan	52° 22′ E	33° 23′ N	1205	10	Maragheh	46° 16 ′ E	37° 21′ N	1477
3	Birjand	59° 21 ′ E	32° 87′ N	1491	11	Rahnan	51° 36 ′ E	32° 41′ N	1545
4	Bojnord	57° 19 ′ E	37° 28′ N	1070	12	Salmas	44° 76 ′ E	36° 19′ N	1398
5	Brojerd	48° 45 ′ E	33° 53′ N	1580	13	Shiraz	52° 22 ′ E	29° 37′ N	1540
6	Drood	48° 70 ′ E	33° 40′ N	1326	14	Sirjan	55° 40 ′ E	29° 27′ N	1735
7	Esfahan	52° 02´ E	32° 32´ N	1525	15	Tabriz	46° 18 ′ E	38° 04′ N	1366
8	Karaj	50° 97 ′ E	35° 82′ N	1300	16	Zabol	61° 29 ′ E	31° 01′ N	475

Materials and methods

Sixteen native Iranian *Spinacia oleracea* L. germplasm collections were collected and evaluated in the field in a randomized complete block design with replicated four repetitions. The geographical properties of the 16 sites of the collected *Spinacia oleracea* L. landraces are given in Table 1. Sowing was done manually in plots contained six rows and plot size was 4.5 m^2. Control by hand weeding was carried out twice when the weed density was high, in the pre-flowering and post-flowering stages. Several quantitative traits consist on leaf length (LL), leaf width (LW), petiole length (PL), petiole diameter (PD), leaf area (LA), leaf numbers in flowering (LN), days to flowering (DF), female plants percent (FP), fresh yield (FY) and dry yield (DY) were measured based on descriptors developed by Bioversity International. The obtained datasets were first tested for normality by Anderson and Darling normality test and analysis of variance was performed to evaluate differences among measured traits. The 16 *Spinacia oleracea* L. accessions were clustered via the measure of dissimilarity was Euclidean distance and the clustering method was un-weighted pair group method using Ward method. The number of clusters was determined using multivariate analysis of variance through Wilks' lambda statistics via SPSS.

Table 2. The quantitative characteristics of 16 clusters of spinach landraces.

	DF	DY	FP	FY	LA	LN	LL	LW	PD	PL
Arak	178.7	3455.7	57.2	38251.0	71.8	20.5	11.4	7.4	12.9	8.2
Ardestan	176.4	2237.7	67.9	25573.2	64.4	17.7	11.1	8.0	10.5	10.4
Birjand	178.8	3402.5	51.2	34826.7	61.1	20.3	11.0	7.2	12.3	10.0
Bojnord	169.0	2160.9	56.4	21936.3	55.2	17.4	10.5	6.6	10.9	8.6
Brojerd	160.4	1433.4	57.2	13767.1	50.1	16.6	10.5	5.9	10.6	8.3
Drood	177.8	1623.8	54.9	15552.0	36.9	12.9	8.5	6.1	10.0	7.4
Esfahan	179.6	2802.8	67.9	28885.5	72.4	18.9	13.1	6.4	13.4	9.2
Karaj	174.3	3325.8	48.6	37154.2	79.8	21.0	11.2	8.6	12.4	10.3

Langrood	170.1	1021.8	56.0	9616.4	33.3	17.5	7.1	4.9	7.9	8.5
Maragheh	155.1	1164.8	62.7	11327.2	50.2	19.2	11.3	5.8	11.2	8.0
Rahnan	179.2	3575.9	64.4	36272.6	63.3	17.9	11.6	6.4	13.5	9.5
Salmas	158.0	764.4	55.8	7825.0	25.3	15.0	7.6	3.9	9.1	5.8
Shiraz	179.6	2865.9	65.4	30065.6	73.5	18.2	13.2	6.9	13.2	8.7
Sirjan	178.0	3258.2	60.8	33287.6	62.8	18.9	11.4	6.6	12.8	9.9
Tabriz	154.0	1610.7	48.3	16572.5	40.5	14.7	9.3	5.2	11.0	7.5
Zabol	173.5	2418.1	58.7	24546.5	61.6	18.2	11.2	7.4	11.0	9.4

LL, Leaf length (cm); LW, Leaf width (cm); PL, Petiole length (cm); PD, Petiole diameter (mm); LA, Leaf area (cm^2); LN, Leaf numbers in flowering; DF, Days to flowering; FP, Female plants percent; FY, Fresh yield (kg ha^{-1}); DY, Dry yield (kg ha^{-1}).

Results and discussion

Accession Arak had the high amounts of dry yield, fresh yield, leaf area and leaf numbers in flowering while Ardestan accession showed the largest values for female plants percent, petiole length, and leaf width (Table 1). Accession Birjand had the high magnitudes of petiole length, dry yield, and leaf numbers in flowering while Rahnan accession indicated the high mean for petiole diameter, days to flowering fresh yield and dry yield. Finally, accession Shiraz was better in days to flowering, leaf area, leaf length and petiole diameter. However, the other accessions consist on Bojnord, Brojerd, Drood, Langrood, Maragheh, Salmas, Sirjan, Tabriz, Zabol were not the best in any of the measured traits. Regarding the importance of dry yield and decreeing the magnitudes of petiole length and petiole diameter for remove the high amount of nitrite content (Shi et al. 2016) as well as days to flowering to cover early maturing, accession Arak followed by accessions Karaj and Birjand were the best ones.

Agglomerative hierarchical clustering methods use the elements of a proximity matrix to generate a tree diagram or dendrogram. The dendrogram generated from accessions distance matrices showed to clear grouping pattern (Fig. 1). In a distance of cutoff point, the examined 16 spinach accessions could be agglomerated into three clusters. The number of clusters was verified by multivariate analysis of variance test through Wilks' Lambda statistics (data not shown). In cluster-I, accessions Arak, Karaj, Rahnan, Birjand and Sirjan were grouped with each other, thus they are similar in the measured traits while in cluster-II, accessions Ardestan, Zabol, Bojnord, Esfahan and Shiraz were grouped with each other, thus they are similar in the measured traits. Finally, in cluster-III, accessions Brojerd, Drood, Langrood, Maragheh, Salmas and Tabriz were grouped with each other, thus they are similar in the measured traits. Regarding the most favorable accessions (Arak followed by Karaj and Birjand), it seems that the all of the accessions of cluster-I are useful for economic targets. The long petiole length is essential for machinery harvesting and genetic improving for having long petiole length is one of the breeding targets of spinach (Sabaghnia et al. 2014). Also, the relative length of petiole is a commercial factor for the producing of spinach canner. The small petiole diameters were observed in the Langrood and Salmas were located in the cluster-III while the short petiole lengths were found in the Salmas, Tabriz and Drood were located in the cluster-III, thus the accessions of this cluster can be used for improving the quality of spinach.

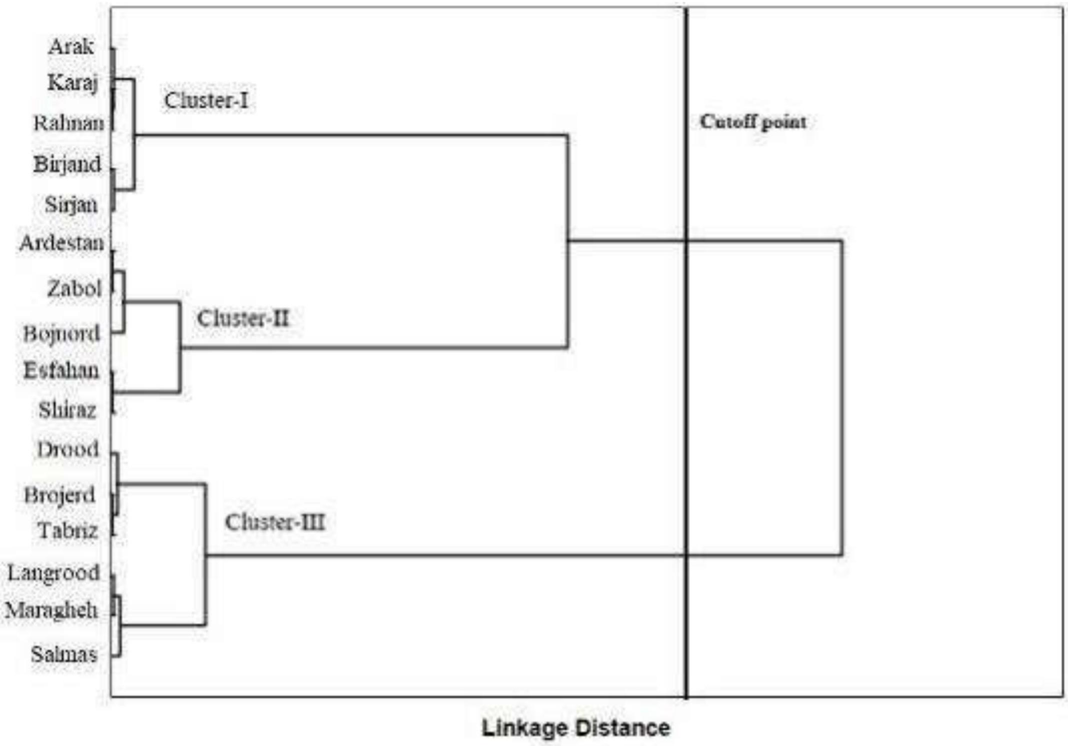

Fig. 1. Hierarchical cluster analysis of the 16 spinach genotypes based on Ward's method

Conclusion

A total of 16 *Spinacia oleracea* L. landraces were collected from different geographical regions of Iran which provided morphological data for the landraces. The dendrogram of cluster analysis for the dataset showed three groups. This investigation provided suitable information that may be useful to breeders who wish to find the most distinct spinach landraces. In conclusion, it was seen that characterization of spinach landraces based on the morphological traits was suitable to assess the diversity among collected spinach landraces. However, a high variability was observed for most measured traits and obtaining more diverse collections especially exotic germplasm is not needed for future preservation of *Spinacia oleracea* L.

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BIOLOGICAL VARIATION OF SOME GARDEN CRESS (*LEPIDIUM SATIVUM* L.) ACCESSIONS

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Abstract

Lepidium sativum L. is one of native vegetable crops from origin of Iran which is a fast-growing edible herb. A study was conducted using 81 genotypes (77 accessions of genebank Department of Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany and four local varieties from different geographical areas of Iran) which were evaluated for phenological and plant structural traits. The genotype× trait (GT) biplot analysis was applied and shoed that the first two principal components of the site regression model accounted for 70% of the total interaction. The vertex accessions were G23, G59, G61, G51, G6 and G12 while important traits consist on seed and biological yield were favorable in sectors of G32 and G12 and these vertex accessions were fell in same sectors. Based on seed yield performance, accession G6 was the best genotype, followed by accessions G29, G32, G48 and G50. The results showed that the studied germplasm may be an important material for improving yield and some yield components which can be used to design efficient breeding strategies in *Lepidium sativum*. By applying GT biplot to our garden cress trials, interrelationships among the garden cress traits were clearly shown, providing more information on these relationships Our results indicated that biological diversity of *Lepidium sativum* L., may be an important source for improving some components such as number of siliques per plant, number of seeds of silique per plant and 1000-seed weight.

Keywords: *accessions, biological variation, germplasm, traits association.*

Introduction

Garden cress (*Lepidium sativum* L.) is an annual herbaceous from Iran origin, fast-growing and edible plant belonging to the Brassicaceae family. It is a plant from Southwest Asia and probably Iran that spread around the world centuries ago. The seeds and leaves are economically important; However, the crop is mostly grown for seed. It is an important green vegetable plant eaten by humans, mostly as a leafy vegetable (Sabaghnia et al. 2015). However, leaves of garden cress are mostly eaten raw in salads, but they have also been cooked with vegetable curries and used as a garnish in some centuries, for example in India (Wadhwa et al., 2012). Garden cress is a cool season annual that is grown as a salad around the world for its long leaves at the base of the stem and small bright green feathers. It can be harvested throughout the year, either indoors or outdoors, and grows abundantly in well-cultivated and well-drained soils (Sharma and Agarwal, 2011). Garden cress grows quickly and can be harvested at seed in many areas; and needs moist soil and some shade in the summer, so that the heat does not flow directly to the seeds. Little is known about the trait relationships between garden cress genotypes, so this study aimed to investigate the biologic diversity of garden cress and reveal their interrelationships and compare genotypes based on multiple traits using GGE biplot methodology that may be candidates for future programs.

Material and methods

A collection of 81 accessions, while 77 accessions were obtained from the Genome Division of the Leibniz Institute for Plant Genetics and Plant Research (IPK) in Gatersleben, Germany, and four of them were Iranian accessions named Birjand, Tabriz, Kerman and Shiraz in Iran, collected. from different geographical regions of Iran. each plot used a 9 × 9 simple grid design with four replicates, consisting of six rows spaced 0.30 m apart, 2 m long, and a plot size of 3.6 m2. Sowing was done in the first week of May, which is the optimal time for sowing garden cress. The following plant traits were at 10 random competing points per plot: height of first branch (HFB), height of first silique (HFS), length of main axis (MAL), number of lateral branches (NLB), number of cylinders per lateral branch (NSL), number of wings on main axis (NSM), number of wings per plant (NSP), number of seeds per lateral branch slice (SLB), number of seeds per main axis silique (SMA), number of silique seeds per plant (NSSP) and plant height (PH). Where possible, days to germination (DE), percent emergence (EP), days to flowering (DF), flowering period (FP) and days to maturity (DM) were also recorded. Flowering days were recorded when 50% of the plants in the experimental area had at least one open flower. A subsample of seeds harvested from each plot was measured for thousand-seed weight (TSW). Four intermediate rows were harvested to determine biological yield and seed yield. The GT biplot method was used to show genotype per trait through the first two principal components retained in the model, which is generally best for extracting structure and rejecting noise from the data. All biplots were generated using GGEbiplot software (Yan, 2001).

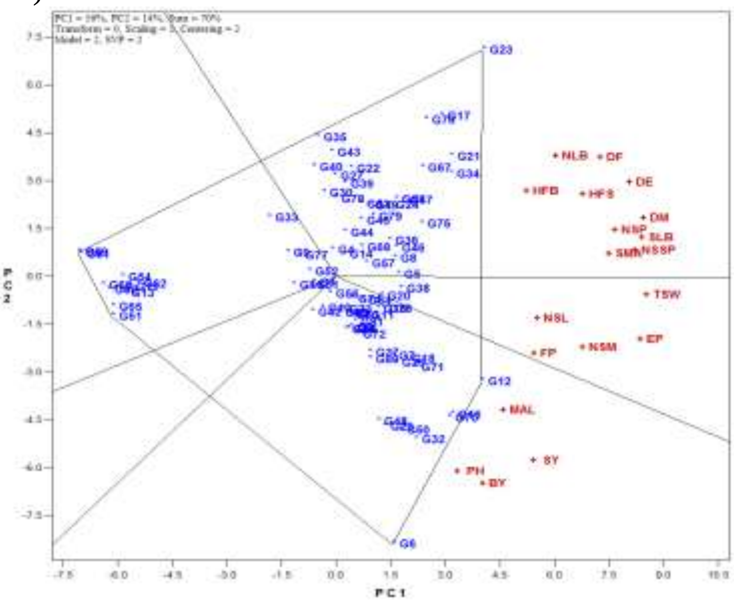

Fig. 1. Polygon-view of GT biplot representing the "which-won-where pattern of traits and genotypes.

Results and Discussion

There were highly significant differences among accessions of *Lepidium sativum* L. and the GT biplot explained 70 of the total variation of the standardized data. This percentage reflects the complexity of the relationships among the measured traits of *Lepidium sativum* L. The fundamental structures among the traits should be captured by the biplots (Sabaghnia et al. 2015). The polygon-view of the GT biplot is the best way to visualize the structure of relations between genotypes and traits. Fig. 1 represents the mentioned biplot generated from data on morphologic traits of *Lepidium sativum* L. accessions in experimental. The following information can be seen from this figure are: the vertex accessions are G23, G59, G61, G51, G6 and G12, and the trait fell into the sectors of G23 and G12. Therefore, accession G12 had the highest values of PH, MAL, SY and BY traits; and accession G23 had the highest values of the other remained traits. Therefore, there are two vertex accessions which were favorable for some traits but the rest of them (four vertex accessions) were not favorable for any *Lepidium sativum* L. traits. In the testing of different interactions for significance, one of the common practices is the tendency to apply multiple mean comparisons while these comparisons do not give extra information about the nature of interactions such as positive or negative interactions. Therefore, the GT biplot procedure, gives the same information as ANOVA as well as additional information of multiple mean comparisons (Bedassa et al. 2013). However, accessions G12, G20, G23, G32 and G53 as the vertex accessions were the most favorable accessions in the most important studied traits of *Lepidium sativum* L. On the other hand, GT biplot explained most of the variation (due to 81 *Lepidium sativum* L. genotypes main effects plus genotype × trait (GT) interaction effects. It can be said that almost all information contained ANOVA and multiple comparisons of genotype means is graphically displayed in a GT biplot. Sabaghnia et al. (2015) in *Lepidium sativum* L., also reported similar findings in the studying of effects of genotype, environment, treatment, trait and their interactions.

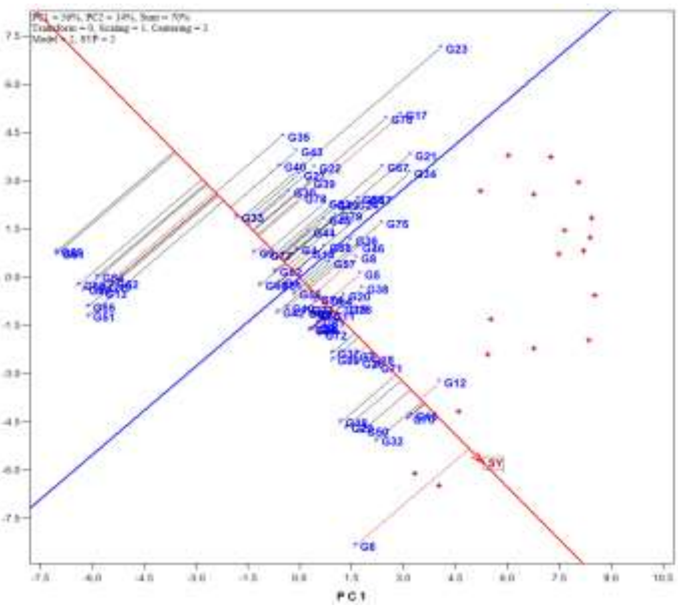

Fig. 2. The GT biplot representing ranking of genotypes based on seed yield.

For accession evaluation based on individual trait which is allows ranking of accessions based on a single trait, the seed yield trait is selected. A line as trait axis is drawn that passes through the biplot origin and the marker of the trait, and the precise position of the marker is at its left end, and the arrow on the selected axis points to increasing trait value. A perpendicular line is drawn that also passes through the origin and is perpendicular to the trait axis. Based on seed yield in year 2012 (Fig. 2) accession G6 is the best genotype, followed by genotypes G29, G32, G48 and G50. All these genotypes had above-average seed yield. Seed yield is forms via number of siliques per plant, number of seeds of silique per plant and 1000-seed weight of *Lepidium sativum* L., and biological diversity in its accessions is very important breeding objectives. Although the GGE biplot was proposed for the analysis of experiments involving multiple environments, it is applicable to any bidirectional set, such as a genotype × trait bidirectional data set. Different features have different units, and the units must be eliminated by standardization before biplot analysis. There is no doubt that the biplot of the first two principal components is more effective than conventional analysis methods if their calculated variance was appropriate. In addition, a biplot shows a graphical representation of the genotype × trait bidirectional pattern, and visualization of the data set is easy with GGEbiplot (Yan and Rajcan, 2002). The two-plot shows a complete picture of the relationships between genotypes and traits. For the above reasons, we recommend using the GGE biplot model to analyze the garden cress dataset. Similar reports showed that GT biplots were an excellent tool for visualizing genotype × trait data and revealing relationships between traits.

Conclusion

Our results indicated that biological diversity of *Lepidium sativum* L., may be an important source for improving some components such as number of siliques per plant, number of seeds of silique per plant and 1000-seed weight. By applying GT biplot to our garden cress trials, interrelationships among the garden cress traits were clearly shown, providing more information on these relationships. Given the importance of biological diversity regarding to seed yield, the high yielding accessions will need to be selected in *Lepidium sativum* L. programs.

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GRAPHIC ANALYSIS OF GENOTYPE BY TRAIT INTERACTION IN SAFFLOWER USING BIPLOT METHOD

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Abstract

Safflower is a neglected crop with many traditional and industrial uses form high quality edible oil to cheaper substitute for saffron. In this study, 81 safflower genotypes were evaluated in a 9×9 simple lattice design for number of plants per plot (NPP), plant height (PH), height of the first lateral branch (HFL), height of the first lateral capitulum (HFC), stem diameter (SD), number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), number of seeds per lateral capitulum (SLC), seed yield (SY) and thousand seed weight (TSW). The genotype by trait (G×T) interaction biplot model was used to indicate the pattern of G×T interaction two-way interaction data in a graph and showed that the first principal component effect explained 49%, and the second principal component, 24%, of the total mean squares interaction. The vector-view displayed that NCP with NMB, and SMC with SY were positively associated while there was a negative association between HFC with TSW, and between NLB with NPP. The polygon-view graph is divided into eleven sectors, and the sector of genotype G80 was winner for most traits. Genotype G58 followed by genotypes G30, G33 and G72, were the most favorable genotypes in the regard of SY while regarding this trait as a reference, SMC was identified as most related trait which is followed by SLC, SD. Applying G×T biplot to the safflower multiple trait data demonstrated that this model graphically displayed the interrelationships among seed yield with number of seeds per main and lateral capitula followed by number of capitula per plant and thousand seed weight, and facilitated visual genotype comparisons and selection. It was found that selection for seed yield alone was not only dependent to number of seeds per main and lateral capitula, but also related to the other traits in safflower breeding.

Keywords: *Yield components, seed yield, principal component analysis.*

Introduction

Safflower (*Carthamus tinctorius* L.) as an oilseed crop from Asteraceae family is a diploid (2n=24) annual herbaceous crop, is originated in the Middle East and cultivated since thousands years ago. The genus *Carthamus* has 25 species and is well adapted to hot and dry climate conditions of arid and semi-arid environments while only C. tinctorius is cultivated as crop worldwide (Rathnakumar and Sujatha, 2022). However, safflower is a drought tolerant crop and can be well adopted in rain-fed agriculture system. Kazakhstan is the largest safflower producer (35%), following to Russian Federation (24%) and United States (10%) in the world with the highest acreage (43%) to Russian Federation (28%) and United States (6%) and seed yield is for the vegetable oil market (FAOSTAT, 2021). According to statistics of FAOSTAT (2021), the highest mean yield performance is belonging to United States (5463 kg ha^{-1}), India (4647 kg ha^{-1}) and Mexico (3110 kg ha^{-1}). Safflower is cultivated on 3700 hectares in Iran, average yielding 1320 kg ha^{-1} and quantity 4889 ton (FAOSTAT, 2021). Regarding the global average seed yield of safflower (1600 kg ha^{-1}), the performance of this

crop is low (about 20% lower) in Iran, and considering the highest mean yield in the world, is very low (about 75% lower), thus it must be improved through a strong breeding program.
Safflower is a good valuable crop which contains flavonoids, lignins, triterpene alcohols and polysaccharides as well as anticoagulant, antioxidant and neuroprotective (Adeleke and Babalola, 2020). It is rich in magnitudes of oleic acid as well as linoleic acid and is very similar to olive oil, and is considered as one of the most ancient important oilseed crops worldwide (Joshan *et al.,* 2019). The press cake or oil cake after industrial oil extraction has good protein content that is used as animal feed while its seed oil yield is about 40% similar to sunflower and olive oil yield performance. Safflower is a multipurpose crop and its products are used as bird feed seed, medicinal plant with special uses, livestock forage, instead of saffron in foods, teabags, or as ornamental cut flowers. In most plant breeding programs, the germplasm is measured to identify the most important lines and the efficiency of a selection project depends on the magnitudes of genetic diversity. Therefore, the genetic variation is evaluated through measuring some characteristics like morphological traits (Gholami *et al.*, 2018). Safflower shows high genetic variation across various environments of the world and there is only a limited researches assessing its genetic variation according to agronomic as well as morphological traits.
Khan *et al.* (2009) assessed genetic variation for 193 safflower accessions from forty countries and eight geographical regions and showed that cluster analysis as well as and principal component analysis demonstrated wide genetic diversity in germplasm and a considerable potential for improving this crop for both agronomic and quality traits. Shinwari *et al.* (2014) evaluated 122 safflower genotypes collected from various eco-geographical regions of the world and indicated that the largest variation was belonged for number of capsules per plant, number of seeds per capsule, seed yield per plant, plant height, days to flowering initiation and days to maturity. They showed that principal component analysis displayed that the traits that mainly distinguish the germplasm were, capsule diameter, number of capsules per plant, number of seeds per capsule, days to maturity, plant height and time of flowering. Gholami *et al.* (2018), studied sixty-four safflower genotypes based on twenty traits and reported high variation for the number of wizened seeds per lateral capitulum, seed weight of lateral capitulum, number of lateral branches, number of capitula per lateral branches, diameter of main capitulum, yield of single plant, thousand seed weight and seed yield. They categorized these safflower genotypes into five clusters but they are not separated based on their geographical origins. This study encompassing 81 various germplasm of safflower from various agro-climatic regions of the world using some of agronomic and morphologic traits will add good information for knowledge of safflower breeders and aid them in future projects.

Material and Methods

In growing season 2021, 81 safflower genotypes from different eco-geographical were cultivated in experimental field in University of Maragheh (37°23′N; 46°14′E), where average rainfall is 322 mm with 12.5 °C annual temperature. It is a highland semiarid zone and the soil texture was sandy loam type with 1.6% organic matter. The trial was carried out in a 9×9 simple lattice design and the size of each experimental plots was 1.0 × 2.0 m^2 with 4 lines per genotype and row width 0.25 m. Hand drill sowing following to thinning was carried out and crop was grown under rainfed circumstances with two supplemental irrigations in the reproductive stage. Number of plants per plot (NPP), plant height (PH), height of the first lateral branch (HFL), height of the first lateral capitulum (HFC), stem diameter (SD), number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), and number of seeds per lateral capitulum (SLC) were measured from ten randomly selected plants from each plant based on safflower (*Carthamus tinctorius* L.) Standard Descriptor of International Board of Plant Genetic Resources (IBPGR), FAO, Rome, Italy. Seed yield (SY) was recorded at physiological maturity and adjusted to 12.5% seed moisture content and then thousand seed weight (TSW) was measured using three random sub-samples of the seed yield from each plot. The dataset was tested for normality by the Anderson and Darling normality test using Minitab version 14.0 (2005) statistical software and phenotypic linear correlation coefficients were calculated for all possible comparisons using the Pearson correlation coefficient to all the measured traits. The genotype by trait (G×T) interaction biplot model (Yan and Frégeau-Reid, 2018) was used to indicate the pattern of G×T interaction two-way interaction data in a graph based on the following formula:

$$\frac{Y_{ij}-\mu_j}{\sigma_j}=\sum_{n=1}^{2}\lambda_n\xi_{in}\eta_{jn}+\varepsilon_{ij}=\sum_{n=1}^{2}\xi_{in}^{*}\eta_{jn}^{*}+\varepsilon_{ij}$$

where Y_{ij} is the mean of genotype i for trait j, μ_j is the mean of all genotypes in trait j, σ_j is the standard deviation of trait j among genotypes, λ_n is the singular value for principal component n (PC_n), ξ_{in} and η_{jn} are scores for genotype i and trait j on PC_n, respectively, and ε_{ij} is the residual value of model related with genotype i in trait j. To obtain symmetric scaling in the scores of the genotypes and traits, the singular value λ_n has to be absorbed by their singular vectors (ξ_{in} and η_{jn}) as the adjusted ones; $\xi_{in}^{*}=\sqrt{\lambda_n}\xi_{in}$ and $\eta_{jn}^{*}=\sqrt{\lambda_n}\eta$. The G×T interaction biplot graphs are drawn by plotting the symmetric scaled scores of the genotypes as well as traits, so that each genotype (entry) or trait (tester) is shown by a special marker in each generated graph. For more details about G×T interaction biplot model and other types and applications of biplots for two-way structures, see Yan and Frégeau-Reid (2018). The ordinary statistical analyses were carried out using IBM-SPSS version 23.0 (IBM-SPSS, 2015) and G×T interaction biplot graphs were generated by GGEbiplot software (Yan, 2001).

Results and Discussion

The genotype by trait (G×T) interaction biplot model indicates that the first principal component effect explained 49%, and the second principal component, 24%, of the total mean squares interaction. The G×T biplot model can evaluate and explore the nature and structure of genotype by trait interaction effect, effectively. Results of analysis of variance is a perquisite option to decide whether a trait has to proceed for t the G×T biplot model or not (Yan *et al.*, 2011), thus in case of significant differences between interaction of main factors i.e. genotypes and traits, one should go for the G×T biplot model while in case of non-significant interaction use of the G×T biplot model is of no use. Analysis of variance for various traits of safflower (results are not shown) verified to proceed for the G×T biplot model as there were highly significant variations among genotypes and traits effects for all safflower genotypes. The vector-view of the G×T biplot model (Fig. 1A), displays the interrelationships among measured traits of safflower whereas the rays connecting the traits to the biplot origin are showed as trait vectors and the associations between traits are estimated by the cosine of the angle between their vectors. Two traits are associated positively if the angle between their vectors is less than 90°, associated negatively if the angle is more than 90°, independent if the angle is 90°, whereas, traits with longer vectors are significant and more responsive while traits with shorter vectors are less significant and responsive as well as those located at the graph origin are not correlated with the other traits or not-responsive (Yan and Fregeau-Reid, 2018). Fig. 1A, shows that the number of capitula per plant (NCP) and number of main branches per plant (NMB) were positively associated as indicated by the small acute angles between their vectors. Also, number of seeds per main capitulum (SMC) and seed yield (SY) were positively correlated as shown by the small acute angles between their vectors. On the other hand, there was a negative association between height of the first lateral capitulum (HFC) with thousand seeds weight (TSW), and between number of lateral branches per plant (NLB) with number of plants per plot (NPP) as shown by the large obtuse angles between their vectors (Fig. 1A). Also, there was a near zero association between TSW with NPP, between HFC with NPP, and between PH with NLB because their near perpendicular vectors. Most of above results are good consistent with those reported by Pearson’s correlation matrix (Table 1), whereas the predictions of the G×T biplot model for traits’ associations can be confirmed from this matrix but some are not consistent because the model described only 73% rather than 100% of the observed mean squares.

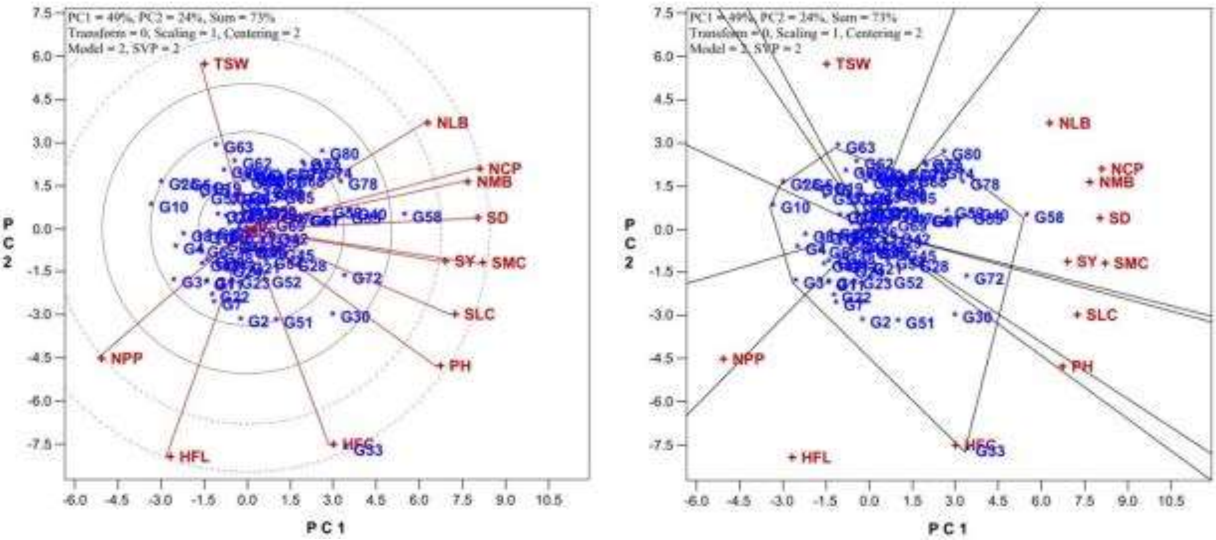

Figure 1. (A) Vector-view of G×T biplot showing interrelationships among traits and (B) Polygon-view of G×T biplot indicating the which-won-where pattern for safflower genotypes.

Among different options of the G×T biplot model, the polygon view provides detection of genotypes with the highest magnitudes for one or more traits and helps to find the best way for graphic interpretation the G×T interaction statures among traits and genotypes. For this purpose, the farthest genotypes from the graph origin are lined with straight lines and a polygon is generated with other genotypes contained within the vertex genotypes. The vertex entries (genotypes) are the most responsive ones as the best or the poorest in some or all of the measured traits while the perpendicular lines are drawn from the graph origin for categorizing the graph into several sections, each having a special vertex entry. In the G×T biplot model, the polygon-view graph (Fig. 1B) is divided into eleven sectors, and the sector contains genotype G80 on the polygon vertex winner for most traits consist on number of lateral branches per plant (NLB), number of main branches per plant (NMB), number of capitula per plant (NCP), number of seeds per main capitulum (SMC), stem diameter (SD), seed yield (SY). While genotype G33 is situated on the vertex of another sector with height related traits as plant height (PH), height of the first lateral branch (HFL) and height of the first lateral capitulum (HFC) (Fig. 1B). Genotype G3 is situated on the vertex of another sector with number of plants per plot (NPP) while genotype G63 is situated on the vertex of another sector with thousand seeds weight (TSW), and genotype G58 is situated on the vertex of another sector with number of seeds per lateral capitulum (SLC). The identified vertex genotypes in each section gave the highest performance for the traits that fall within that section while the other vertex genotypes did not give the highest performance in any of the traits and were the poorest genotypes in some or all of the traits.

The center of the circles in Fig. 2A indicates the position of an ideal entry, which is determined by a projection on to the section axis that is equivalent to the largest vector of the entries that had grater performance than mean and by a low projection on to the vertical axis while a genotype is more desirable if it is closer to this position. Thus, genotypes G72 and G30 following to genotypes G58 and G33 were more desirable than other genotypes while the low desirable genotypes were G10 and G21 because they were far away from the position of ideal entry. An ideal tester should be differentiating of the genotypes and representative of the main goal trait and regarding that the testers or traits are representative items of the main goal trait, the ideal tester should be on the mean traits coordinate axis. The center of the circles shows the ideal tester, which has the largest vector of the traits that had positive projections onto the mean traits coordinate axis (Fig. 2B). A measured trait is more desirable if it is closer to the ideal tester, therefore, number of seeds per lateral capitulum (SLC) was desirable trait followed by number of seeds per main capitulum (SMC) and seed yield (SY). In the next step, plant height (PH) and stem diameter (SD) were relatively desirable traits, whereas thousand seeds weight (TSW) and number of plants per plot (NPP), were relatively undesirable traits.
We found highly significant association between seed yield with number of seeds per main and lateral capitula as well as number of capitula per plant which are in agreement with report of Shinwari *et al.*, (2014) and Gholami *et al.*, (2018), who recorded that number of seeds per capitulum had highly significant positive contribution with seed yield performance. To improve yield, number of seeds per capitulum and number of capitula per plant are important traits following to thousand seeds weight based on report of Ahmadzadeh *et al.* (2012). The higher seed production of safflower was mostly associated with number of capitula per plant and number of seeds per capitulum (Johnson *et al.*, 2012). In our study high branching and more capitula or more seeds per capitulum were found as the most influencing traits on yield performance. However, it seems that for improving seed yield performance in safflower, it is better to focus on more number of seeds per capitulum and then try to increase number of capitula per plant and thousand seeds weight. Also, we found genotype G80 following to genotype G58 as the best for most traits especially number of main and lateral branches, number of capitula per plant, number of seeds per capitulum and seed yield while genotype G63 is a good source for high thousand seeds weight. We found that, seed yield, number of seeds per main and lateral capitula and plant height had the high discriminating potential as well as the high representative ability based on ideal trait biplot. Thus, the mentioned traits indicated both properties and can be advised for screening of genotypes reliably in a large scale, because it was good representative of all measured traits and cold discriminated studied genotypes (Sabaghnia *et al.*, 2016).

Conclusions

Although, the genotype (G) plus genotype by environment interaction (G×E), GGE, biplot methodology was originally used for multi-environment trials interpretation, but it is applicable to two-way type as an entry by tester pattern, such as a genotype by trait (G×T) two-way matrix. The main difference between two above procedures is that in GT data, various traits have different units, so a standardization is needed before analyze. Successful safflower genotypes need to be improved based on effective selection indices in order to ensure their yield performance and economic profitability. It is clear that the G×T biplot method is an excellent procedure for graphic analysis. Compared with common methods, the G×T biplot method has some advantages, including graphical presentation, increase breeders' ability to understand the data pattern, interpretative potential of the G×T biplot method, facilitating pair-wise genotype comparisons, ranking genotypes based on the target trait, and identification of possible groups of genotypes as well as traits.

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MECHANICAL PEAR HARVESTING WITH THE "MULTIBASKET" SYSTEM

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Abstract

Modern fruit growing requires technical innovations to ensure sustainability, reduce labor costs and overcome lack of skilled harvest employees. To solve these issues, CREA-IT researchers designed an innovative plant-machine system for mechanical fruit harvesting, both for fresh market or processing. The “Multibasket” prototype was developed for harvesting apples and pears from narrow, 45° inclined, “X” shaped trellises. The self-propelled machine has a harvesting frame equipped with multiple baskets to catch the fruits detached by means of four impact rods from the fruit-bearing surface. A soft-roller conveyor belt carries the fruit to the rear end with an automatic bin-filler to store the fruits. This paper presents the results obtained by the prototype in mechanized pear harvesting on an experimental field. Yield and quality parameters of the crop were measured. The machine operating parameters and work quality were evaluated by establishing detachment efficiency, fruit losses and fruit damage. The innovative prototype design proved to be efficient, including the self-propelled frame and the hydraulic system to move the mechanical parts. The rods for detaching the fruit were effective and damage to branches was negligible. The automatic controls allowed for an optimal alignment of the vehicle to the rows and to the fruit-bearing surface. The "Multibasket" prototype showed good harvest efficiency, achieving detachment rates higher than 85%. However, recorded fruit losses to the ground were high (24%), requiring the need to optimize the design of the “multi-basket” intercepting frame. Fruit damage was acceptable and lower than approximately 20%.

Keywords: *orchard mechanization, harvest quality, fruit damage, picking efficiency.*

Introduction

Modern fruit growing requires continuous technical-economic innovations to rationalize farm management, improving production and revenue. Profits can be increased both by improving fruit quality and thereby increasing the price of the product, even if restrained by the global market competition, and by reducing cultivation costs. In economically advanced countries, however, it is necessary to limit fruit production costs, in particular by reducing the use of labor, which, in addition to accounting for up to 50% of cultivation costs, is often difficult to find, especially in the peak harvesting season. To achieve this goal, CREA-IT has long since proposed an integrated plant-machine system for a fully mechanized harvesting of fruit for processing and in some cases, depending on the species and variety, also appropriate for fruit proposed for fresh market (Colorio, 1993).
The *Multibasket* prototype, which was designed, built and tested in CREA's experimental fields for several years, especially for apple harvesting, demonstrated optimal interaction with 45º inclined fruit-bearing trellises. In order to achieve these inclined walls, Y- and X-shaped training forms were proposed and tested (fig. 1 and 2), specially designed for this integrated plant-machine production system (Colorio, 1986). With a first innovative prototype, hereafter referred to as *Multibasket 1*, promising results were obtained and the indications from numerous experimental harvesting trials made it possible to design a second prototype,

referred to as *Multibasket 2*, in order to increase the efficiency of the system. This new machine (Multibasket 2) was later constructed in order to improve the efficiency of the integrated harvesting system. A further opportunity to expand the field of use of the Multibasket system arose from the experimental harvesting trials with pears of *cv.* Decana del Comizio and *cv.* Williams, destined for the agro-food industry. However, it was possible to establish that the quality of the work carried out in the harvest trials by both machines, which are compared in the present paper, allows for a product that is also suitable for the fresh-consumption market (Colorio *et al*, 1994).
The aim of the present paper was to evaluate the operative performance of the two prototypes, carrying out mechanical harvesting trials in the experimental pear orchard established at CREA-IT Extension Field. In particular, the purpose was to assess the improvements achieved with the second prototype, to confirm the validity of the design modifications.

Figure 1. "X" shaped trellis

Figure 2. "Y" shaped trellis

Materials and Methods

Based on tests carried out over several years of research and development, the study was set up to increase the efficiency of the integrated machine-crop system with the adoption of improved modifications to the Multibasket prototype (Colorio and Beni, 1995b).
Compared to the Multibasket 1, the Multibasket 2 model has undergone modifications in the following components: self-propelled frame with integral hydraulic drive; hydraulic system for operating the various mechanisms of the harvesting machine; automatic system for approaching and positioning the Multibasket interceptor frame to the trees (with associated detachment rods and harvesting devices); innovative system for detaching the fruit by means of a dynamic impulse at the base of the rod (for each of the 4 horizontal rods); conveyor belt system for transferring the fruit to the storage bins located at the rear of the machine; automatic system for filling the plastic pallet bins with fruit (fig 3 and 4) (Colorio and Tomasone, 2002).

Figure 3. side view of Multibasket 1

Figure 4. side view of Multibasket 2

Particularly significant innovations were made to improve the functionality and efficiency of four systems that had shown limitations in the first design. Each innovation realized is analyzed and described below.

A first important improvement was made to the design of the fruit detachment system, allowing a reduction in the level of damage to the branches, which had been observed in the harvest trials with the first prototype. The fruit detachment system consists of four rods mounted on the multi-basket frame. Each rod is kept in contact with the fruit-bearing wall surface at all times by means of a spring mechanism. Fruit detachment is achieved using dynamic impulses produced by a beating mass inserted at the base of each rod (fig. 5 beater schematic). This mass is loaded by a spring mechanism, accumulating elastic potential energy which, when released, imparts a strike to the base of the rod itself.

This operating mode makes it possible to indirectly transfer acceleration (via the interposed rod) to the fruit walls without a direct impact on the vegetation, thus avoiding injuries to the branches and twigs that occurred with the first prototype in which the rod directly struck the inclined fruit wall.

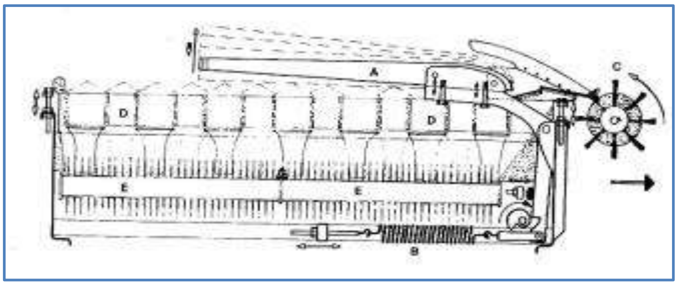

Figure 5. schematic of rod mechanism: A) impact rod; B) spring; C) brush roller; D) intercepting frame; E) roller conveyer

A second change to the prototype regarded the arrangement of the line of conveyor belts, which carry out the transfer of the detached and intercepted fruit from the inclined 'multi-basket' frame to the rear bins filler.

This system of conveyor belts, equipped with soft rollers, is divided into two contiguous sectors. The first sector is placed underneath the 'multibasket' frame, and intercepts the fruit released from the baskets and consists of three transversal belts placed side by side.

Figure 6.: a) transversal belts; b) longitudinal belt.

Figure 7. Rear view Multibasket 2

This first group of three side-by-side conveyer belts (fig. 6a) allow the fruit to be transferred and discharged evenly onto a subsequent longitudinal belt, mounted on the opposite side of the inclined frame (fig. 6b). In the first machine model, the transversal belt was a single belt as wide as the intercepting frame and the fruit was discharged onto the central area of the longitudinal belt. This solution caused the accumulation of fruit along the center line of the longitudinal belt, resulting in subsequent unevenness in the filling of the bin. The subdivision into three belts, with attachment in three different zones of the longitudinal belt, avoids the accumulation of fruit in the central part, which is unfavourable for optimal bin filling. The second belt arranged longitudinally with respect to the machine, transports the harvested fruit towards the rear of the machine, where the automatic palletized bin filler is mounted (fig. 7). The latter is equipped with a

series of pairs of counter-rotating rollers that gently accompany the fruit into the box, which is simultaneously moved forwards and backwards on two rear forks, aligned parallel to the longitudinal axis of the machine. The filling mechanism lowers the bin as it fills, for depositing the fruit in stratified layers (Colorio and Beni, 1995a).
Compared to the first prototype, the system for automating the alignment of the multi-basket intercepting frame to the inclined fruit-bearing surface has also been optimized. The system was implemented using three contact-rods mounted on the frame, which control its movement automatically. The first rod controls the lateral distance of the frame from the fruit wall, the second controls the height of the frame from the ground, and the third controls the angle of inclination of the frame so that it is perfectly aligned with the inclined fruiting surface (fig. 8).
Harvest trials were conducted in an experimental field with two pear cultivars, *cv.* Williams and *cv.* Decana del Comizio, trained in the X shape trellis, inclined at 45º, planted in 2010 at CREA-IT Research Centre (Monterotondo, Italy). The characteristics of the pear orchard are shown in Table 1. A new "X-shaped" support system was designed to hold an inclined fruit-bearing surface, utilizing freely jointed iron bars (Fig. 9). The special joint allows for a certain independent freedom of movement of the opposite bars, so that the impact applied by the rods on one side is not transmitted to the opposite side. The lower rigidity of the support structure improves the shaking action of the rods. Instead, the previous trellis designs where not optimal, because the action of the machine on one side caused fruits to fall on the opposite side to a certain degree.

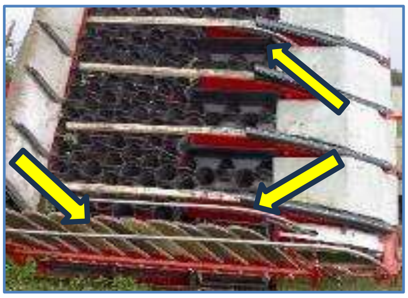

Figure 8. Three contact-rods (arrows)

Figure 9. Joint between trellis beams

In 2019, mechanical harvesting trials were carried out using both Multibasket prototypes, to harvest *cv.* Williams in August and *cv.* Decana del Comizio in September. In the harvesting trials, production and harvesting parameters, operational performance and the quality of the work done by the prototypes were measured. The detachment efficiency, fruit interception capacity, fruit plant and ground losses, and the extent and type of fruit damage were measured. Unfortunately, the small size of the orchard did not allow changes in forward speed during the tests of the two machines.
The comparison of the machines in the harvesting trials made it possible to assess the mechanical efficiency and validity of the modifications made to the new prototype

Table 1 Characteristics of the pear orchard studied for the field tests

Characteristics	units	*cv.* Williams	*cv.* Decana del Comizio
Trellis form	--	X	X
plant distribution	--	binary rows	binary rows
planting spacing	m	5 x 1.5 (0.75 x 0.75)	5 x 1 (0.50 x 0.50)
trunk section area	cm^2	41.0	37.1
maximum tree height	m	2.93	2.87
trees/hectare	n°	2222	3333

Results and Discussion

All the modifications of the new prototype were found to be an improvement on the previous machine. The self-propelled machine and the hydraulic system for the motion of the mechanical parts are more efficient and allow for better machine maneuvering. The 4 impact rods for detaching the fruit are kept properly in contact with the fruit-bearing surface at all times, and the new impact mechanism enables more effective fruit detachment while reducing damage to branches. The modified conveyor belts are more effective in transferring the fruit, although the solution adopted of dividing the sector under the multi-basket frame into three transverse belts instead of one whole belt resulted in a higher loss of fruit due to falling between the gaps.
The new contact-rod automatisms allow the optimum alignment of the machine to the row and the alignment of the multi-basket frame to the inclined fruiting surface. In the X-trellised pear orchard, the *cv.* Williams showed very good productivity, while the *cv.* Decana del Comizio was quite good (Tab 2). The new support structure for the X-shaped training system made it possible to independently harvest the fruit wall on the side of the machine without transferring the impacts to the opposite side. This arrangement reduces the fall of fruit from the branches on the opposite side of the row.

Table 2. Experimental pear orchards yield characteristics

Characteristics	units	*cv.* Williams	*cv.* Decana de1 Comizio
yield	kg/plant	23.34	7.72
average fruit weight	g	153	270
fruit detachment force	kg	4.0	4.6
pulp consistency	kg	8.7	7.7
Sugars	°Brix	13.3	16.3
Production/hectare (theoretic)	t/ha	51.778	25.725

In the field trials, using an equal 1 km/h forward speed for both prototypes, the Multibasket 2 showed a higher production efficiency in comparison to Multibasket 1, for both cultivars harvested (Tables 3 and 4). The new impact-rods detached more than 96% of the pears from the branches as compared to only 82% with the previous prototype. Both machines detached the fruit of cv. Decana del Comizio, with larger fruit mass, more easily than those of cv. Williams. Most of the un-detached fruit remaining on the plant were placed near the trellis support beams, which absorb and attenuate the impacts of the rods, reducing their effectiveness. The fruit loss on the ground appeared significantly lower with Multibasket 1 with respect to Multibasket 2, due to the partially ineffective protection for the gaps between the 3 transversal belts in the second model. Finally, fruit damage observed for both prototypes (Table 5) was very small and characterized by small holes caused mainly by broken fruit peduncles. However, the new prototype proved to be gentler on the fruit than the first one. In fact, the greater efficiency in detaching the fruit decreased the danger of reciprocal impacts with the branches, together with the delicacy of the conveyor belts, reduced damage by about 20%.

Table 3. Prototype efficiency during *cv.* Williams pear harvest

Prototype	Multibasket 1		Multibasket 2	
units of measure	kg/tree	%	kg/tree	%
detached fruits	14.89	67.4	23.73	96.5
fruits left in plant	7.20	32.6	0.86	3.5
fruits stored in bins	12.46	56.4	20.51	83.4
fruits fallen to the ground	2.43	11.0	3.22	13.1
total production	22.09	100.0	24.59	100.0

Table 4. Prototype efficiency during *cv.* Decana del Comizio pear harvest

prototype	Multibasket 1		Multibasket 2	
units of measure	kg/tree	%	kg/tree	%
detached fruits	6.55	81.6	7.18	96.9
fruits left on plant	1.48	18.4	0.23	3.1
fruits stored in bins	5.77	71.9	5.79	78.1
fruits fallen to the ground	0.78	9.7	1.39	18.8
total production	8.03	100.0	7.41	100.0

Table 5. Fruit quality characteristics picked with the two harvesters

Cultivar	*cv.* Williams		*cv.* Decana del Comizio	
prototype	Multibasket 1 %	Multibasket 2 %	Multibasket 1 %	Multibasket 2 %
fruits with peduncle	63.2	73.9	47.8	50.7
fruits without a peduncle	36.8	26.1	52.2	49.3
healthy fruits	75.9	93.5	54.9	76.8
fruits with minor damage	22.4	6.0	31.4	27.7
fruits with severe damage	1.7	0.5	13.7	5.5

Conclusion

Although the Multibasket 2 prototype needs to be fully completed and fine-tuned, it has shown a significant technical and operating improvement in comparison to the previous Multibasket 1 prototype. Interesting results are expected from the next trials on apricot, plum and peach orchards provided with the new X-shaped training structure. Eventually, more extensive orchard trials need to be carried out to determine the limits of the machine's operating performance. However, the Multibasket system is presented as a viable alternative to conventional orchard cultivation for industry food-processing, with estimated savings in harvesting costs of over 80%.

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DEVELOPING A DECISION TREE MOBILE APPLICATION TO CONTROL A SMART GREENHOUSE IN THE JORDAN VALLEY

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Abstract

The agriculture sector in Jordan faces significant challenges related to water scarcity and resource management. In the arid climate of the Jordan Valley, and varying temperatures between summer and winter, efficient farming practices are essential for sustainable crop production. To address this, we propose a smart greenhouse system that utilizes artificial intelligence (AI) and sensor technology to optimize water usage and enhance productivity. Our smart greenhouse integrates a suitable number of four types of sensors placed at several places. These are moisture sensors to continuously measure soil moisture content, temperature sensors to monitor ambient temperature both inside and outside the greenhouse, a humidity sensor to monitor humidity inside the greenhouse to adjust ventilation and irrigation to maintain an ideal environment, and a luminosity sensor to assess light intensity, allowing precise control of shading and artificial lighting if needed. Our system uses the Decision Tree (DT) machine learning algorithm. Trained on historical sensor data, the DT model predicts irrigation needs based on real-time inputs. By dynamically adjusting irrigation schedules and environmental parameters, our system achieves remarkable water efficiency and crop yield improvements. Our research contributes to sustainable agriculture practices in water-scarce areas like the Jordan Valley. The integration of AI and sensor technology holds promise for optimizing resource utilization and enhancing greenhouse productivity. This study shows that our DT algorithm produced a simulation of a smart greenhouse with a 94.76% overall system accuracy of correct system reactions to different surrounding circumstances.

Keywords: *Smart greenhouse, Decision tree algorithm, Sustainable Agriculture, Water management, Ventilation controller.*

Introduction

The Jordan Valley faces several agricultural challenges such as water scarcity, soil salinity, climate variability, land degradation, and limited arable land. Addressing these challenges requires comprehensive strategies encompassing sustainable water management practices, soil conservation measures, climate-resilient agricultural techniques, land-use planning, and infrastructure development (Al-Ghussain, 2017). Building smart greenhouses in the Jordan Valley presents a promising solution to address some of these challenges. The Jordan Valley, characterized by its arid climate and water scarcity, poses significant constraints to traditional farming practices. However, with advancements in technology and agricultural innovation, smart greenhouse systems offer viable pathways towards efficient resource utilization and optimized crop production (Qatarneh, 2018).
Despite formidable challenges, agriculture remains crucial to the Jordan Valley's economy, providing substantial employment and significantly contributing to food security. The government is committed to enhancing the agricultural sector through initiatives focused on improving water management and productivity (Jordanian Agricultural Statistics, 2019). The Jordan Valley, producing about 40% of the nation's agricultural output, primarily cultivates

tomatoes, cucumbers, eggplants, melons, bananas, and citrus fruits, alongside extensive dairy, and poultry farming. Water scarcity is a major challenge, prompting government efforts to develop efficient irrigation and water conservation measures. Additionally, there is a strong push towards sustainable agricultural practices, reflecting a commitment to long-term environmental stewardship and resilience (Jordanian Agricultural Statistics, 2019)
A smart greenhouse integrates various cutting-edge technologies such as sensor networks, automated climate control systems, precision irrigation, and data analytics to create an optimal growing environment for plants. By employing these technologies, farmers can mitigate the adverse effects of environmental factors, maximize crop yields, and minimize resource wastage. The key components of a smart greenhouse are climate control systems, precision irrigation, remote monitoring and control, and crop management and automation (Badji, 2022).
The Ritchie et al. (2022) article on "Crop Yields," published online at OurWorldInData.org, offers a comprehensive exploration of global crop production and yield trends. By analyzing data from various sources, including national agricultural statistics and research studies, the authors provided insights into the variability of crop yields across different regions and crops (Hannah, 2022). Using the Offered "Crop Yields" tool, Figure 1 gives an insight into potato and tomato crops respectively in several regions. the cultivation of potato, tomato, and cucumber crops in Jordan plays a crucial role in meeting dietary needs, supporting rural livelihoods, and contributing to the country's agricultural economy. This research focuses on tomato, potato, and cucumber crops which are integral to the food security and nutritional well-being of the population, underscoring their significance within Jordan's agricultural sector.

Figure 1. Potato and Tomato Yields measured in tones/hectare. (Hannah, 2022)

Smart greenhouse technology has seen notable advancements recently, focusing on enhancing agricultural productivity and sustainability. Alpay and Erdem (2018) pioneered the use of fuzzy logic-based control systems integrated with wireless sensor networks for efficient environmental monitoring and management in greenhouses. Kumar et al. (2020) expanded on this foundation by reviewing sensor network technology in greenhouse farming, emphasizing real-time data collection, automated decision-making, and resource optimization. Karanisa et al. (2022) explored smart greenhouse technology's role in precision agriculture within the food-energy-water nexus, particularly in Qatar, highlighting its potential to optimize resource use, improve yields, and address food security in arid regions. Viswanathan et al. (2024) examined IoT technology's application for real-time monitoring, predictive analysis, and automated control in greenhouses. Collectively, these studies illustrate the evolution of smart

greenhouse technology, emphasizing its innovative solutions for agricultural production, resource management, and environmental sustainability.
These interconnected studies demonstrate samples of the development of smart greenhouse technology and its increasing significance in modern agriculture. From the utilization of fuzzy logic and wireless sensor networks to the integration of IoT technology for real-time monitoring and control, smart greenhouses offer innovative solutions to address key challenges in agricultural production, resource management, and environmental sustainability.
Our research proposed the first attempt to implement a smart greenhouse project in the Jordan Valley. This project aims to propose a smart greenhouse system that utilizes artificial intelligence (AI) and sensor technology to optimize water usage and enhance productivity. A Decision Tree (DT) machine learning algorithm was developed to control smart greenhouses. It was trained on historical sensor data; the DT model predicts irrigation needs based on real-time inputs. Decision Tree is considered a small-scale software, and it can be used on mobile phones other than fuzzy Logic. DT models following AI principles were more suitable than IoT to manage large scale greenhouses.

Materials and methods

Decision trees

The Decision-making algorithm was implemented in this research. Most common algorithms for decision making include **the Decision Trees algorithm** which is a type of supervised learning algorithm that can be used to classify data or make predictions. They work by dividing the data into smaller and smaller groups until each group can be classified with a single decision. Where **Random Forests algorithm** provides an ensemble learning algorithm that combines multiple decision trees to make a more accurate prediction. They work by training multiple decision trees on different subsets of the data and then averaging the predictions of the trees. Also, **Support Vector machines** (SVMs) are types of supervised learning algorithms that can be used for classification or regression. They work by finding the hyperplane that best separates the data into two classes. In addition, **Neural networks** are examples of machine learning algorithms that can be used to learn complex relationships between data. They work by simulating the human brain's neural network. **Bayesian networks**, which represent a probabilistic graphical model that can be used to represent uncertainty in data, work by modeling the relationships between variables and their probabilities (Wikipedia, 2024).
The best algorithm for a specific problem will be controlled by the following factors: size and complexity of the data set, the number of possible decisions, the degree of uncertainty in the data, the desired accuracy of the decision (Zharmagambetov, 2022).
The best decision-making algorithm for a farmer depends on the factors involved, such as climate, soil, and the degree of uncertainty in weather. For decisions with high uncertainty, a Bayesian network might be suitable. For decisions with large amounts of data, a decision tree or random forest could be effective. Experimenting with different algorithms can help determine the best fit. In conclusion, **Decision Trees (DT)** are often ideal due to their simplicity, low uncertainty, and minimal parameter requirements. The DT are a popular choice for smart greenhouse climate control due to several advantages that make them well-suited for this application: **Interpretability:** Unlike some machine learning models, decision trees are inherently interpretable. The tree structure clearly shows the decision-making process, making it easy to understand how the model arrives at a specific control action (e.g., increasing ventilation or adjusting irrigation). This transparency is crucial in greenhouses where growers need to be confident in the system's logic, especially when unexpected situations arise. Also, **Real-time decision making:** Decision trees are efficient algorithms

that can make predictions quickly based on sensor data. This is essential in greenhouse environments where prompt adjustments to temperature, humidity, or irrigation are necessary to maintain optimal growing conditions. In addition, **handling multiple sensor inputs:** Greenhouses rely on various sensors to monitor factors like temperature, humidity, light intensity, and soil moisture. Decision trees can effectively handle these multiple inputs and create rules based on their combined influence. And **adaptability to new data:** As growers gain experience and collect more data, decision trees can be easily updated by incorporating the new information. This allows the model to continuously improve its performance and adapt to changing environmental conditions or crop requirements. Finally, **it works well with missing data:** Sensor readings in a WSN (Wireless Sensor Network) can sometimes be missing due to various reasons. Decision trees are relatively robust to the missing data provided by input and can still make predictions using the available information (Zharmagambetov, 2022).

Technical features

Temperature and air humidity Sensors (four of each) were placed 2 meters away from each other, while the single luminosity sensor was placed at the center of the greenhouse. Two soil moisture sensors were placed at the center and one end of the greenhouse. The prototype (Figure 2) of the simulated model was for a greenhouse 10 meters long and 3 meters wide.
Drip irrigation pipes were placed on the soil, about 30 cm away from the roots and stem of the plants. Heating fans were placed at each end of the greenhouse while motors were connected to the ventilators on the sides to allow air flow and lower the internal heat inside the greenhouse, and motors were placed on top panels to allow more sunlight (during the day) to enter the greenhouse for optimal luminosity for optimum plant growth. Using an Artificial Intelligence (AI) tool by Figma (2024), an edit to a generated sketch map of the suggested greenhouse is shown in Figure 2.

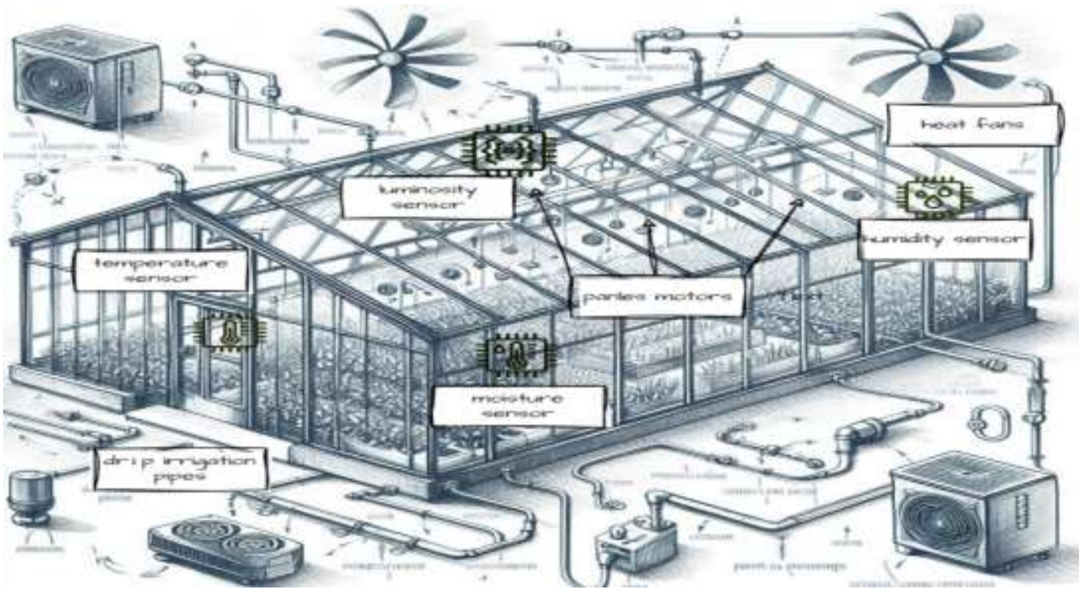
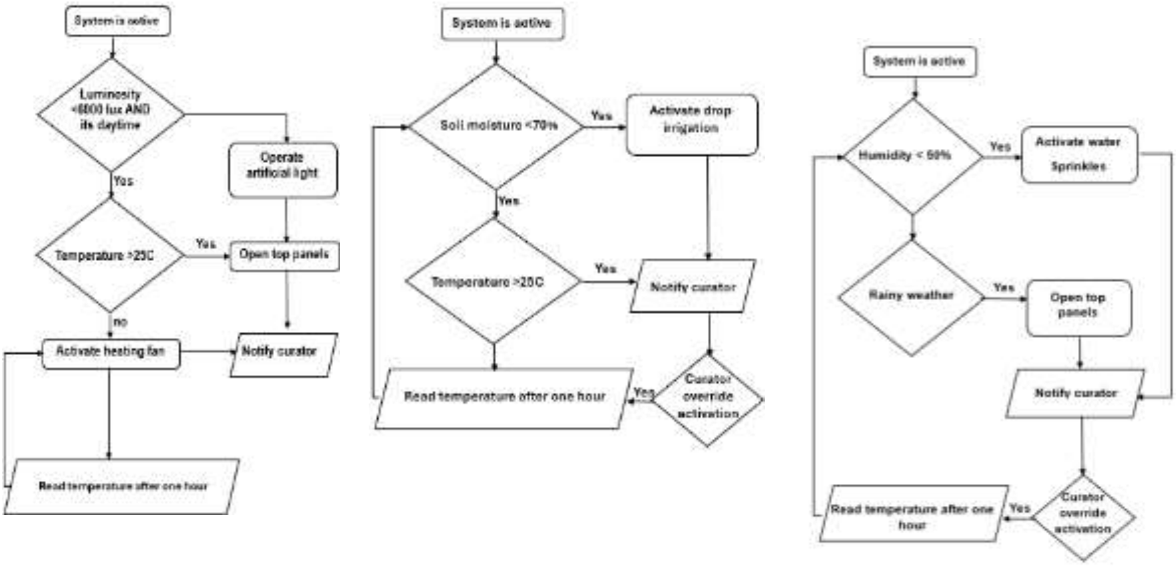


Figure 2. Edited AI generated WSN in the greenhouse under study. (Figma, 2024)

The workflow of actions was taken depending on readings from sensors. Each sensor triggers a certain action to create the optimal environment inside the greenhouse. Figure 3 demonstrates the flow of all sensor types used. Figure 3a shows the flowchart for readings from luminosity and temperature sensors, while Figure 3b shows the flowchart of actions for soil moisture sensors, and finally, Figure 3c the flowchart of actions for humidity sensors.

The DT program was implemented using ***Python*** programming language 3.0 in anaconda ***JupyterLab*** (Project Jupyter, 2024) which is recently considered the most suitable programming language for machine learning and artificial intelligence especially in Mobile Phones Applications, because ***Python*** is flexible and it has simple and readable syntax, a variety of libraries to implement AI, and it is considered an independent platform (Python Land, 2024). The DT program was trained on simulated data that generated random values for all 4 types of sensors twice a day: 10:00AM and 10:00PM. The program used a random library to simulate sensor readings and ***NumPy***, which is a ***Python*** library used for working with arrays, for the DT work. Actions according to the workflow mentioned earlier were added manually to facilitate the training and testing process.



a. b. c.

Figure 3. Flowchart of different types of sensors used in simulation.

Results and discussion

The simulator produced data from **2 moisture sensors, 1 luminosity sensor, 4 humidity sensors, and 4 temperature sensors**. Weather forecast data for the next day was also added to help the decision-making process on the action to be taken. The data set was divided into 80:20 training ratios; where 80% of the data was used for training the decision tree, and the remaining 20% was used for testing the system. The system will aim to maintain a good balance of moisture, humidity, luminosity, and temperature to make crop yields better, and avoid loss, while providing sustainability of the greenhouse's resources (water and electricity mainly).

According to this research, optimal humidity ranges for tomato, cucumber, and potato crops were set between **50% to 70%**, while the temperature to keep the crops in good growth were specified between **18° C – 26**° C. Best soil moisture levels were set between **70% - 80%** for the crops at the fruit stage, and between 75% - 90% at the seeding stage, according to Ministry of Agriculture (Jordanian Agricultural Statistics, 2019). A data set was simulated for the entire month of March in 2024 which is known for weather fluctuations in Jordan. Results were collected for 31 days twice a day resulting in a total of 62 records. Table 1 shows the obtained results, with an overall system accuracy equaling 94.76%.

Table 1. Applications of the developed DT algorithm in 62 trials and their accuracy percent.

Actual action	Predicted action				**Total number of trials**	Accuracy
	Open air ventilation	Turn on heating fans	Turn on drip irrigation	Turn on sprinklers		
Open air ventilation	58	2	1	1	**62**	0.935484
Turn on heating fans	2	59	0	1	**62**	0.951613
Turn on drip irrigation	0	0	61	1	**62**	0.983871
Turn on sprinklers	2	1	2	57	**62**	0.919355

We developed an android mobile application as a **Mobile controller** named **<u>S</u>mart<u>G</u>reen<u>H</u>ouse App** (SGH). It was built to give the farmer the chance to monitor his greenhouse and control all its aspects. This app offers the following services: action taken with an override button, Remaining energy for each sensor (map of sensors with energy for each sensor, alert time to change (or charge batteries), and History of sensors' readings every day.

Conclusion

Luminosity values vary according to the plant's growth stage; while plants at seedling and Vegetative stages require less sunlight, in the flowering and fruit production they need more. We are focused in this research on the fruit production stage, where the most suitable luminosity values vary between **6000–9000** lux for tomatoes and cucumber crops. As at night there is no sunshine, in future, the greenhouse could be improved to install light panels that are activated during short-day months, and the plants don't get enough sunshine. An additional feasibility study needs to be conducted to evaluate the cost in terms of energy consumption to crop yields and find the optimal conditions to incorporate light panels.

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APPROACHES TO ASSESSING THE ENERGY POTENTIAL OF BIOMASS OBTAINED FROM CROP RESIDUE IN REPUBLIC OF NORTH MACEDONIA

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Abstract

The energy potential of plant residues (leaf, stem) from field crops (wheat, barley, maize, rice, sunflower, rye, tobacco and oats) is the basis for a different focus on biomass in plant production in our country, now as a source of energy. For this purpose, we used statistical data for the period 2014-2023 (total arable and sown area ha, total production t, kt/y, kg/ha), literature data (residue product ratio - RPR, higher calorific value - CV, dry matter - DM), and the basic mathematical and biomass energy potential estimation method (BEP) for obtaining energy potential for crop residues. From the average total arable area (515983 ha), the field crops were represented at 34.9% (180229 ha), of which the most represented were wheat (14.1%), barley (8.6%), maize (6.1%), and tobacco (3.1%), and the rest of the crops were represented below 1%. The total amount of biomass from the studied crops was estimated at 722 kt/y, or from 291.2 kt/y for wheat to 8.3 kt/y for oats, on the sown areas. The total energy potential of all crops according to the basic mathematical model showed 12 081 GJ and was correspondingly higher than the BEP method (10 622 GJ) by 12%, where DM was also included in the assessment. The energy potential of the crops corresponding to the sown areas was realized for wheat at 4926 GJ according to the basic mathematical model, or 4335 GJ according to the biomass energy potential estimation method.

Keywords: *residual biomass, energy potential, field crops.*

Introduction

The global economy is mainly based on fossil fuels, which account for 81% of the total primary energy supply, nuclear energy for 5% and renewable energy sources for 14%, of which biomass is represented at around 70% (Popp et al., 2021). Historically, this was already visible in 2018 when the share of bioenergy exceeded 13.5% (Havrysh et al., 2021). The EU Renewable Energy Directive (2018/2001/EU) of December 2018, as part of the “Clean Energy for All Europeans” package, set a new binding renewable energy target for the EU of at least 32% for 2030, revision in 2023, raises the target to 42.5% (https://energy.ec.europa.eu). Agriculture has optimal conditions for the use as renewable energy sources, with great demand for low-temperature energy sources, relatively low atmospheric pollution, and a large energy potential of renewable energy sources, especially biomass resources (Pabis et. al., 2015). Biomass from agricultural production is provided as crop residues from field crops (stalk, straw, husk, cob, boll, shell and pod) can be used as by-products - residues from agricultural production (Ertugrul et al., 2024). On the other hand, plantation biomass - energy crops (sugarcane, switch grass, sorghum, eucalyptus, poplar, and oil palm) refers to grown whole plants through traditional agricultural practices (Gravalos et al., 2016). The advantage of using crop residues compared to energy crops is that their use leads to minimal impacts on land use change (Batidzirai et al., 2016). Besides the differences, at community level, farmers can cultivate energy crops that fetch more income while meeting their food needs (Dimov et al., 2018). Bioenergy is an important component of the bioeconomy. EU concept who includes economic activities concerning the use of biomass, with strategy focused on the balance of environmental, social, and economic benefits through the sustainable use of renewable resources, for realization of jobs and profit as a goal (Havrysh et al., 2021). Enhancing renewable energy portfolios is crucial to ensure the sustainability of energy since renewables are set to remain in forward thinking of energy security (Ertugrul et al., 2024). Having in mind above mentioned, the primary aim of this research is to assess agricultural residual biomass and its energy potential in the Republic of North Macedonia, using two different approaches for evaluating renewable energy resources.

Material and methods

The accurate assessment of biomass energy potential is crucial for initial evaluations of agricultural residue from field crops. To determine this two different approaches for potential assessments were adapted from studies by Guzmán-Bello et al., (2023), and Batista et al., (2023). These were incorporated into a mathematical procedure essential and the BEP method. The first formula used is: $E = m\times CV$, where: E - energy potential (J or MJ), m - mass of the biomass (kg or t), and CV - calorific value of the biomass (MJ/kg). The second equation is Biomass Energy Potential Estimation Method (BEP), which determines the theoretical energy potential of biomass: $BEP=\sum i=1(Mi\times RRPi\times HHVi\times DMCi)$ where: BEP - the Biomass Energy Potential, expressed in MJ or GJ; n - the total number of agricultural products; M - the annual production; RRP - the residue ratio for the product; HHV - the value of higher calorific value; DMC - the dry matter content (%). Data for total arable and sown areas, total production, and yields from: wheat, barley, maize, rice, sunflower, rye, tobacco and oat from the period 2014 - 2023 were utilized from the Macedonian Statistical Office (https://makstat.stat.gov.mk). Data sets from the literature for crop residue ratio (RPR), calorific value (CV) of biomass conversion and dry matter content (DMC) were also used. The results from the two methods were compared using ANOVA and the LSD test, with a significance level of $p>0.05$.

Results and discussion

In following tables, the literature datasets are presented for factors such: residue-to-crop production ratio (Table 1), biomass calorific value (Table 2), and dry matter content (Table 3).

Table 1. Literature datasets used for determining residue to crop production ratios

Wheat	Barley	Maize	Rice	Sunflower	Rye	Tobacco	Oats	
				2.4				Aboudradc Cl al. (2006)
				1.45				De la Vega et al. (2001) and
1.30	1.30	1.00					1.30	Ericsson and Nilsson (2006)
		1.00						Glassner et al. (1998)
	1.50						2.00	Craham et al. (2007)
1.20		0.90		1.50			1.40	Johnson et al. (2006)
1.34		1.00						Kadam and McMillan (2003)
1.75	1.75	2.00	1.76		1.75		1.75	Koopmans and Koppejan (1997)
0.60		1.30		2.60				Koukios (1998)
		0.87						Linden et al. (2000)
	1.00	1.00					1.00	Nelson (2002)
1.00	1.24	0.70	1.00	1.40			1.27	Panoutsou and Labalene (2006)
	1.65						1.57	Patterson et al. (I995)
	1.25				1.70		1.50	Petersen et al. (1995)
				2.40				Rinaldi et al. (2003)
	1.20						1.20	Kaltschmltt and Hartmann (2000)
		1.00						Sokhansanj et al. (2002)

					2.80				Soriano et al. (2004)
				1.55					Summers et al. (2003)
		1.00	1.00					1.00	Walsh et al. (2000)
			1.00						Wilhelm et al. (2004)
							0.51		Sheen S. J. (1983)
							2.27		Ozturk and Bascetincelik (2006)
	1.2	1.19						1.16	Cukaliev (2014)
	1.3								Maznevska (1998)
x̄	1.21	1.31	1.06	1.44	2.08	1.73	1.39	1.38	

Table 2. Literature datasets for determining content in CV of biomass conversion

		Mj/kg		± Mj/kg	
Wheat	16.54	17.30		16.92	Jóvér et al., (2018), Piskier (2017)
Barley	16.10	16.80		16.45	Piskier (2017), Tyminski (1997)
Maize	17.50	16.80		17.15	Jóvér et al., (2018), Piskier (2017)
Rice	15.09	16.47		15.78	Gravalos et al., (2016)
Sunflower	14.37	15.85		15.11	Jóvér et al., (2018), Kucukbayrak et al., (1991)
Rye	16.90	17.50		17.20	Górnicki et al., (2018), Harasim (2011), Górnicki et al., (2018)
Tobacco	11.30	15.41	16.47	14.39	Popovski et al., (2010), Arsov et al., , (2020)
Oats	18.50	17.60		18.05	Pabis et al., (2015), Górnicki et al., (2018)

Table 3. Literature datasets for dry matter content

	Wheat	Barley	Maize	Rice	Sunflower	Rye	Tobacco	Oats
DMC %	88%	88%	88%	88%	79%	88%	90%	88%
DMC num	0.88	0.88	0.88	0.88	0.79	0.88	0.90	0.88

* Wirsenius (2000); Ronzon et al. (2015); Drackley et al. (1985)

In assessing the distribution of total arable land nationwide (515983 ha) from 2014 to 2023, field crops constitute 34,9%. This significant portion of arable land represents potential biomass derived from eight primary field crops, with wheat accounting for 14,1%, barley 8,6%, maize 6,1%, and tobacco 3,1%, being the most prominent among them (Tab 4). The biomass should be considered as biological, technical, economic and available potential. The assessment of biomass potential in the energy sector focuses primarily on available potential represents the biomass energy stream that can be utilized for the energy purposes (Piskier, 2017). The energy content of the above-ground biomass was significantly by the species and year (Fuksa et al., 2013), and includes harvestable crop residue, primary economy yield part, non-harvestable crop residue, and below ground biomass (Kishore et al., 2021; García-Condado et al., 2017). The minimum quantity of above-ground biomass required for soil function is 2,5t/ha above-ground of not harvestable biomass and roots (Cativa et al., 2024). According our investigation, the mean yearly total above-ground harvestable biomass from examined field crops during the period 2014 - 2023 was 721924 t. Among these, wheat contributed the highest biomass production at 291155 t, while oats production yielded the lowest at 8305 t (Table 4).

Table 4. Total biomass production, yield and % of sum

x̄ 2014 - 2023	Wheat	Barley	Maize	Rice	Sunflower	Rye	Tobacco	Oats	Sum
Total Arable Land ha	72606	44126	31664	3809	4691	3933	16154	324	180229
Total prod. t	24062	13437	14181	2213	6752	8761	25206	601	58568
kg/ha	3326	3073	4485	5905	1462	2232	1562	185	
% of total arable land	14.1	8.6	6.1	3.1	0.9	0.8	0.7	0.6	34.9
RPR	1.21	1.31	1.06	1.44	2.08	1.73	1.39	1.38	
Total biomass prod./t	29115	17602	15032	3187	14044	1515	35036	830	72192
Yield biomass kg/ha	4025	4026	4754	8503	3041	3861	2172	256	32944
% total prod. biomass t	40.3	24.4	20.8	4.4	1.9	2.1	4.9	1.2	100.0
% biomass yield kg/ha	12.2	12.2	14.4	25.8	9.2	11.7	6.6	7.8	100.0

*Source: https://makstat.stat.gov.mk and literature datasets for RPR residue to crop production ratios

The distribution of biomass production is highest in wheat (40.3%), followed by barley (24.4%), and maize (20.8%), while oats, sunflower, and rye contribute the lowest percentages - 1.2%, 1.9%, and 2.1% respectively from total biomass production and total arable land. Conversely, rice (4.4%) and tobacco (4.9%) are inversely proportional to total arable land. Regarding biomass yield relative to production, rice achieves the highest percentage at 25.8%, followed by wheat at 14.4%. In contrast, oats account for 9.2% and tobacco for 6.6%, representing the lowest percentages in this regard. To accurately assess the biomass potential, it was essential to include both the caloric value and dry matter content. In our research, the net CV for all examined field crops has amounts of 16.6 Mj/kg (Table 5). The caloric value of fresh straw for wheat range between 12.9 - 14.9 MJ/kg for barley 12.0 - 13.9 MJ/kg and 3.3 - 7.2 MJ/kg for maize, and the CV of dry straw is 15 - 17 MJ/kg (Tymiński 1997, Piskier 2017). Jóvér et al., (2018), confirmed CV of wheat at 16.55 MJ/kg, corn at 17.51 MJ/kg and sunflower at 14.39 MJ/kg respectively. For comparision, the caloric value of bio-oil, which is approximately 50-55% of that of diesel oil is 16-18 MJ/kg (Bal and Piechocki, 2006). Wood harvested annually has a CV of 18.55 MJ/kg, and wood harvested every three years has a value 19.56 MJ/kg (Szczukowski et al., 2006). The gross CV of different agroforestry species and bio-based industry residues range from 14.3 - 25.4 MJ/kg (Gravalos et.al, 2016). The second measurement point used in energy potential estimation method are dry matter content, whose average value for the eight field crops is 87%. DM content directly affecting combustion efficiency, and is chosen instead of ash content or chemical composition due to direct impact on extractable energy from biomass (Guzmán-Bello, et al., 2023).

Table 5. Energy potential of biomass from field crops (MJ, GJ)

		Wheat	Barley	Maize	Rice	Sunflower	Rye	Tobacco	Oats	Sum
Total biomass production	t	291155	176029	150321	31877	14044	15157	35036	8305	721924
	kt/y	291.2	176.0	150.3	31.9	14.0	15.2	35.0	8.3	722
Yield biomass	kg/ha	4025	4026	4754	8503	3041	3861	2172	2562	
Caloric Value	x̄ Mj/kg	16.92	16.45	17.15	15.78	15.11	17.20	15.84	18.05	x̄ 16,6
Dry Matter	DMC %	88.0	88.0	88.0	88.0	78.6	88.0	90.0	88.0	x̄ 87.0
Approach I EP	MJ/t	492634	289567	257800	503023	212207	26069	554976	14990	1208081
	MJ/kt/y	4926	2896	2578	503	212	261	555	150	12081
	MJ/kg/ha	68100	66226	81536	13418	45949	66415	34400	46246	543054
	GJ	4926	2896	2578	503	212	261	555	150	12081
	GJ/kt/y	4.9	2.9	2.6	0.5	0.2	0.3	0.6	0.1	12
	GJ/kg/ha	68.1	66.2	81.5	134.2	45.9	66.4	34.4	46.2	543
Approach II BEP	MJ	433518	254819	226864	442661	166795	22940	499478	13191	1062227
	MJ/kt/y	4335	2548	2269	443	167	229	499	132	10622
	MJ/kg/ha/	59928	58279	71752	11807	36116	58446	30960	40697	474256
	GJ	4335	2548	2269	443	167	229	499	132	10622
	GJ/kt/y	4.34	2.55	2.27	0.44	0.17	0.23	0.50	0.13	11
	GJ/kg/ha/	59.9	58.3	71.8	118.1	36.1	58.5	30.96	40.70	474
I-II / LSD	GJ	591*	347*	309*	60	45	31	55	18	1459

* Literature datasets for CV and DM (Tab 2,3), biomass potential from used approaches (Guzmán-Bello et al., 2023; Batista et al., 2023), differences between approaches and LSD test at level of $p>0.05$.

The theoretical energy potential of the biomass from the examined field crops, shows a total potencial of 12081GJ in the first approach and whare dry matter contet is includ in the assessment the potential is lower at 10622 GJ. The difference in the mesure sum of total energy production between first and secund aproach was 1495 GJ. The energy potential of individual field crops varies based on the amount of total arable land (Tab. 4). Wheat, grown on 72606 ha total arable land, has energy potential of 4926 GJ in first and 4335 GJ in second approach, resulting in a differences of 591 GJ. The other two most common field crops, barleys and maize, achieve energy potential of 2896 and 2578 GJ in first, and 2548 GJ and 2269 GJ in

second approach, with 347 GJ i.e. 309 GJ respectively. Comparing two approaches at a level of 0.05, significant differences were only found in wheat, barley, and maize, or crops that are grown on larger arable land (wheat, barley) and produce higher biomass (Table 5).
Significant amounts of agricultural residues contributing to bioenergy production are available at EU level (1,530 PJ), with the largest quantities available in Germany, Romania, France, Spain and Italy (Bădan et al., 2020). The amount of electrical energy calculated per capita is about 70 GJ per year, which is equivalent to 2.5 t of coal or 1.6 t of crude oil (Pabis et al., 2015). In our case this energy requirement can be met from the annual harvest of maize which provides 71.8 GJ, and rice 118.1 GJ (Table 5).

Conclusion

The potential of the biomass from the primary field crops represented in Macedonia, which some of them are used in the livestock sector as fodder, can also be analyzed from another perspective as a significant energy potential. By applying and comparing two different approaches, we estimated a theoretical energy potential of 12,081 GJ in the first and 10,622 GJ in the second approach. The significant difference of 1495 GJ between the approaches, and at wheat, barley and maize, highlights the greater relevance of the BEP Method, for measuring the energy potential of field crops based on total biomass production.

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CORRELATION ANALYSIS OF CORN BIO-MORPHOLOGICAL TRAITS IN THE INITIAL GROWTH PHASE UNDER DIFFERENT TEMPERATURE CONDITIONS

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Abstract

Germination and seedling development are essential phases in a plant life cycle that are significantly influenced by non-optimal temperatures. This study aimed to examine the bio-morphological traits of various corn hybrids in the initial growth phase, the seeds of which were exposed to non-optimal temperatures. Some of the seeds of super sweet corn (P300), semi-flint corn (P402) and popcorn (P398) were germinated for seven days at +10^0 C (cold-test), after which they were transferred to optimal conditions, and some of the seeds were kept for 30 minutes at +50^0 C before germination. The bio-morphological traits, such as overall seed germination, root and shoot length, root and shoot biomass, root and shoot vigor and metabolic efficiency of tested seeds were compared with those of control seeds, which germinated in optimal conditions. An assessment of the relationship between metabolic efficiency and other traits to predetermine the limits of corn resistance to abiotic factors, especially to non-optimal temperature was carried out. Metabolic efficiency was strongly correlated with overall germination (0.8609-0.9603), root and shoot length (0.8392-0.9131), root and shoot biomass (0.9131-0.9911) of control seeds germinated in optimal conditions. The metabolism of seeds exposed to non-optimal temperatures before germination changes significantly. The correlation coefficients decreased, and the correlation in the P402 cold-test and P300 high temperature experiments became inverse. Metabolic efficiency of semi-flint corn P402, seeds exposed to +50^0 C was highly correlated with root and shoot length (0.9305-0.9883); and root and shoot vigor (0.8118-0.9696). The data obtained make it possible to differentiate corn hybrids according to their resistance to temperature.

Keywords: *corn, seed, germination, bio-morphological traits, correlation.*

Introduction

Each varieties of corn has the ability to adapt to changing environmental conditions within limits determined by his genotype. The most cultivated high-yielding corn hybrids with a tooth-shaped and flinty endosperm structure, from which is produced flour, alcohol and animal feed, less demanding on temperature and humidity, than hybrids of popcorn and sweet corn. Popcorn, which is used in the production of flakes and other similar products, is the most sensible type of corn and is characterized by lower biological adaptability to various growing conditions. Sweet corn is a vegetable crop that uses in the canning industry and needs irrigation for growing like all vegetable crops. Many breeders make special efforts to find corn lines and hybrids characterized by more vigorous germination, more powerful seedlings, high initial growth rate in cold and wet soils and normal growth at relatively low and high temperatures (Miedaner and Juroszek, 2021; Khaeim *et al.*, 2022). The abiotic stress hazards seriously affect the yield of corn; however, the genetic basis of temperature adaptation, especially to low temperature, in corn is still poorly understood (Jiao *et al*., 2022).
The higher ability to produce metabolic substances necessary for plant life under stress, the wider the reaction norm of a given plant and the better its capacity to adapt (Borovskij, 2013;

Gupta *et al*., 2013). This property is especially important for edible corn hybrids, since they are more demanding on growing conditions. Changes in corn traits due to temperature stress should be used for diagnostic purposes to realize the potential of plant resistance. Among the variety of physiological traits, the intensity of growth reactions as an integral indicator of the metabolic activity of plants at extreme temperatures is most closely related to the general resistance of plants to stress and is methodically accessible for determination.
Knowledge of interactions between features related to physiological traits and yield is very important in the breeding and cultivation of corn to determine the degree of influence of growing conditions on them (Jayakumar *et al*., 2007; Amin *et al*. 2013). Early it was reported (Kinfe *et al*., 2015; Sharma et al., 2021) that the grain yield of the corn hybrids was significantly positively correlated with yield attributing traits (cob diameter, number of kernels per row, number of kernel rows per cob, etc.). Establishing correlations between highly heritable valuable traits of hybrids for a more complete analysis of their resistance to temperature conditions of germination and development could be powerful for increasing the efficiency of technological methods. This study aimed to determine the bio-morphological traits and examine their relationships in the initial growth phase of various corn hybrids seeds exposed to non-optimal temperatures.

Materials and methods

The studies were carried out in 2024 in the Institute of Genetics, Physiology and Plant Protection (IGFPP), Moldova State University, Republic of Moldova.
Corn seeds of hybrids: Porumbeni 300 sh-2 (P300), super sweet corn, FAO 300, mid ripening; Porumbeni 398 (P398), popcorn FAO 400, mid-late-ripening; and Porumbeni 402 (P402), semi-flint corn, FAO 400, mid-late-ripening were generously contributed by the National Center of Research and Seed Production (Institute of Crop Science „Porumbeni”).
Laboratory testing. Seeds pre-soaked in water for 24 hours were subjected to heat stress at 50°C for 30 minutes (heat test). Germination of intact (control) and heated seeds was carried out in accordance with the provisions of international rules (ISTA. 2017). To conduct the cold test, seeds were germinated for seven days at temperature of 10°C, and then 4 days at 25°C (Goggi *et al.*, 2009; Zhao *et al.*, 2022). After germination, the overall seed germination, root and shoot length, root and shoot biomass, root and shoot vigor and metabolic efficiency of tested seeds was assessed. The biomass of reserve substances that was spent for respiration during seed germination was calculated (Dascaliuc *et al.*, 2020; Borovskaia *et al.*, 2023).
Statistical analysis. Pearson correlation coefficients of relationships between studied traits were determined by Excel and Statistica 7 software.

Results and discussion

The seeds of tested hybrids germinated in optimal conditions (control) demonstrated the highest positive correlation (≥0.8609) between different traits, conjoint of which were seed germination -metabolic efficiency; root length – shoot length; and root vigor – shoot vigor (Table 1). At the same time, other strong relationships between the traits of each individual hybrid in initial growth phase were identified. The number of positive correlated relationships (≥ 0.6029) varied depending on the corn hybrid: in super sweet P300, correlation was observed for 17 traits, popcorn P398 for 8 traits, semi-flint P402 for 16 traits (Table 1). Strong correlation (≥ 0.9297) between root biomass and its length was evaluated for super sweet corn (P300) and semi-flint corn (P402); and between shoot biomass and its length – only for semi-flint corn. The metabolic efficiency of corn seeds had a direct dependence on the root biomass accumulated during germination under optimal conditions and a strong negative dependence on the biomass of seed reserve substances spent on respiration (Table 1).

Table 1. Pearson correlation coefficients of corn traits in the initial growth phase, control

Trait	Metabolic efficiency	Seed germination	Length		Biomass		Vigor	
			Root	Shoot	Root	Shoot	Root	Shoot
P300								
Seed germination	*0.8609*	*1*						
Root length	*0.9131*	*0.8210*	*1*					
Shoot length	*0.5331*	*0.1857*	*0.7127*	*1*				
Root biomass	*0.9911*	*0.9179*	*0.9297*	*0.4861*	*1*			
Shoot biomass	*-0.3510*	*-0.3639*	*0.0444*	*0.4851*	*-0.3263*	*1*		
Root vigor	*0.9268*	*0.9130*	*0.9822*	*0.5685*	*0.9593*	*-0.0746*	*1*	
Shoot vigor	*0.9393*	*0.8959*	*0.9900*	*0.6029*	*0.9646*	*-0.0747*	*0.9984*	*1*
Biomass spent for respiration	*-0.9998*	*-0.8710*	*-0.9134*	*-0.5207*	*-0.9934*	*0.3553*	*-0.9306*	*-0.9420*
P398								
Seed germination	*0.9450*	*1*						
Root length	*-0.0707*	*0.1748*	*1*					
Shoot length	*-0.4688*	*-0.3021*	*0.8677*	*1*				
Root biomass	*0.7723*	*0.9037*	*0.1115*	*-0.3964*	*1*			
Shoot biomass	*0.1917*	*0.1331*	*0.6169*	*0.3770*	*0.4265*	*1*		
Root vigor	*0.7673*	*-0.8745*	*0.5847*	*0.1845*	*0.6814*	*0.2179*	*1*	
Shoot vigor	*0.4379*	*0.5752*	*-0.8300*	*0.5859*	*0.3414*	*0.2611*	*0.8998*	*1*
Biomass spent for respiration	*-0.9499*	*-0.9262*	*-0.1967*	*0.1769*	*-0.6756*	*0.1507*	*-0.9032*	*-0.6855*
P402								
Seed germination	*0.9603*	*1*						
Root length	*0.8392*	*0.9575*	*1*					
Shoot length	*0.9074*	*0.9869*	*0.9868*	*1*				
Root biomass	*0.9131*	*0.9903*	*0.9874*	*0.9949*	*1*			
Shoot biomass	*0.9180*	*0.9880*	*0.9779*	*0.9834*	*0.9966*	*1*		
Root vigor	*0.2480*	*-0.0302*	*-0.3154*	*-0.1815*	*-0.1616*	*-0.1276*	*1*	
Shoot vigor	*0.1949*	*-0.0840*	*-0.3700*	*-0.2350*	*-0.2137*	*-0.1784*	*0.9984*	*1*
Biomass spent for respiration	*-0.9991*	*-0.9711*	*-0.8610*	*-0.9236*	*-0.9293*	*-0.9334*	*-0.2081*	*-0.1548*

The metabolism of seeds exposed to non-optimal temperatures before germination changes significantly. The relationships between traits were modified both qualitatively and quantitatively (Table 2). Correlation coefficients for traits of corn development in the initial phase of growth from seeds exposed to $+50^0$ C before germination decreased. However, popcorn (P398) and semi flint (P402) hybrids showed a high positive correlation between root length and dry biomass (0.6660-0.7791), as well as between root and shoot vigor (0.8686-0.9673). The number of traits which had strong positive relationships (coefficients of correlation $\geq$0.75) diminished by 4 times for P300, 1.6 times for P398 and 2.3 times for P402 compared to control corn (Table 1, 2). Considering that the metabolic efficiency of the P402 hybrid was strong correlated with root/shoot length and root biomass at the level of control seeds, it can be assumed that this hybrid is thermotolerant. Therefore, acceleration of root and shoot growth of semi flint corn after heat stress was predermined by high metabolic efficiency of its seeds. Seeds of the P300 corn were more sensitive to heat stress. The metabolic efficiency of super sweet corn exposed to heat stress was negatively correlated with all studied traits (Table 2).

Table 2. Pearson correlation coefficients of corn traits in the initial growth phase, heat test

Trait	Metabolic efficiency	Seed germination	Length		Biomass		Vigor	
			Root	Shoot	Root	Shoot	Root	Shoot
P300								
Seed germination	*-0.0435*	*1*						
Root length	*-0.3948*	*0.9334*	*1*					
Shoot length	*-0.5556*	*-0.4792*	*-0.2790*	*1*				
Root biomass	*-0.0851*	*-0.2122*	*-0.2179*	*0.7995*	*1*			
Shoot biomass	*0.7532*	*-0.6869*	*-0.9013*	*-0.0467*	*0.1390*	*1*		
Root vigor	*-0.0846*	*0.9991*	*0.9468*	*-0.4503*	*-0.2012*	*-0.7157*	*1*	
Shoot vigor	*-0.4222*	*0.1719*	*0.2600*	*0.7480*	*0.8717*	*-0.3620*	*0.1952*	*1*
Biomass spent for respiration	*-0.9572*	*-0.1297*	*0.1359*	*-0.1100*	*-0.6808*	*-0.3976*	*-0.1104*	*-0.4747*
P398								
Seed germination	*0.5067*	*1*						
Root length	*0.0795*	*0.7358*	*1*					
Shoot length	*-0.0215*	*-0.1000*	*0.4272*	*1*				
Root biomass	*-0.1124*	*0.4215*	*0.9214*	*0.7647*	*1*			
Shoot biomass	*0.9832*	*0.4504*	*0.1352*	*-0.1574*	*0.0020*	*1*		
Root vigor	*0.5700*	*0.9842*	*0.7876*	*-0.0598*	*0.5125*	*0.5429*	*1*	
Shoot vigor	*0.5993*	*0.9077*	*0.8400*	*-0.2998*	*0.6407*	*0.6177*	*0.9673*	*1*
Biomass spent for respiration	*-0.9963*	*-0.4951*	*-0.0200*	*0.1053*	*0.1887*	*-0.9653*	*-0.5454*	*-0.5561*
P402								
Seed germination	*0.1889*	*1*						
Root length	*0.8156*	*0.3881*	*1*					
Shoot length	*0.8888*	*0.4427*	*0.6002*	*1*				
Root biomass	*0.8768*	*-0.2085*	*0.7791*	*0.5661*	*1*			
Shoot biomass	*-0.3691*	*0.1491*	*-0.7237*	*0.0619*	*-0.6733*	*1*		
Root vigor	*0.2460*	*0.9645*	*0.5585*	*0.3873*	*-0.0653*	*-0.1136*	*1*	
Shoot vigor	*0.4796*	*0.9316*	*0.4900*	*0.7354*	*0.0382*	*0.1863*	*0.8696*	*1*
Biomass spent for respiration	*-0.9685*	*-0.1993*	*-0.6628*	*-0.9556*	*-0.7698*	*0.1294*	*-0.1901*	*-0.5263*

It should be noted that in all tested hybrids, both control (Table 1) and those exposed to heat/cold stress (Table 2, 3), metabolic efficiency had a strong correlation with the biomass of reserve substances spent on respiration during seed germination (r = 0.9572-0.9999). The shoot vigor and root vigor correlated positively for all studied hybrids: control, heat and cold tested seeds.

The relationship between the traits of corn exposed to cold germination was significantly different from that of the control and heat-treated corn. Common to all cold-tested hybrids was a positive correlation between root/shoot vigor and their biomass, which was not found for control and heat-stressed corn seeds (Table 3). The root/shoot vigor of the P300 and P 398 hydrides, seeds of which were germinated in cold conditions, strong correlated with their root/shoot length and biomass (0.7902-0.9749). In addition, root/shoot vigor of these hybrids had the good correlation with all studied traits. The strong correlation (0.8470-0.9848) was also established between shoot vigor and shoot length/biomass of hybrid P402 (Table 3). Since the cold test is considered most important for the vigor of corn seed (Sena *et al.*, 2017), it can be concluded that the seeds of the P300 and P398 hybrids were more resistant to cold treatment compared to the P402 hybrid. The resistance to temperature stress is controlled by the corn’s own genetic constitution, ambient temperature and their interaction. Zhao *et al.* (2022) showed that the low temperature imposed higher influences on growth and yield traits of corn. Thus, according changes in traits relationships of corn in the initial growth phase under influence non-optimal temperatures, the P300 super sweet corn was the most sensible to heat stress and P402 semi-flint – to cold stress.

Table 3. Pearson correlation coefficients of corn traits in the initial growth phase, cold test

Trait	Metabolic efficiency	Seed germination	Length		Biomass		Vigor	
			Root	Shoot	Root	Shoot	Root	Shoot
P300								
Seed germination	*0.5316*	*1*						
Root length	*0.6007*	*0.8071*	*1*					
Shoot length	*0.7292*	*0.3539*	*0.7978*	*1*				
Root biomass	*0.9738*	*0.5713*	*0.7435*	*0.8567*	*1*			
Shoot biomass	*0.7025*	*0.3767*	*0.8235*	*0.9983*	*0.8398*	*1*		
Root vigor	*0.7100*	*0.9308*	*0.9460*	*0.6713*	*0.7902*	*0.6883*	*1*	
Shoot vigor	*0.8522*	*0.7239*	*0.9300*	*0.8916*	*0.9406*	*0.8946*	*0.9229*	*1*
Biomass spent for respiration	*-0.9968*	*-0.5863*	*-0.6113*	*-0.6933*	*-0.9650*	*-0.6683*	*-0.7394*	*-0.8521*
P398								
Seed germination	*0.6800*	*1*						
Root length	*0.4854*	*0.8788*	*1*					
Shoot length	*0.5482*	*0.2021*	*0.4550*	*1*				
Root biomass	*0.5976*	*0.7875*	*0.4030*	*-0.2839*	*1*			
Shoot biomass	*0.4961*	*0.5502*	*0.8279*	*0.8618*	*-0.0523*	*1*		
Root vigor	*0.5734*	*0.9623*	*0.9749*	*0.3323*	*0.5967*	*0.7144*	*1*	
Shoot vigor	*0.7397*	*0.6271*	*0.7800*	*0.8892*	*0.1317*	*0.9496*	*0.7185*	*1*
Biomass spent for respiration	*-0.9966*	*-0.6712*	*-0.4402*	*0.4830*	*-0.6406*	*-0.4252*	*-0.5442*	*-0.6816*
P402								
Seed germination	*0.3290*	*1*						
Root length	*-0.7069*	*0.0503*	*1*					
Shoot length	*-0.5115*	*0.6333*	*0.7015*	*1*				
Root biomass	*0.1388*	*0.3349*	*0.5971*	*0.3148*	*1*			
Shoot biomass	*-0.0575*	*0.8799*	*0.5188*	*0.8776*	*0.5673*	*1*		
Root vigor	*0.4695*	*0.0341*	*0.1898*	*-0.2316*	*0.8489*	*0.1149*	*1*	
Shoot vigor	*0.0236*	*0.9409*	*0.3800*	*0.8470*	*0.4485*	*0.9848*	*0.0173*	*1*
Biomass spent for respiration	*-0.9999*	*-0.3167*	*0.7038*	*0.5210*	*-0.1450*		*-0.4805*	*-0.0124*

Conclusions

Based on the results of correlation analysis, it was established that the strong relationship between the metabolic efficiency of corn seeds and other traits of its development in the initial phase depends on both the temperature conditions of germination and type of corn hybrid. Our results confirmed the possibility of using corn seeds growth traits and their relationships to study the characteristics of the reaction of seeds during germination and for a comparative assessment of the resistance of corn hybrids to non-optimal temperature.

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EVALUATING STRAWBERRY CULTIVAR PERFORMANCE AND RUNNER HEALTH ACROSS VARIED CLIMATIC ZONES IN POONCH DISTRICT, AZAD JAMMU AND KASHMIR IN PAKISTAN

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Abstract

Strawberries are a popular fruit valued for the outstanding flavor and nutritious composition. The current climate in the area where the runners are grown has a major impact on the productivity of strawberry nurseries. The climate in Azad Jammu and Kashmir is suitable for growing superior strawberry runners; nevertheless, uniform standards on site selection and growth techniques for various cultivars are required. In order to close this gap, we conducted three separate studies in the Poonch district to evaluate the runner health of three different strawberry cultivars: ‘Chandler’, ‘Sea Scape’, and ‘Tribute’. During this study the performance of these cultivars was assessed at Chotta Galla, Khai Galla, and Hajira (Azad Jammu & Kashmir) using a randomized complete block design (RCBD). Our results showed that temperature affected the number of runners produced; cooler climates encouraged vigorous runner growth, while warmer climates were associated with larger yields and higher fruit quality. Furthermore, differences in flower and yields among cultivars were recorded; some showed fruit yields that were limited, which was directly related to the ratio of fruit to leaf. All in all, our research highlights the significance of taking site-specific factors into account while growing strawberries, with a focus on how temperature affects the dynamics of runners and yield. These discoveries aid in the creation of customized plans for maximizing strawberry yield under various climate scenarios.

Keywords: *Strawberry, Fruit, Quality, Runners.*

Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is a member of the *Rosaceae* family. It is among the sweetest and tastiest fruits. Strawberry has a unique place among all berries due to its charming red colour and its nutritive value. Though, it is a fruit of temperate region, but it can be grown in tropical and sub-tropical regions as well. Botanically, it is a highly perishable aggregate fruit. According to Finn and Strike (2008), in temperate climates, the plants exhibit the characteristics of a small perennial herb with a shallow root system, but in sub-tropical climates, they behave more like annuals. It is one of the delicious fruit due to its flavor, aroma and nutritional properties. It possesses a unique place among all the berry fruits due to its charming red color. This small berry fruit is fully loaded with phytochemical, volatile compounds, sugars and acids. Additionally, they contain high concentrations of phenolic compounds, including as phenolic acids, hydrolyzable tannins, and anthocyanins. The fruit has a lot of nutrients and vitamins. The two main components of strawberries are water (91%) and carbohydrates (7.7%). They have a negligible 0.7% fat content. Less than 50 calories are found in one cup (150 g) of whole strawberries. Less than 6 g of digestible carbohydrates are included in every 100 g of strawberries. Fruit's flavor is primarily influenced by three types of components: sugars, acids, and aromatic compounds. The fruit of the strawberry has 0.5%

total sugar and between 0.90% and 1.85% organic acids, with citric and malic acids predominating.
There are more than 20 species and 600 varieties of strawberries in the world (Mondal, 2010). All these varieties vary in their nutritional composition, size, flavor and color. The world production of strawberry is around 9.2 million tons (FAO, 2017). The largest producer of strawberries in the world is China with annual production of 3.7 million tons followed by USA and Mexico (FAO, 2017). Strawberry production in Pakistan is very low as compared to other areas of the world as Pakistan produced 609 t strawberries per year. This less yield of strawberry is mainly due to lack of research, improper cultivation practices and dependence on only one variety 'Chandler' (Aziz et al., 2018).
Since last few years, area under strawberry production is increasing rapidly in sub-continent countries (Singh et al., 2007). However, its production in Pakistan is limited due to certain environmental factors and lack of knowledge. Our preliminary investigation has revealed that at least eight cultivars 'Chandler', 'Sequoia', 'Camino Real', 'Sea Scape', 'Tribute', 'Corona', 'Stuff' and 'Mark' are available in Pakistan. However, it has been reported that 'Chandler' is the only cultivar which is grown commercially throughout the country (Anwar *et al*., 2018). This might be due to the lack of quality runners which only produced in Mangora and Dir districts, and supposed to be the main which hinders strawberry production (Rajwana et al., 2016).
The climate of Rawalakot suits the nursery production of strawberry and provides good quality runners of different strawberry cultivars. Rawalakot, located at 33°51'32.18"N latitude, 73° 45'34.93"E longitude and 1638 m altitude, is a small town of district Poonch, which seems an ideal place for growth and production of strawberry crop (Hayat et al., 2022). However, till-to-date no trial for nursery production of strawberry has been conducted at different locations of district Poonch. Exploring different areas for strawberry runner production may not only provide multi-sourcing opportunities for farmers/suppliers but may also ensure sustainable supply of healthy runners of different cultivars. Considering the above facts, the present work has been designed to evaluate the runner health of three strawberry cultivars 'Chandler', 'Sea Scape' and 'Tribute' in different areas of Poonch district.

Materials and Methods

Study area

This study was performed at three different locations: Chota Galla (Latitude: 33° 48' 59.21"; Longitude: 73° 49' 19.74" ˚E; 1918 m.a.s.l.), Khai Galla (Latitude: 33° 84' 65" N; Longitude: 73° 82' 87" E; 1736 m a.s.l.) and Hajira (Latitude: 33° 46' 18.12" N; Longitude: 73° 53' 45.96" E; Elevation 3168 m a.s.l.) of Azad Jammu and Kashmir in three different strawberry varieties ('Chandler, 'Sea Scape', 'Tribute'). The study was carried out during consecutive seasons of year 2022 and 2023. Land was prepared according to standard management practices and soil analysis was conducted before the start of experiment. Beds (30 cm above soil level) were prepared. Width of each bed was 45 cm having two strawberry rows and distance between plants was30 cm. Bed to bed distance was maintained at 45 cm.
For runner production, diseased-free mother plants were planted at a distance of 30.48 cm and runners were harvested and propagated. After plug plant production, these plants were planted in field for fruit yield and quality.

Number of runners per plant

Numbers of runner per plant were counted and their average was recorded.

Leaf area (cm2)

With the aid of a leaf area meter, the leaf area of three fully developed leaves from each plant was measured and data were showed in . After that, average leaf area per plant was calculated.

Number of flowers per plant
Flowers per plant were counted from selected plants of each cultivar from first flower opening to full bloom.

Number of fruits per plant
From randomly chosen plants, the number of fruits per plant was counted.

Statistical Analysis
Three treatments and three replications were used in the randomized complete block design (RCBD) experiment setup. SAS software was used to do statistical analysis utilizing the analysis of variance (ANOVA) technique. The Least Significant Difference (LSD) test was used to examine treatment mean differences at a 5% probability level.

Results and Discussion

Number of runners per plant
It was observed that results regarding number of runners per plant were significantly different among cultivars and locations (Table 1). The highest number of runners per plant was found in strawberry ‘Chandler’ (8.55) which was followed by strawberry ‘Sea Scape’ (6.00), while the lowest number of runners per plant was noted in strawberry ‘Tribute’ (2.55). Whereas, the strawberry plants transplanted at Chotta Galla location showed the highest number of runners per plant (9.77) which was followed by Khai Galla location (6.44), while the lowest number of runners per plant were recorded at Hajira location (0.88) . Current study revealed that runner production is high in the areas where temperature is low and flower and fruit production is more in the areas where temperature is high. Our results are in line with the previous findings that runners, are high in the area (Chotta Galla) where flower and fruit production is low. However, it was observed that flowering and runner production in strawberry depends on the temperature of the cropping year (Sønsteby et al., 2022).

Table 1. Effect of different locations on number of runners per plant of three strawberry cultivars.

Cultivar	**Locations**			**Means**
	Chotta Galla	Khai Galla	Hajira	
‘Chandler’	15.0 a	9.00 bc	1.66 e	**8.55 A**
‘Sea Scape’	11.0 ab	6.33 cd	0.66 e	**6.00 B**
‘Tribute’	3.30 de	4.00 de	0.33 e	**2.55 C**
Means	**9.77 A**	**6.44** B?	**0.88 C**	

* different lettering indicate statistically differences at $P < 0.05\%$.

Leaf area
It was observed that results regarding leaf area were significantly different among cultivars and locations under study (Table 2). The maximum leaf area was found in strawberry ‘Chandler’ (144.51 cm^2) which was followed by strawberry ‘Sea Scape’ (119.90 cm^2), while the minimum leaf area was noted in strawberry ‘Tribute’ (89.40 cm^2). Whereas, the strawberry plants transplanted at Hajira location showed the maximum leaf area (125.33 cm^2) which was followed by Khai Galla location (115.46 cm^2), while the minimum leaf area were recorded at Chotta Galla location (113.02 cm^2). It was reported that in some strawberry cultivars flower and fruit production is source limited and is directly proportional to leaf area (Sønsteby et al., 2021). These findings are notably evident that high temperature and low rainfall at Hajira location increased microbial activity in the soils results in availability of

nitrogen to plants helps in increased leaf area (Purbajanti et al., 2019). In our results, highest leaf area was obtained at Hajira location, with high temperature and less rainfall than Chotta Galla nad Khai Galla location which is helpful in increased microbial activities.

Table 2. Effect of different locations on leaf area (cm^2) of three strawberry cultivars.

Cultivar	**Locations**			**Means**
	Chotta Galla	Khai Galla	Hajira	
‘Chandler’	139.2 ab	136.0 b	158.3 a	**144.51 A**
‘Sea Scape’	110.4 cd	127.2 bc	122.0 bc	**119.90 B**
‘Tribute’	89.4 de	83.1 e	95.6 de	**89.40 C**
Means	**113.02 B**	**115.46 B**	**125.33 A**	

* Different lettering indicate significant statistically differences at $P < 0.05\%$.

Number of flowers per plant

It was observed that results regarding number of flowers per plant were different among cultivars and locations (Table 3). The highest number of flowers per plant was found in strawberry ‘Chandler’ (11.9) followed by strawberry ‘Sea Scape’ (7.8), while the lowest number of flowers per plant was noted in strawberry ‘Tribute’ (5.7). Whereas, the strawberry plants transplanted at Hajira location showed the highest number of flowers per plant (11.3) which was followed by Chota Galla (7.2), while the lowest number of flowers per plant were recorded at Khai Galla (6.8). High temperatures cause the organic matter in the soil to decompose, increasing the amount of nutrients available and stimulating microbial activity in the soil (Broadbent, 2015). Plant growth patterns are enhanced by this nutrient availability (Mohamed et al., 2021). Consequently, plants growing in the Hajira location have the greatest number of flowers and fruits, which may be related to the amount of N, P, K, and other hormones in the soil. These were essential for the synthesis of gibberellic acid in the roots, which broke the dormant state of the buds. This led to a rise in flowering sites, an increase in bud development, and an early commencement of the reproductive stage (Kumar et al., 2018).

Table 3. Effect of different locations on number of flowers per plant of three strawberry cultivars.

Cultivar	**Locations**			**Means**
	Chotta Galla	Khai Galla	Hajira	
‘Chandler’	11.3 ab	10.37 ab	14.0 a	**11.9 A**
‘Sea Scape’	5.7 cd	6.07 cd	11.6 ab	**7.8 B**
‘Tribute’	4.7 cd	4.07 d	8.3 bc	**5.7 C**
Means	**7.27 B**	**6.8 B**	**11.3 A**	

* Different lettering indicate significantly statistically differences at $P < 0.05\%$.

Number of fruits per plant

It was observed that results regarding number of fruits per plant were significantly different among cultivars and locations (Table 4). The highest number of fruits per plant was found in strawberry ‘Chandler’ (8.9) followed by. ‘Sea Scape’ (4.8), while the lowest number of fruits per plant was noted in strawberry ‘Tribute’ (2.8). Whereas, the strawberry plants transplanted at Hajira location showed the highest number of fruits per plant (7.4) followed by Chota Galla (4.8), while the lowest number of flowers per plant were recorded at Khai Galla (4.3). According to Broadbent (2015), elevated temperatures cause microbial activity in the soil to rise, which in turn breaks down organic matter in the soil and makes nutrients available. Plants develop more efficiently when these nutrients are available (Mohamed et al., 2021). The abundance of N, P, K, and other hormones in the soil may be the reason why plants cultivated in the Hajira area bear the most blooms and fruits. These were essential for the synthesis of gibberellic acid in the roots, which broke the dormant state of the buds. This brought about a rise in flowering sites, an early commencement of the reproductive stage, and an increase in bud production (Kumar et al., 2018). Thus the increased bud production and increased flowering sites results in increased yield. Nevertheless, yield might be cultivar and species specific (Mardanluo et al., 2018).

Table 4. Effect of different locations on fruits per plant of three strawberry cultivars.

Cultivars	**Locations**			**Means**
	Chotta Galla	Khai Galla	Hajira	
'Chandler'	8.7 ab	7.0 bc	11.0 a	**8.8 A**
'Sea Scape'	3.6 d	3.6 d	7.3 b	**4.8 B**
'Tribute'	2.0 d	2.3 d	4.0 cd	**2.7 C**
Means	**4.77 B**	**4.33 B**	**7.44 A**	

* Different lettering indicate significantly statistically differences at P < 0.05%

Conclusions

The findings of this study indicate that strawberry plants transplanted in cooler locations exhibit a higher capacity for producing abundant and robust runners. On the other hand, plants transplanted in slightly warmer locations demonstrated superior performance in terms of fruit production.

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ENHANCING APPLE QUALITY ASSESSMENT THROUGH ANOMALY DETECTION WITH AUTOENCODERS

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Abstract

A more accurate assessment of apple quality is paramount for various industries, notably in agriculture and food sectors, ensuring economic efficiency and consumer satisfaction. The conventional assessment methods encounter obstacles related to labor intensity, speed, and maintaining quality control standards. This paper presents an alternative perspective, focusing on anomaly detection employing autoencoder techniques to enhance apple quality assessment in a cost-effective manner. Autoencoders are trained on a dataset comprising over 2700 images of healthy apples, offering an economical means of learning representations of healthy apples. These autoencoders proficiently identify anomalies, particularly those indicative of spoiled or damaged apples, streamlining their removal from the sorting process. Additionally, compared to typical CNN architectures, autoencoders provide the advantage of unsupervised learning, as they do notn't require labeled data for training, making them more versatile and adaptable to various datasets and scenarios. The study explores a range of autoencoder architectures and hyperparameter configurations to optimize anomaly detection performance. Initial findings indicate promising results in leveraging autoencoders for identifying spoiled apples, offering potential improvements in sorting efficiency, decreased labor costs, and strengthened quality control procedures.

Keywords: *autoencoder, fruit sorting, deep learning, neural network, convolutional network.*

Introduction

Autoencoders are neural networks designed to learn a compressed representation of input data through an encoder-decoder architecture. The encoder compresses the input into a latent space representation, while the decoder reconstructs the input from this representation. By learning to accurately reconstruct the original images, the autoencoder develops a robust understanding of typical fruit characteristics, including color, texture, and shape. Consequently, when presented with anomalous or spoiled fruit images during inference, the reconstructed outputs deviate significantly from the original inputs, thereby signaling potential defects.
Compared to conventional Convolutional Neural Networks (CNNs) typically utilized for image classification tasks (Taner *et al.*, 2024), autoencoders offer several advantages in the context of fruit quality detection. Firstly, autoencoders operate in an unsupervised manner, eliminating the need for labeled data during training, which can be costly and time-consuming to acquire. Secondly, autoencoders inherently prioritize feature extraction and representation learning, focusing on capturing the most important aspects of the input data (Ventura *et al.*, 2022).

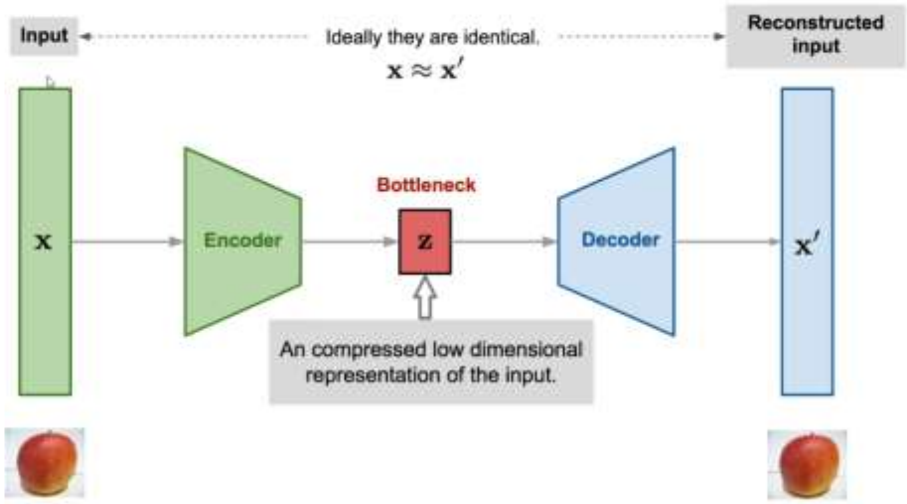

Figure 1: Autoencoder model architecture
(adapted from https://lilianweng.github.io/posts/2018-08-12-vae - Weng, 2024)

Moreover, the compact latent space representation learned by autoencoders facilitates efficient storage and inference, making them suitable for deployment in resource-constrained environments such as agricultural facilities or mobile inspection units. When an apple image deviates significantly from this learned representation - indicative of potential defects or poor quality - the autoencoder's output will exhibit greater reconstruction errors. These errors can then be quantified to flag substandard apples efficiently (Adari *et al.*, 2024).
In this article, we explore the advantages offered by autoencoders and present experimental results demonstrating the efficacy of autoencoder-based models in real-world fruit quality inspection scenarios.
Previous researches have demonstrated the effectiveness of CNNs, especially in classification and sorting operations, and similarly highlighted the potential of autoencoders.
Xue *et al.* (2020) detail the development of a novel deep learning framework specifically designed for the classification of fruit images. This framework, named CAE-ADN, integrates a convolution autoencoder (CAE) and an attention-based densely connected convolutional network (DenseNet) to effectively pre-train and classify fruit images, enhancing operational efficiency across various stages of the fresh produce supply chain. The model achieved an impressive classification accuracy of 95.86% on the Fruit 26 dataset and 93.78% on the Fruit 15 dataset.
Aiadi *et al.* (2022) introduce an advanced approach for categorizing date fruits, a prominent agricultural commodity in the Middle East.Recognizing the nutritional benefits of dates and their role in disease prevention, the authors emphasize the need for efficient sorting methods in the date industry, which is traditionally labor-intensive and costly.
Chen *et al.* (2021) describe a vision system tailored for the citrus industry to address the significant inefficiencies observed in traditional manual sorting processes. The proposed system offers a high-tech solution that integrates advanced deep learning techniques to optimize sorting on a citrus processing line. By analyzing the historical tracking data, the system is able to accurately determine the true categories of the citrus fruits, achieving a high detection precision reported at 93.6%.
The research conducted by Liu *et al.* (2024) critically examines the application of deep learning algorithms, particularly Convolutional Neural Networks (CNNs), in this sector. Their study conducts an extensive evaluation of several popular CNN architectures such as DenseNet, InceptionV3, ResNet, VGGNet, Xception, MobileNet, NASNet, EfficientNet, and

SqueezeNet. The paper tests these models against key performance indicators like sensitivity, specificity, and accuracy, providing a detailed analysis of their capabilities and shortcomings.

Materials and methods

In our research, we explored the following types of autoencoders to enhance the apple sorting process by analyzing and improving image data:

1. Basic Convolutional Autoencoder is the simplest form of this architecture, comprising an encoder and a decoder. The encoder reduces the dimensionality of the input images, extracting essential features, while the decoder reconstructs the images from these features.
2. Deep Convolutional Autoencoder consists of multiple layers, allowing it to learn more complex features. This architecture is more capable of capturing subtle anomalies in apple quality, providing a deeper understanding of intricate patterns within the image data.
3. Variational Autoencoder (VAE) introduces a probabilistic approach, where the encoder outputs mean and variance for the latent space. This helps in capturing more generalized features and improving robustness to variations.
4. Denoising Autoencoder is designed to reconstruct the input from a corrupted version, enhancing its ability to identify anomalies by focusing on key features and disregarding noise. This type of autoencoder is beneficial for improving the quality of images with imperfections, making it easier to detect defects in apples.
5. Contractive Autoencoder (CAE) includes a regularization term that penalizes the norm of the Jacobian matrix of the encoder's activations. This results in more robust feature learning, making the model less sensitive to small changes in input. CAEs are useful for creating stable and reliable representations of apple features, ensuring consistency in quality assessment.

For the purpose of training we utilized a dataset consisting of 2,782 images of apples, all classified as being in good condition. These images were sourced to train the model on recognizing the characteristics of high-quality apples. Each image was resized to a uniform dimension of 128x128 pixels to maintain consistency and improve computational efficiency during model training. The images were preprocessed using the class ImageDataGenerator from the Keras library, which included rescaling the pixel values to a range of 0 to 1 to facilitate model training by normalizing the data.

The authors researched the five type of autoencoder described above. Each type is characterized by its specific encoder and decoder architectures, latent space, and key features:

1. Basic Convolutional Autoencoder - encoder: Conv2D (32 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (64 filters, 3x3, ReLU) → MaxPooling2D (2x2); latent space: Dense layer with 128 units; decoder: Conv2DTranspose (64 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (32 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (1 filter, 3x3, Sigmoid); key features: simplest architecture, basic feature extraction.
2. Deep Convolutional Autoencoder - encoder: Conv2D (32 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (64 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (128 filters, 3x3, ReLU) → MaxPooling2D (2x2); latent space: Dense layer with 256 units; decoder: Conv2DTranspose (128 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (64 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (32 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (1 filter, 3x3, Sigmoid); key features: deeper architecture, captures more complex features

3. Variational Autoencoder (VAE) - encoder: Conv2D (32 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (64 filters, 3x3, ReLU) → MaxPooling2D (2x2); latent space: Dense layers for mean and variance with 128 units each; decoder Architecture: Conv2DTranspose (64 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (32 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (1 filter, 3x3, Sigmoid); key features: probabilistic approach, captures generalized features.
4. Denoising Autoencoder - encoder: Conv2D (32 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (64 filters, 3x3, ReLU) → MaxPooling2D (2x2); latent space: Dense layer with 128 units; decoder: Conv2DTranspose (64 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (32 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (1 filter, 3x3, Sigmoid); key features: reconstructs from corrupted input, focuses on key features.
5. Contractive Autoencoder (CAE) - encoder: Conv2D (32 filters, 3x3, ReLU) → MaxPooling2D (2x2) → Conv2D (64 filters, 3x3, ReLU) → MaxPooling2D (2x2); latent space: Dense layer with 128 units with contraction penalty; decoder: Conv2DTranspose (64 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (32 filters, 3x3, ReLU) → UpSampling2D (2x2) → Conv2DTranspose (1 filter, 3x3, Sigmoid); key features: regularization for robust feature learning, less sensitive to small input changes.

These architectures have been evaluated for their effectiveness in improving apple quality assessment. For training and testing the autoencoder models, we utilized Python scripts with TensorFlow and Keras.

The autoencoders were compiled with the Adam optimizer and mean squared error (MSE) loss function, which quantifies the difference between the original and reconstructed images. The final models were trained for 120 epochs using a batch size of 8. The validation was performed using a separate set of images to monitor the model's performance and avoid overfitting. During training, the loss and validation loss were recorded for each epoch to track the models' learning progress. The trained autoencoders were employed to differentiate between normal apples and anomalies. This was achieved by comparing the reconstruction error between the input and output images. Images with higher reconstruction errors were flagged as potential anomalies, indicative of defects or diseases. To automate the detection process, thresholds for density and reconstruction error were empirically set based on the distribution of these metrics across known good and anomalous samples. These thresholds help in distinguishing normal apples from those with defects (Sayed, 2023).

The effectiveness of the model in identifying anomalies was assessed by evaluating it on a separate dataset specifically curated to contain anomalous apple images. The models’ performance metrics, such as reconstruction error and density estimates from a Kernel Density Estimator (KDE), were calculated and compared against the set thresholds to determine the presence of anomalies.

Results and discussion

In assessing the performance of various autoencoder models for our apple quality assessment project, key performance indicators were utilized. These metrics provided insights into the models' abilities to reconstruct images and identify anomalies effectively.

The evaluation criteria included (Sayed, 2023):

1. Low Reconstruction Error - a measure of reconstruction quality for normal apples.
2. High Anomaly Detection Accuracy - reflecting the model's capability to identify defects accurately.

3. Balanced Precision and Recall - ensuring unbiased performance in distinguishing between normal and anomalous apples.
4. High F1 Score and AUC-ROC - indicating overall effectiveness in classifying normal and anomalous apples.

Based on the results presented in Table 1, which outlines the performance of various autoencoder models, the Contractive Autoencoder (CAE) emerges as the optimal architecture for our project. Its superior performance across multiple metrics suggests its suitability for a more accurate apple quality assessment.

Table 1. The performance of various autoencoder models

Model	**Reconstruction Error (Mean ± SD)**	**Anomaly Detection Accuracy**	**Precision**	**Recall**	**F1 Score**	**AUC-ROC**
Basic Convolutional Autoencoder	0.05 ± 0.02	0.85	0.80	0.75	0.77	0.82
Deep Convolutional Autoencoder	0.04 ± 0.01	0.88	0.83	0.78	0.80	0.85
Variational Autoencoder (VAE)	0.06 ± 0.02	0.82	0.78	0.70	0.74	0.80
Denoising Autoencoder	0.05 ± 0.02	0.87	0.82	0.76	0.79	0.84
Contractive Autoencoder (CAE)	0.04 ± 0.01	0.89	0.85	0.79	0.82	0.87

Conclusions

The Contractive Autoencoder (CAE) emerges as a standout performer in enhancing apple quality assessment through anomaly detection. It demonstrates a very good reconstruction accuracy with one of the lowest reconstruction errors (0.04 ± 0.01), indicative of its superior ability to faithfully reconstruct normal apples. Moreover, the CAE exhibits remarkable efficacy in detecting anomalies, achieving the highest accuracy (0.89) among all evaluated models. Its precision (0.85) and recall (0.79) values underscore its capability to accurately identify anomalies while minimizing false positives. Furthermore, the CAE achieves a high F1 score (0.82), reflecting a balanced performance between precision and recall. With the highest AUC-ROC value (0.87), the CAE showcases its proficiency in effectively distinguishing between normal and anomalous apples.

In analyzing the outcomes of alternative autoencoder models, the following observations are evident in comparison to the performance of the Contractive Autoencoder (CAE), acknowledged for its better efficacy:

- the Deep Convolutional Autoencoder (DCAE) and CAE demonstrate the lowest mean reconstruction error (0.04 ± 0.01), closely followed by the Basic Convolutional Autoencoder (BCAE) and Denoising Autoencoder (DAE). The Variational Autoencoder (VAE) exhibits a slightly higher reconstruction error at 0.06 ± 0.02.
- while the DCAE closely approaches with an accuracy of 0.88, the CAE achieves the highest anomaly detection accuracy of 0.89, surpassing all other models. BCAE, DAE, and VAE have accuracies ranging from 0.82 to 0.87.

- CAE demonstrates the highest precision (0.85) and commendable recall (0.79), indicating its superior ability to accurately identify anomalies while minimizing false positives. DCAE also displays competitive precision and recall values. In comparison, BCAE, DAE, and VAE present slightly lower precision and recall values.
- CAE attains the highest F1 score (0.82), indicating a well-balanced performance between precision and recall. The next closest is DCAE with an F1 score of 0.80, while BCAE, DAE, and VAE have slightly lower F1 scores.
- CAE demonstrates the highest AUC-ROC (0.87), signifying its superior capability in distinguishing between normal and anomalous apples. DCAE also exhibits a high AUC-ROC value of 0.85, while the other models have AUC-ROC values ranging from 0.80 to 0.84.

In conclusion, while all autoencoder models exhibit competitive performance metrics, the Contractive Autoencoder (CAE) stands out in its effectiveness in apple quality assessment through anomaly detection, consistently outperforming other models across various evaluation metrics.

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EXPLORING INSECTS DETECTION PERFORMANCE IN CORN PEST CONTROL WITH MOBILENET-SSD NEURAL NETWORK AND HIGHER-RESOLUTION INPUT

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Abstract

Building upon previous research in the domain of corn pest detection from some species of insects (Coleoptera Order) using Convolutional Neural Networks (CNNs), this study investigates the impact of a higher input resolution on the performance of the MobileNet-SSD neural network architecture. Our initial study focused on optimizing a deep-learning model for low-power mobile devices to detect various corn pests while preserving beneficial species. In order to further improve the detection capabilities of MobileNet-SSD, the present research explores the effect of a higher resolution of 512x512 pixels, as input for the MobileNet-SSD-v1 and v2-Lite models. These adjustment aimed to provide a more detailed input representation to facilitate enhanced detection performance. The evaluation metrics include average accuracy per class and mean average precision (mAP), as used in the previous study, to ensure consistency in performance assessment. Our findings indicate that training the networks on the augmented dataset at a higher resolution resulted in improved detection performance, with both models demonstrating enhanced mAP scores (MobileNet SSD v1: mAP = 0.912; MobileNet SSD v2 Lite: mAP = 0.919). Additionally, MobileNet SSD v2 Lite consistently outperformed MobileNet SSD v1 in terms of mAP across both training phases. This research contributes to a broader understanding of the relationship between input resolution and detection performance in CNNs, providing valuable insights on refining the approach to further enhance the precision and efficiency of modern pest detection systems in agricultural contexts.

Keywords: *Pest control, insects, Coleoptera, smart agriculture, neural network.*

Introduction

The demand for effective pest management solutions in agriculture is an important objective alongside the need for sustainable and eco-friendly practices. Traditional methods relying heavily on chemical pesticides face environmental concerns and diminishing effectiveness over time. As a response, there has been a shift towards integrating artificial intelligence (AI) technologies into agricultural practices, offering innovative solutions for pest detection and management.

Previous research has demonstrated the potential of AI-driven techniques in various agricultural domains, including disease management in sunflower crops (Gulzar *et al.*, 2023), fruit classification in horticulture (Gulzar, 2023), and citrus fruit disease detection (Dhiman *et al.*, 2023). These studies showed the effectiveness of advanced algorithms, particularly neural networks, in addressing diverse agricultural challenges.

Insect pest detection and identification have been particularly emphasized in recent research, with studies employing a range of neural network models and methodologies. The most popular object detection algorithm was YOLO, followed by Faster R-CNN and VGG16 (Maican *et al.*, 2023). In terms of mean average precision (mAP), some of the most notable results are presented in Table 1.

Table 1. Ranking of some insect identification neural networks by maximum mAP

Model	Dataset	Maximum mAP
YOLOv5m	Dataset from Maryland Biodiversity Project (Sava *et al.*, 2022)	99.2%
YOLOv5-X	7046 images, 23 pest classes (Ahmad *et al.*, 2022)	98.3% (mAP@0.5)
YOLOv7	4865 images of coccinellids, seven classes (Wang *et al.*, 2023)	Up to 97.31% (AP@0.50)
Faster R-CNN	36,000 images of harmful and non-harmful beetles (Butera *et al.*, 2022)	92.66%
YOLOv5	100,000 annotated images of small insects (Kranthi *et al.*, 2002)	92.4% (mAP@0.50)
Improved Faster R-CNN	AgriPest21 dataset: 21 pest classes, 25,000 images (Wang *et al.*, 2021)	78.7%
AgriPest-YOLO	Pest24 dataset: 25,000 images of small pests (Zhang *et al.*, 2022)	71.3% (mAP@0.50)

However, most existing research faces challenges when applied to low-power agricultural mobile systems, which require lightweight neural networks both to conserve battery life and to accommodate their limited processing capabilities. In this context, MobileNet networks have received limited attention despite their potential advantages in resource-constrained environments.

Our previous research (Maican *et al.*, 2023) focused on developing optimized MobileNet SSD networks, for crop pest detection. Utilizing a two-step transfer learning approach, we aimed to enhance the accuracy of these networks, particularly MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite. Despite the reduced size of the training set (2648 images), both models demonstrated similar and good overall accuracies (MobileNet-SSD-v2-Lite: mAP = 0.8923; MobileNet-SSD-v1: mAP = 0.908), highlighting their potential for real-time pest control.

Building upon these findings, the main objective of our current study is to investigate the impact of input image resolution on the performance of MobileNet-SSD networks for crop pest detection. By increasing the input resolution from 300 to 512 pixels, we seek to assess whether higher-resolution images yield improved accuracy in pest identification. This adjustment aimed to provide a more detailed input representation to facilitate enhanced detection performance.

Materials and methods

In this study, we modified the MobileNet SSD v1 and MobileNet SSD v2 Lite networks to process images with a resolution of 512x512 pixels. This adaptation aimed to enhance the networks' ability to detect and classify objects with finer details, ultimately improving their performance. The training process was conducted on the Nvidia Jetson AGX Orin platform using PyTorch 2.0.0 for JetPack 5.1.1. Additionally, we utilized Nvidia TensorRT for real-time inference optimization on Nvidia GPUs.

The training dataset comprised 2648 images after augmentation, sourced from the iNaturalist web portal (iNaturalist, 2023). These images represented four beetle species that are pests known to cause substantial damage to crops such as corn, wheat, sunflower, and beans (*Anoxia villosa*, *Diabrotica virgifera virgifera*, *Opatrum sabulosum*, and *Zabrus tenebrioides*). In addition to pest species, the dataset includes images of the beneficial *Coccinella* sp., which is an aphid predator in agricultural ecosystems, in order to test the ability of the network to distinguish it from pest species.

The training process involved both a common transfer learning procedure, as well as a novel two-step transfer learning procedure, which consisted of the following steps:

- Initial Transfer Learning: in the first step, we performed transfer learning using the Open Images dataset to recalibrate the pre-trained MobileNet-SSD models for insect detection task. This involved retraining the models on an Open Images subset of 2605 images, containing only two classes: Beetle and Ladybug.
- Refinement Transfer Learning: in the second step, we further refined the pre-trained models using our custom dataset, organized according to the Pascal VOC format. This dataset included images of the five insect species mentioned earlier, allowing the models to learn specific features relevant to our pest detection task.

The training and evaluation procedures were conducted using two PyTorch scripts: 'train_ssd.py' and 'eval_ssd.py', respectively. These scripts were used for model training, validation, and performance evaluation, including metrics such as mean average precision (mAP). Further optimization for real-time inference was achieved using Nvidia TensorRT and detectNet – an object detection framework that performs optimized real-time inferencing on Nvidia Jetson GPUs. Detailed information on the training and evaluation procedures can be found in our previously published work (Maican *et al.*, 2023).

To summarize, in order to ensure consistency in performance assessment between the results obtained by increasing the image resolution and those from our previous research, experiments were conducted under identical conditions:

- The same two MobileNet-SSD models (MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite) were trained, but modified to process images with resolutions of 512x512 pixels;
- Both models were trained on the same dataset, with the same images in the training, validation, and test sets;
- Identical values were employed for hyperparameters such as batch size, number of workers, and epochs;
- The same evaluation metrics were employed (average accuracy per class, and mAP@.50).

Results and discussion

In our previous work, we demonstrated that training the MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite networks on the unaugmented dataset yielded inferior results compared to training on the augmented dataset. Therefore, in this research, all training was conducted on the augmented dataset. For experimentation, we downloaded pre-trained versions of the two neural networks: MobileNet-SSD-v1 pre-trained on the PASCAL-VOC dataset, and MobileNet-SSD-v2-Lite pre-trained on the COCO dataset. We conducted the following experiments (Figure 1):

1. Single transfer learning training:
   - 1.1. Training the downloaded MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite directly on the augmented dataset (5 classes, 2648 images, input resolution: 512x512 pixels);
   - 1.2. Comparing the results from step 1.1 with the results obtained through the same procedure in the previous research, but with an input resolution of 300x300 pixels;
2. Two successive transfer learning trainings, as described in the Materials and Methods section (Two-step Transfer Learning):
   - 2.1. Training the downloaded MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite on the Open Images subset of 2605 images (2 classes: Beetle and Ladybug, input resolution: 512x512 pixels);
   - 2.2. Training the MobileNet-SSD-v1 and SSD-v2-Lite networks pretrained at 2.1. on the augmented dataset (5 classes, 2648 images, input resolution: 512x512 pixels);

2.3. Comparing the results from 2.2. with the results obtained through the same procedure in the previous research, where the input resolution was 300x300 pixels.

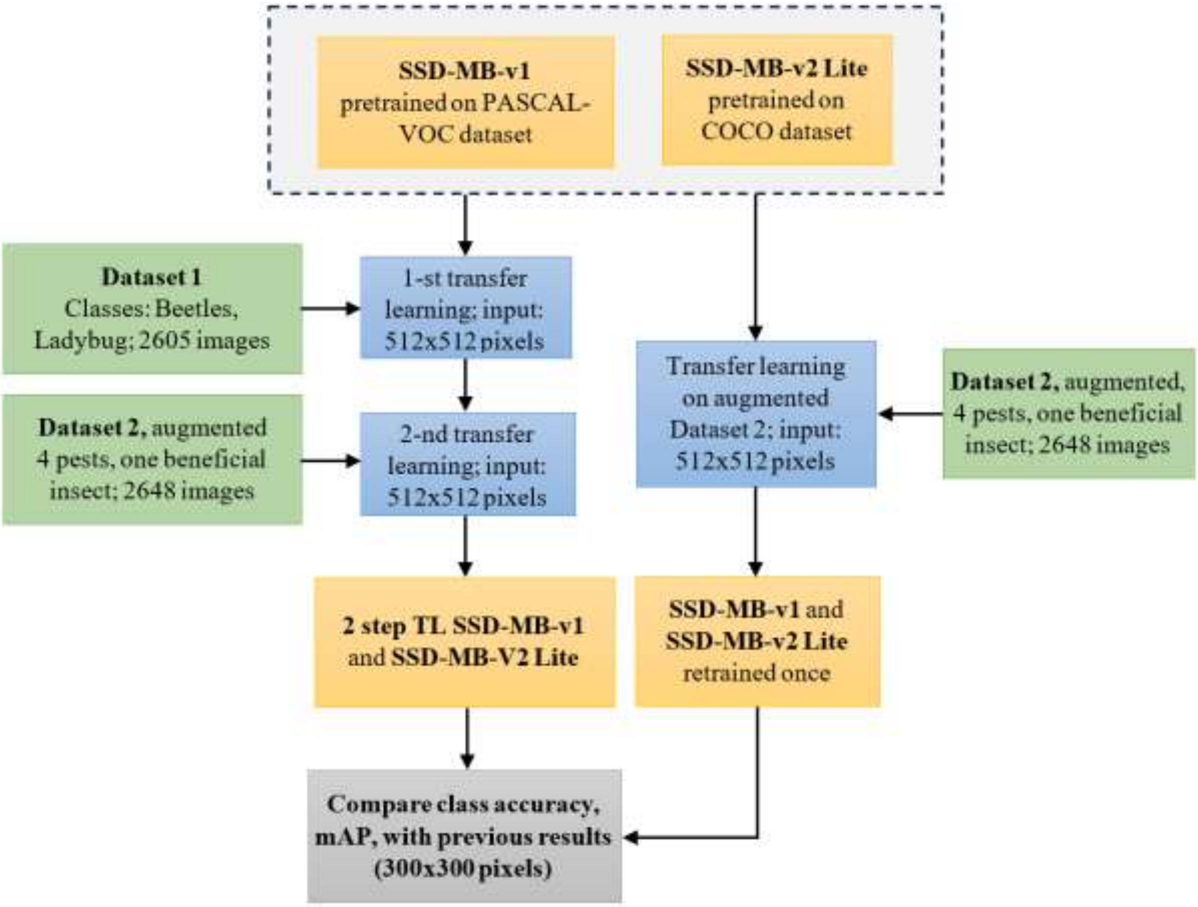

Figure 1. Schematic representation of the training and performance evaluation procedures; 2 step TL: two-step transfer learning; color scheme: orange - neural network models; blue - training procedures; green - datasets; grey - evaluation (adapted from Maican *et al.*, 2023)

To compare the results obtained from the experiments conducted in the current work, using a resolution of 512x512 pixels, with those from the previous work, where a resolution of 300x300 pixels was employed, we analysed the mean average precision (mAP), and the accuracy per class, for both MobileNet SSD v1 and MobileNet SSD v2 Lite.
The mAP metric provides an overall measure of the performance of the object detection models across all classes, capturing their ability to detect objects accurately and localize them within the image.
Conversely, the accuracy per class is a measure of the performance of the object detection neural networks for each individual class, showing their ability to differentiate between various pest species, thus allowing for a nuanced evaluation of their capabilities across different categories.
In case of the Single Transfer Learning training at the resolution of 512x512 pixels, both MobileNet SSD v1 and MobileNet SSD v2 Lite achieved higher mAP values compared to the previous work at 300x300 pixels (Table 2). Specifically, for MobileNet SSD v1, the mAP increased from 0.887 in the previous work to 0.912 in the current work. Similarly, for MobileNet SSD v2 Lite, the mAP increased from 0.884 to 0.891. This indicates that training the networks on the augmented dataset at a higher resolution improved detection performance.

Table 2. Average accuracy per class and mAP for single transfer learning training

Resolution: 512 x 512 (current work)				Resolution 300 x 300 (previous work)			
Mobilenet SSD v1		**Mobilenet SSD v2 Lite**		**Mobilenet SSD v1**		**Mobilenet SSD v2 Lite**	
Anoxia	0.998	Anoxia	0.977	Anoxia	0.987	Anoxia	0.991
Diabrotica	0.768	Diabrotica	0.766	Diabrotica	0.726	Diabrotica	0.727
Ladybug	0.977	Ladybug	0.909	Ladybug	0.874	Ladybug	0.891
Opatrum	0.909	Opatrum	0.909	Opatrum	0.924	Opatrum	0.909
Zabrus	0.909	Zabrus	0.895	Zabrus	0.947	Zabrus	0.903
mAP	0.912	mAP	0.891	mAP	0.887	mAP	0.884

Examining the results from the Two-step Transfer Learning training pipeline, at the resolution of 512x512 pixels, both networks showed better performance compared to the previous work (Table 3). The mAP values increased from 0.908 to 0.915 for MobileNet SSD v1 and from 0.892 to 0.919 for MobileNet SSD v2 Lite. This further confirms the advantage of using the augmented dataset at a higher resolution for training.

Table 3. Average accuracy per class and mAP for two-step transfer learning training

Resolution: 512 x 512 (current work)				Resolution 300 x 300 (previous work)			
Mobilenet SSD v1		**Mobilenet SSD v2 Lite**		**Mobilenet SSD v1**		**Mobilenet SSD v2 Lite**	
Anoxia	0.989	Anoxia	0.986	Anoxia	0.907	Anoxia	0.985
Diabrotica	0.787	Diabrotica	0.795	Diabrotica	0.777	Diabrotica	0.807
Ladybug	0.988	Ladybug	0.998	Ladybug	0.944	Ladybug	0.894
Opatrum	0.909	Opatrum	0.906	Opatrum	0.906	Opatrum	0.951
Zabrus	0.902	Zabrus	0.907	Zabrus	0.903	Zabrus	0.905
mAP	0.915	mAP	0.919	mAP	0.908	mAP	0.892

Comparing the performance of the two networks at the resolution of 512x512 pixels, one can notice that MobileNet SSD v2 Lite consistently outperformed MobileNet SSD v1 in terms of mAP. This suggests that MobileNet SSD v2 Lite is more effective in handling object detection tasks at the higher resolution. The accuracy per class analysis demonstrates consistent improvements in detection performance when using higher input resolutions. MobileNet SSD v2 Lite generally has slightly higher accuracies compared to MobileNet SSD v1, particularly at 512 pixels, indicating its suitability for object detection tasks in agricultural environments.

Conclusions

This study investigated the impact of input resolution on the performance of two MobileNet-SSD neural network architectures for crop pest detection, focusing on the Coleoptera order. By increasing the input resolution from 300x300 to 512x512 pixels, we aimed to assess whether higher-resolution images would yield improved accuracy in pest identification. Our findings suggest several key conclusions:

1. Both MobileNet SSD v1 and MobileNet SSD v2 Lite demonstrated enhanced mAP scores when trained on datasets with a resolution of 512x512 pixels compared to 300x300 pixels. This improvement indicates that higher-resolution images provide more detailed input representations, facilitating better detection performance.
2. The use of a two-step transfer learning approach resulted in improved detection performance for both MobileNet SSD v1 and SSD v2 Lite at the higher resolution.

3. Most of the time, MobileNet SSD v2 Lite slightly outperformed MobileNet SSD v1 in terms of mAP across both training phases at the resolution of 512x512 pixels.
4. The findings of this study have practical implications for pest control in agriculture. By using higher-resolution images on neural networks for limited-power devices such as MobileNet SSD, farmers and agricultural professionals can benefit from more accurate and efficient pest detection systems, leading to improved crop management practices and reduced environmental impact.

Overall, this research contributes to a deeper understanding of the relationship between input resolution and detection performance in convolutional neural networks, offering valuable insights for optimizing pest detection systems in agricultural contexts. Future studies may explore additional factors such as dataset size, augmentation techniques, and model architecture modifications to further enhance the precision and efficiency of crop pest detection systems.

Acknowledgement

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RESEARCH ON DETERMINING THE PERFORMANCE OF A VARIABLE WIDTH PLOUG DESIGNED FOR HIGH-POWER TRACTORS (180-240 HP)

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Abstract

It is known that plowing remains a task difficult to replace with other alternative soil processing works, due to its importance. At the same time, plowing remains work that involves the highest fuel consumption. On the other hand, it is known that the physical and mechanical characteristics of the processed soil can vary greatly from one plot to another, and the plow intended for plowing work must be properly adjusted so that the tractor operating in aggregate works under optimal conditions. Variable plowing width prove to be the most suitable for adapting to soil conditions and tractor traction performance. For large agricultural operations, the use of high-power tractors is recommended to ensure that work is carried out with a minimum number of passes. In this paper, the results obtained from experimental trials conducted in Romania using a variable width plow equipped with five moldboards and powered by a 200 HP tractor are presented. The trials have been conducted for different working widths/moldboards (30, 35, 40, 45 cm), different working depths (20, 25, 30 cm), and different working speeds, which allowed, on one hand, the determination of qualitative work indices, and on the other hand, obtaining the energy indices of the tractor-plough unit (fuel consumption, working capacity, slippage, specific fuel consumption, etc.), which are very useful for those who operate such equipment, as well as for agricultural tractor dealers.

Keywords: *Plow, Agricultural tractor, Experimental testing, Quality indices, Energy indices.*

Introduction

Plowing is an essential, yet fuel-intensive agricultural task that requires careful adjustment of the plough to match the varying physical and mechanical characteristics of the soil across different plots, with variable plowing width and high-power tractors being recommended to optimize the adaptation to soil conditions and minimize the number of passes needed for large-scale operations. Enhancing the traction performance and power transfer efficiency of farm tractors during cultivation can lead to optimized energy utilization and reduced fuel consumption and greenhouse gas emissions (Md-Tahir, H., et. al., 2021), (Lekavičienė, K., 2019). Over the years, there have been numerous attempts to develop mathematical models that quantify the influence of the constructive and functional parameters of the plough on soil processing performance, considering its varying physical properties (Ghereș, M.I., 2013), (Gheorghiță, N.E., et. al., 2022), (Meca, V.A., 2012), (Augustin, K., 2019). Soil-machine interaction studies aim to provide scientific insights into how tillage tools and traction devices interact with the terrain, investigating variables such as pulling or pushing forces, vertical and lateral forces, soil failure patterns, displacement of soil particles, interface force between wheel and soil, wheel slippage, rolling resistance, estimation of wheel contact area, and soil stress at various depths. Soil bin facilities serve as ideal laboratories for tillage and traction

experiments, comprising components such as a soil bin simulating the ground, a mobile tool carrier representing tool frames, soil-engaging devices simulating tillage tools or traction elements, a power source and drive system emulating prime movers, soil conditioning equipment for soil preparation, motion control systems for component movement, measurement and data acquisition systems for real-time analysis, and a lifting system for heavy component handling (Ani, O. A., 2018), (Kheiralla, A.F., 2004), (Varani, M., 2023).The operational performance of the plough and the aggregate formed by it with a high-power tractor can be quantified in terms of qualitative and energetic indices, which are as follows (Gill, W.R., Vanden Berg, G.E., 1968):
The average working depth:

$$a_m=\frac{\sum_1^n a_i}{n} \quad [\text{cm}] \tag{1}$$

where: a_i -represents the measured working depth, (cm); n -the number of measurements.
Squared average deviation of working depth:

$$\sigma_a=\pm\sqrt{\frac{\sum_1^n (a_i-a_m)^2}{n-1}} \quad [\text{cm}] \tag{2}$$

The working depth variation index:

$$V_a=\frac{\sigma_a}{a_m}100 \quad [\%] \tag{3}$$

The average working width:

$$B_m=\frac{\sum_1^n B_i}{n} \quad [\text{m}] \tag{4}$$

where: B_i - is the measured working width, (m); n -the number of measurements.
Squared average deviation of working width:

$$\sigma_B=\pm\sqrt{\frac{\sum_1^n (B_i-B_m)^2}{n-1}} \quad [\text{cm}] \tag{5}$$

The working width variation index:

$$V_B=\frac{\sigma_B}{B_m}100 \quad [\%] \tag{6}$$

The degree of crop residue coverage: It is determined by the ratio, expressed as a percentage, between the amount of plant material remaining on the soil surface and the plant mass present on the field surface before the passage of the implement, using the following relationship:

$$G_v=\frac{\sum_1^n \frac{G_{ti}-G_{Si}}{G_{ti}}}{n}\times 100 \quad [\%] \tag{7}$$

where: G_{Si} - is the measured mass of plant residues remaining on the soil surface, on the sample taken, after the passage of the machinery, (g); G_{ti}- is the total measured mass of plant residues on the soil surface before the passage of the machinery, (g); *n* - the number of measurements.
The degree of soil pulverization: It represents the weight proportion of soil fractions with satisfactory fragmentation, namely with clod sizes of maximum 100 mm, relative to the total mass of the soil sample. It is calculated using the following relationship:

$$G_{ms}=\frac{\sum_1^n \frac{M_{ci}}{M_{ti}}}{n}\times 100 \quad [\%] \tag{8}$$

where: M_{ci} - the measured mass of soil clods with dimensions smaller than 100 mm, (kg); M_{ti} - the measured weight of the entire soil sample, (kg); *n* - the number of measurements.
Viteza de lucru:

$$v_e=\frac{3{,}6\cdot s}{t} \quad [\text{km/h}] \tag{9}$$

where: *s* - linear distance traveled, (m); *t* - time taken to traverse the distance, (s).
Fuel consumption:

$$Q=\frac{V}{3{,}6t} \quad [\text{l/h}] \tag{10}$$

where: V -is the volume of fuel recorded by the consumption device, (ml); t -sampling time, (s).
Fuel consumption per hectare:

$$q=\frac{Q}{W_{ef}} \quad [1/\text{ha}] \tag{11}$$

where: Q -hourly fuel consumption, (l/h); W_{ef} -hourly effective working capacity, (ha/h).
The hourly working capacity during actual time:

$$W_{ef}=0{,}1\cdot B_m \cdot v \quad [\text{ha/h}] \tag{12}$$

where: B_m -is the average working width, (m); v -is the actual working speed, (km/h).

Material and Methods

The variable-plowing width for high-power tractors is designed to perform plowing on flat or sloping terrain of up to 6°, on all types of soil, at a maximum working depth of 30 cm. The plough operates in conjunction with 180-240 HP wheel tractors equipped with Category III SR ISO 730-1+C1 suspension mechanism. Field trials, under operating conditions, of the PP5VM plough in combination with a "Zimbru" type tractor with 195 HP (Fig. 1), were conducted in August 2022, on the lands of a family farm in Argeș County (Romania). The experimental field tests were carried out under the following conditions:

- soil type: brownish red with loamy-clay texture;
- previous crop: mechanically harvested wheat, with straw baled and transported immediately after harvesting; height of crop residues: 15 cm;
- mass of crop residues: 70 g/m²;
- soil moisture at a depth of 25-30 cm: 18%.
- The main characteristics of the plough used for experimental trials are: number of bodies: 5; diameter of the metal wheel: 500 mm; working width per body: 30-45 cm; distance between bodies in the forward direction: 830 mm; clearance under the frame: 780 mm; length: 4815 mm; width: 2670 mm; height: 1425 mm; constructive weight: 1400 kg.
- The main functional characteristics of the plough are: maximum working depth: 30 cm; clearance in transport: 600 mm; working speed: 4.5-9 km/h; transport speed: max 10 km/h. The constructive characteristics of the body are: share type: helical; maximum working depth: 30 cm; angle formed by the share edge with the furrow wall: 44^0 ; minimum angle of the generatrices with the furrow wall: 29^0 ; maximum angle of the generatrices with the furrow wall: 46^0 ; maximum height of the share above the furrow edge: 435 mm.

Fig. 1. The 195 HP ”Zimbru” tractor and the plough with variable working width with 5 mouldboards.

Results and Discussion

In Table 1, the performance indicators of the aggregate consisting of the T195 tractor and the PP5VM plow are presented for plowing work at a depth of 30 cm and a furrow width of 40 cm on brown-red soil with average moisture of 18% in the plowed layer. In Table 2 and in Figure 2, qualitative indices of the plowing work performed with the aggregate consisting of tractor T195 and plough PP5VM on brown-reddish soil with an average moisture content of 18% in the plowed layer are presented. The meaning of the terms in the table is as follows: Working width on the mouldboard b_t(cm); Effective working speed v_e(km/h); Average working depth a_m(cm); Maximum deviation from $a_m \pm \Delta_a$(cm); Standard deviation σ_a(cm); Depth working inconsistency V_a(%); Average working width B_m(cm); Standard deviation σ_B(cm); Width working inconsistency V_B(%); Soil fragmentation degree G_{ms}(%); Vegetative residue coverage degree G_v(%).

Table 1. Performance indicators

Exploitation index name	**Symbol**	**U.M.**	**Value**
Operational time utilization coefficient	K_{02}	-	0.939
The coefficient of utilization of total operating time	K_{03}	-	0.890
Production time utilization coefficient	K_{04}	-	0.866
The utilization coefficient of exchange time	K_{07}	-	0.731
Return coefficient	K_{21}	-	0.942
Technological safety coefficient	K_{41}	-	0.993
Technical safety factor	K_4	-	0.970
Hourly working capacity at actual time	W_{ef}	ha/h	1.165
The hourly operational working capacity	W_{02}	ha/h	1.094
Hourly work capacity at total operating time	W_{03}	ha/h	1.037
Hourly work capacity during production time	W_{04}	ha/h	1.009
The hourly work capacity during the shift time	W_{07}	ha/h	0.851
The working capacity for an 8-hour shift	W_{sch}	ha/sch	6.813
Specific fuel consumption	Q	l/ha	27.56

Table 2. Qualitative indicators

b_t (cm)	v_e (km/h)	a_m (cm)	$a_m \pm\Delta_a$ (cm)	σ_a (cm)	V_a (%)	B_m (cm)	σ_B (cm)	V_B (%)	G_{ms} (%)	G_v (%)
30	8.04	19.7	2.4-3.6	1.88	6.65	156	11.8	5.49	85.8	85.6
	7.85	25.1	1.8-3.2	1.77	6.23	154	12.3	4.83	83.4	89.3
	7.38	29.7	1.7-2.9	1.73	5.95	153	11.5	4.39	80.2	93.4
35	7.87	20.1	2.1-3.2	1.85	6.53	186	10.9	5.93	84.3	88.6
	7.45	24.9	1.8-3.1	1.79	6.11	182	11.2	5.22	82.4	91.5
	6.83	30.2	2.2-3.3	1.76	5.84	181	10.5	4.11	80.3	94.3
40	7.42	19.9	2.3-3.7	1.89	6.66	220	11.6	6.08	82.3	94.3
	6.36	24.8	1.9-4.1	1.78	6.13	211	11.4	5.45	81.6	94.6
	5.42	30.1	2.4-3.6	1.83	5.81	215	11.3	4.77	79.8	95.2
45	7.01	20.2	2.5-3.8	1.92	6.84	243	12.6	6.37	79.6	96.5
	6.17	25.3	2.2-4.2	1.83	6.45	241	12.8	5.85	78.9	96.8
	5.14	29.6	2.7-4.5	1.87	5.98	234	12.5	5.32	78.3	95.9

In Table 3 and in Figure 3, the energy indices resulting from the experimental trials of the unit consisting of the T195 tractor and the PP5VM plough are presented for plowing work on brownish-red soil with an average moisture content of 18% in the plowing layer. The significance of the terms in the table is as follows: Working width per mouldboard b_t(cm); Average working depth a_m(cm); Average total working width B_m(cm); Average slippage δ

(%); Effective working speed v_e(km/h); Hourly fuel consumption q (l/h); Working productivityat effective time W_{ef}(ha/h); fuel consumption per hectare Q (l/ha).

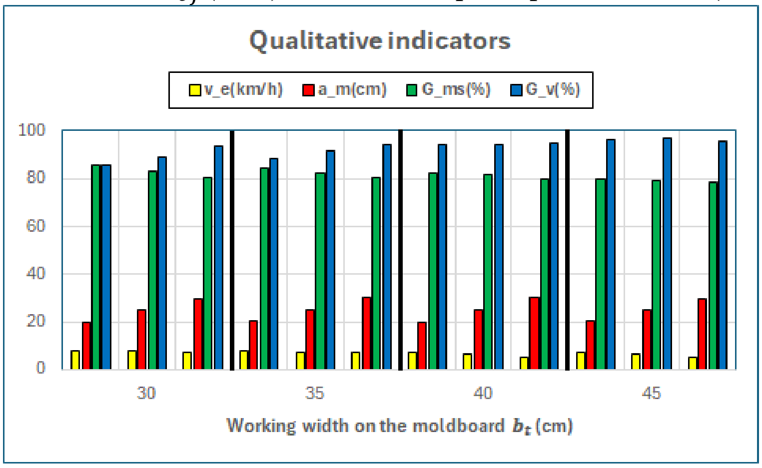

Fig. 2. The variation of quality indices depending on the width of the mouldboard

Table 3. Energy indices

b_t (cm)	Indices of working regime		The characteristic indices for adhesion, dynamics, and energetics			Productivity and consumption indices	
	a_m (cm)	B_m (cm)	δ (%)	v_e (km/h)	q (l/h)	W_{ef} (ha/h)	Q (l/ha)
30	19.7	156	11.8	8.04	29.53	1.254	23.54
	25.1	154	11.7	7.85	29.57	1.209	24.45
	29.7	153	12.1	7.38	30.15	1.129	26.70
35	20.1	186	11.9	7.87	29.85	1.464	20.39
	24.9	182	12.2	7.45	31.18	1.356	22.99
	30.2	181	12.3	6.83	31.79	1.236	25.72
40	19.9	220	12.8	7.42	30.12	1.632	18.45
	24.8	211	13.1	6.36	31.97	1.342	23.82
	30.1	215	13.5	5.42	32.11	1.165	27.56
45	20.2	243	13.9	7.01	32.25	1.703	18.93
	25.3	241	14.3	6.17	33.75	1.487	22.69
	29.6	234	15.5	5.14	34.55	1.203	28.72

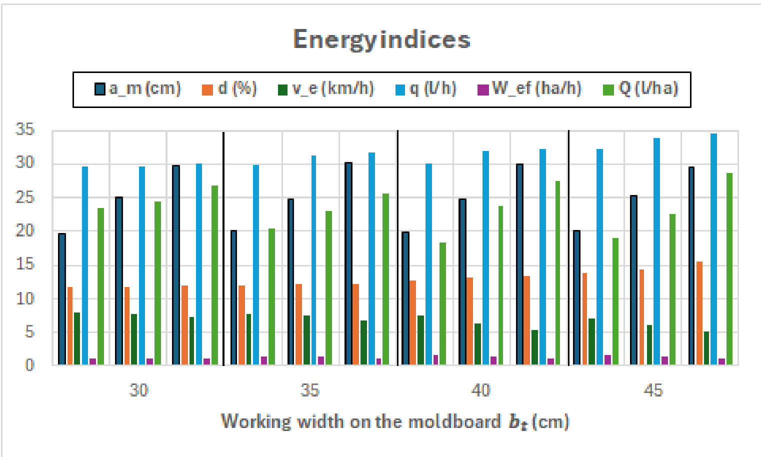

Fig. 3. The variation of energy indices depending on the width of the mouldboard

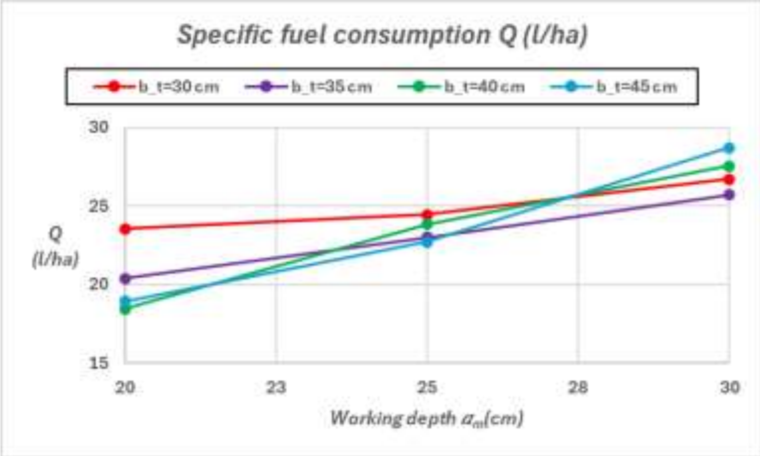

Fig. 4. The variation of specific fuel consumption depending on the working depth

In Figure 4, the graph shows the variation of specific fuel consumption as a function of plowing depth and adjusted working width for a single mouldboard. It can be observed that, as expected, the greatest variation in specific fuel consumption occurs when the working width on the mouldboard is the largest, i.e., b_t=45 cm. This variation is significantly smaller when the plough is adjusted so that the working width on the plow body is 30 cm. However, as indicated in Table 3 and Figure 3, the adjustments made to the plough and operating conditions allow for plowing work to be carried out such that wheel slip remains reasonable (within the range of 11-15%).

Conclusions

This paper emphasizes the essential role of plowing in agriculture, highlighting its irreplaceable nature due to high fuel consumption compared to other tillage methods. The effectiveness of variable plowing width is underscored, adapting efficiently to varying soil physical and mechanical characteristics and tractor traction performance. It recommends high-power tractors to minimize passes over fields, enhancing operational efficiency. Results from experimental trials in Romania using a variable plough width attached to a 200 HP tractor provide valuable data on qualitative and energy indices, such as fuel consumption and tractor efficiency, beneficial for equipment operators and agricultural tractor dealers.

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DIGITAL TECHNOLOGIES IN AGRARIAN SCIENCE AND EDUCATION

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Abstract

The article analyzes statistics on the number of sown areas and cultivated crops in Russia and Siberia, the total sown area being 81.2 and 14.5 million hectares, respectively. The most common crops are grain and leguminous, including corn, occupying 48 and 9.3 million hectares of sown areas in Russia and Siberia, respectively. An analysis of digital technologies in Russian agriculture is provided. Elements of precision farming are used over an area of more than 15,5 million hectares, the main of which include digitization of fields, soil sampling, parallel driving, variable rates of seed sowing and fertilizer application, monitoring the condition of plants using satellite and UAV images, yield mapping and others. Information is provided on the need to develop interdisciplinary specialists in agriculture as part of the digital transformation of society. The need for close participation of agricultural science in the training of personnel for the agricultural industries was noted. Using the example of the experience of the Novosibirsk State Agrarian University, algorithms for the interaction of science, agricultural production and education are reflected. Examples of our own research in the field of precision farming in Siberian conditions are given. The experience of interaction between enterprises producing agricultural machinery and digital services with an educational institution is shown, as well as the experience of introducing elements of precision agriculture into the educational process of a university and the experience of creating educational laboratories that meet all modern requirements of agricultural production in Russia and it`s regions.

Keywords: *Russia, Siberia, digital agriculture, precision planting, digital education.*

Introduction

Currently, agricultural production in Russia is developing quite rapidly, both technically and technologically (Decree of the President of the Russian Federation, 2016). The main constraint on development is the shortage of labor. We are already faced with a shortage of qualified specialists in all areas of agriculture (Pauschinger and Klauser, 2022). The use of digital technologies makes it possible to solve these problems. Following the example of related industries, we see that the introduction of such technologies brings a certain effect. In turn, we come to the conclusion that in the field of agriculture we need completely different specialists. This is not only an agronomist, an engineer, a livestock breeder, a livestock specialist or an information technology specialist. Today, we need a person who will have the basic skills and competencies inherent in all of the listed specialties. Most often, such a vacancy is referred to as a specialist in the implementation of digital technologies in agricultural production. In connection with the current situation, it is necessary to train such specialists within the framework of existing educational programs (Yakushev, 2022). To do this, teachers and researchers need to be at the peak of production, engage in advanced research related to the introduction of digital technologies in agriculture, and then, based on this, develop the material and technical base of universities. Modern equipment and technologies will allow students to master the necessary advanced competencies. In connection with the upcoming transformation of modern universities, this issue is very

relevant. As part of the new formation of educational programs, students will be integrated into scientific activities from the initial courses, which in turn will be based on the established modern laboratories within the framework of the main strategic projects of universities. Such specialists will be sharpened not only by the ability to operate previously developed technical means or apply previously developed technologies. New specialists will be able to independently create fundamentally new solutions based on scientific research. Upon arrival at a new workplace, future graduates will be able to quickly find their bearings and get involved in the production process and benefit the rapidly developing agro-industrial complex.
The aim of this article is to review the state of the agro-industrial complex of the Siberian Federal District of the Russian Federation and the features of training highly qualified personnel capable of introducing digital technologies into agricultural production.

Materials and methods

The article analyzes open data obtained by the Federal State Statistics Service of the Russian Federation on the number of sown areas in the country. An analysis of statistical data from the center for forecasting and monitoring of scientific and technological development of the agro-industrial complex of the Ministry of Agriculture of the Russian Federation in the field of precision farming, automation and robotization was carried out. Research conducted by the Novosibirsk State Agrarian University from Russia is carried out using modern instruments and equipment located both at the university and at partner enterprises. Also, during research and as part of training programs, modern digital services with elements of geoinformation technologies for agriculture, such as ExactFarming, GEOMIR, Cropio, SmartSoil, etc., are used.

Results and discussion

The area of the Russian Federation is 1713 million hectares, which is the first indicator in the world, in turn, the Siberian Federal District, which includes 10 subjects (regions) with a total area of 436 million hectares, is the second largest in the country after the Far Eastern Federal District. The country's cultivated area is 81.2 million hectares, of which 14.5 million hectares are in Siberia. The most common cultivated agricultural crops are grains and legumes, including corn, and occupy 48 and 9.3 million hectares in the country as a whole and in Siberia, respectively (The Federal Service for State Statistics (Rosstat), 2024).

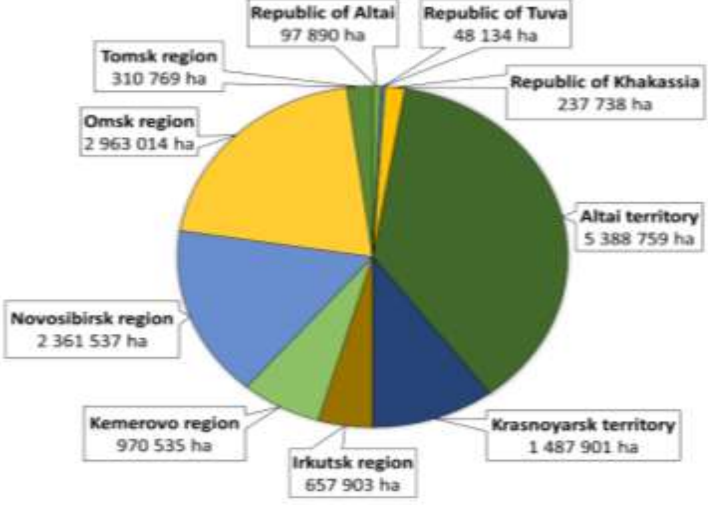

Figure 1. Sown areas of Siberia

From figure 1 it is clear that the territory of Siberia does not have many cultivated areas in relation to a fairly large total area. This fact is due to the fact that most of Siberia is occupied by mountains and forests. Nevertheless, out of 9.3 million hectares, the largest cultivated area is occupied by the Altai Territory with 5.4 million hectares, being the most developed agricultural region of Siberia and one of the leading in Russia, and the Republic of Tuva has the smallest area, due to the fact that it is located mainly in mountainous areas where it borders neighboring Mongolia. It should also be noted that the areas of the Omsk and Novosibirsk regions with 2.9 and 2.4 million hectares of cultivated area, respectively, stand out against the general background.
It goes without saying that in order to increase the economic efficiency of crop production, it is necessary to introduce digital technologies, the basis of which is precision farming, which includes the following elements:
- digitization of fields;
- sampling and tests of soils in the coordinate system;
- parallel driving;
- satellite monitoring of vehicles;
- differentiated spraying/fertilizing/seeding/irrigation/soil cultivation;
- monitoring the condition of crops using remote sensing (UAV, Satellite data);
- yield mapping, etc.

Across the country, one or another of the elements listed above is used on an area of 15.5 million hectares. From Figure 2 it can be seen that in Siberia elements of precision farming are used on an area of 2.9 million hectares (Trufliak, 2020).

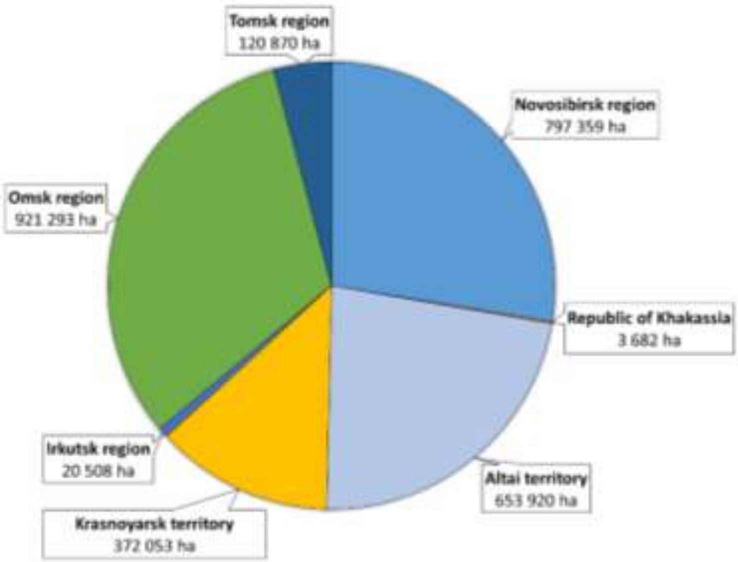

Figure 2. Sown areas cultivated with some elements of precision farming

Having analyzed the areas of Siberia where elements of precision farming are used, we see that the leading regions in the development of modern technologies are the Omsk and Novosibirsk regions with areas of 921.3 and 797.4 thousand hectares. respectively. Elements of precision farming are used on only 30% of the cultivated areas of Siberia, which indicates huge potential for further development.
In turn, the development of modern technologies is impossible without the training of qualified personnel this, in turn, requires practical and scientific experience, which is acquired by the staff of our university in close interaction with agricultural production.

Currently, the main scientific direction of our department is precision agriculture, which, as is known, is based on the concept of an individual approach to each field, and the identification of soil heterogeneities that form depending on many factors (Anfimov et al., 2022; Anfimov, 2022). Subsequently, the calculated areas of heterogeneity are transformed into maps of assignments (prescriptions), using which we can vary the rates of application of seeds, fertilizers, irrigation, and also perform soil cultivation differentiated by depth (Fig. 3). It should also be noted that, taking into account the determined soil differences, it is possible to regulate the operating costs of machine-tractor units when cultivating crops. Since the properties of the soil have a significant impact on such indicators as the traction resistance of implements, and as a result, fuel consumption, and possible disruptions in technological processes that entail losses in yield (Kostić et al., 2014; Iakovlev et al., 2020; Iakovlev, et al., 2023).

Figure 3. Research of soil heterogeneities

We also monitor weather conditions using networks of weather stations (Fig. 4), which allow us to make operational decisions when managing the production cycle (Iakovlev and Polyakov, 2023).

Figure 4. Research of weather conditions

The result of these studies is a reduction in the costs of technological operations, an increase in the yield of cultivated crops and their quality, and as a consequence, an increase in the economic efficiency of agricultural enterprises. And most importantly, our teachers gain enormous practical experience, which they can later pass on to their students, who will soon go to work in agriculture. Modern trends tell us that today's specialists in agriculture must be multidisciplinary, because, firstly, the labor market is experiencing a kind of hunger, and secondly, modern technologies require a specialist to make operational decisions in several areas of specialization. For example, to make decisions in agronomy, a future graduate must have the skills to use geographic information technologies and understand their relationship with engineering technologies. The scientific and industrial experience we have accumulated is reflected in the educational process of our university; in close cooperation with our partners, we successfully introduce elements of agricultural digital technologies into the educational process, modernizing our laboratories, so that our future graduates can master advanced knowledge and skills and apply them in further work (Fig. 5).

Figure 5. Laboratories of the Engineering Institute of NSAU

Conclusion

Agriculture in the Russian Federation and Siberia is currently in a stage of intensive development, as evidenced by constant growth in both technical and technological terms. The total cultivated area in Russia and Siberia is 81.2 and 14.5 million hectares, respectively. The most common crops are grains and leguminous crops, including corn, occupying 48 and 9.3 million hectares of cultivated area in Russia and Siberia, respectively. Elements of precision agriculture are applied over an area of more than 15.5 million hectares, the main of which include field digitization, soil sampling, parallel driving, variable rates of seed sowing and fertilizer application, monitoring of plant health using satellite images and UAVs, yield mapping and others. It is a fact that today it is necessary to train interdisciplinary specialists for agriculture as part of the digital transformation of society. This is possible with the close participation of agricultural science in training personnel for the agricultural industries. Using the example of the experience of the Novosibirsk State Agrarian University, algorithms for the interaction of science, agricultural production and education are reflected. With proper interaction between science, education and agricultural production, the pace will only increase, which is certainly the vector direction of the industry.

Acknowledgement

I express my gratitude to my colleagues from the department of machine and tractor fleet operation and our partner enterprises in the Novosibirsk region and Altai Territory, since everything we do is the merit of each member of our friendly team, working for the benefit of the development of agriculture in our country.

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INFLUENCE OF STEM CUTTING HEIGHT ON THE NUTRITIONAL VALUE OF CORN SILAGE

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Abstract

With an increase in the cutting height of the corn stalks, the crude protein content increases, and thus the nutritional value of the corn silage. During Treatment I, the highest crude protein content was achieved by silage harvester B (7.18%), and the lowest by harvester C (7.03%). The lowest sensitivity during Treatment I was recorded in harvesters A (b = 0.0451) and B (b = 0.0460), and the highest in harvester C (b = 0.0733). It was found that this dependence was very strong for all types of tested silage harvesters, C (R^2 = 0.9973), B (R^2 = 0.9907), A (R^2 = 0.9847) respectively. In Treatment II, silage harvester B also achieved the maximum crude protein content of 8.17%, and harvester C the minimum (7.97%). The minimum sensitivity during Treatment II was recorded in harvester C (b = 0.0171), and higher in harvesters A (b = 0.0208) and B (b = 0.0213). The R^2 factor values showed a strong dependence in all three cases A (R^2 = 0.9960), B (R^2 = 0.9655) and C (R^2 = 0.9903). The content of crude fiber decreased with the increase in the cutting height of the stems. The maximum values of crude fiber content were achieved by harvester C (24.05% in Treatment I and 21.63% in Treatment II). The lowest crude fiber content was achieved during the operation of harvester B (23.03% in Treatment I and 21.08% in Treatment II). Harvester B showed the lowest sensitivity during Treatment I (b = -0.1705) and during Treatment II (b = -0.1486) compared to other types of silage harvesters.

Keywords: *Silage harvester, Corn silage, Cutting height, Crude proteins, Crude fiber.*

Introduction

The preparation of silage is one of the most important processes in the preparation of bulk fodder. The main goal of silage preparation is to produce food with high energy content and highly digestible nutrients (Kung et al., 2018). Good digestibility of corn silage is ensured by the high content of carbohydrates, and the lower content of crude protein and crude ash (Szymańska et al., 2018). The nutritional value of ensiled material is the most important factor affecting the quality of silage. The high nutritional value of the corn plant makes it very suitable for ensiling (Neylon and Kung, 2003), and corn silage itself is one of the most important components of forage used in animal nutrition (Khan et al., 2014). During ensiling, the process of anaerobic conservation of the chopped silage mass by lactic acid fermentation takes place. Zicarelli et al., (2023), state that knowledge of the chemical composition of the whole corn plant is important because it enables the preparation of a balanced meal of high nutritional value. The studies of Mandić et al., (2020) and Bumb et al., (2016) showed that the time of the beginning of the harvest and the cutting height significantly affect the quality of the prepared silage. Maize cutting height affects the chemical composition and nutritional value of silage (Aoki et al., 2013). With higher cutting heights, the nutritional value of silage increases because the content of crude proteins increases, and the content of crude ash decreases, which is a consequence of the increased share of grains in corn silage. Due to the increase in the share of grains, the content of starch is increased, and the content of crude fiber is reduced (Horst et al., 2021), which significantly affects the increase in the nutritional value of silage (Diepersloot et al., 2022). Silage with a high crude protein content has a high energy value (Zhang et al., 2018). Zhao et al., (2018), recommend higher cutting heights of high-protein corn varieties because this improves the nutritional value of the prepared silage. Hulse et al., (2017), recommend harvesting corn at 40 cm height. Higher heights are not recommended, and this is proven by the research of Diepersloot et al., (2022), which states that cutting heights of stems over 60 cm result in a decrease in crude protein content in silage. The choice of appropriate machinery for preparing corn silage (silage harvesters) is an important factor that affects the quality of the silage. The production of quality fodder in animal husbandry depends on the efficiency and quality of work of silage harvesters (Valge et al., 2021). A high correlation dependence is observed between the speed of movement of self-propelled silage harvesters and the cutting height of the stalks. As the movement speed increases, the cutting height increases. The goal of our research is to examine the influence of the cutting height of the stalks on the nutritional value of the silage in dry land conditions during the preparation of corn silage, with the use of different types of self-propelled silage harvesters.

Material and methods

Tests on the influence of cutting height on the nutritional value of corn silage were carried out under dry land conditions during 2020 in central Serbia (43º 33' 35.02" N; 21º 29' 55.61" E) in the municipality of Kruševac. The silage is prepared from whole KWS Mikado hybrid corn plants, of high nutritional value. The harvest was carried out on the same plot with three types of tested self-propelled forage harvesters: New Holland FX 28 (Type A), Claas Jaguar 850 (Type B) and John Deere 6810 (Type C), with a theoretical cutting height of 10 cm (Treatment I) and 30 cm (Treatment II). Using the standard chronometric method (Al-Gadi, 2018), by measuring the time interval of the harvester's movement on an experimental track 500 m long, the working speed of the silage harvester was determined. The length of the trail was measured with a PCE LDM 50 laser range finder. Chronometric measurements were made with a digital chronometer TFA Dostman Triple Time XL-Digitaler 3-fach Timer. The working speed and cutting height of corn stalks was determined in three tests (three repetitions within the test). Using the random sample method, each sample was divided into three parts, which constituted a repetition, and the content of crude proteins, crude fiber and crude ash was determined by appropriate laboratory methods. The content of crude proteins (total nitrogen) was determined according to the standard Kjeldahl method (Thiex, 2009), by decomposition of organic matter with concentrated sulfuric acid (H2SO4) in the presence of catalysts (K2SO4 and CuSO4) and titration of the resulting ammonia. The obtained values were multiplied by a coefficient of 6.25. The crude fiber content was determined by the method (Thiex, 2009), which is based on treating the samples with a solution of sulfuric acid and sodium hydroxide. The rest is separated by filtration and weighed after drying. The method for determining of crude ash is based on decomposition of samples by annealing at 550^0C and measurement of residues (Thiex et al., 2012). Experimental data were processed using the free software package R-Statistics. Tukey's multiple range test was used to test the effects of treatment. A linear regression model was used to analyze the influence of the movement speed of the silage harvesters on the cutting height of the stalks and the effect of the cutting height on the content of nutritional elements.

Results and Disscusion

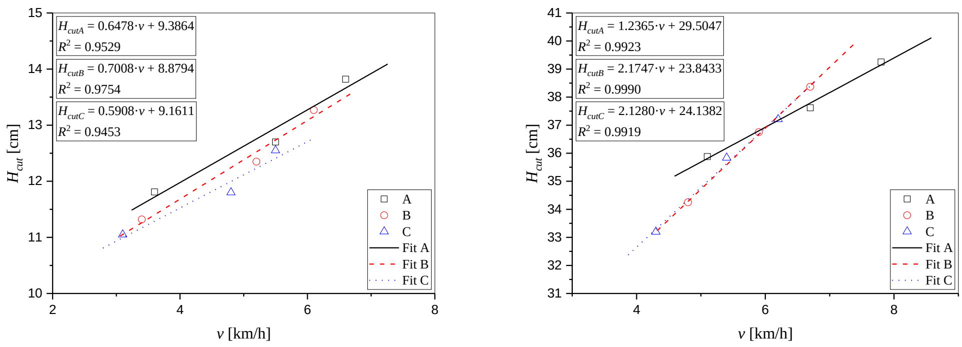
Tests showed that the operating speeds of the investigated silage harvesters influence the cutting height of the corn stalks, which was confirmed by the Tukey test, where a statistically significant difference ($p \leq 0.05$) was found for all the investigated traits except for CP (%) in Treatman II (Table 1). Harvester A achieved the maximum cutting height in the third trial of 13.82 cm at a working speed of 6.60 km h^{-1} in Treatment I and 39.25 cm at a speed of 7.80 km h^{-1} in Treatment II. We recorded the minimum cutting height values with harvester C in the first trial (11.05 cm at a working speed of 3.10 km h^{-1} in Treatment I, i.e. 33.20 cm at a speed of 4.30 km h^{-1} in Treatment II). The achieved results are comparable with the results of Ramm et al., (2023).

Table 1. Results of experimental measurements of silage harvesters (type A, B, C)

Cutting height (cm)		Treatment I				Treatment II			
Param.	Mower	Repetition			$\overline{X}$	Repetition			$\overline{X}$
		1	2	3		1	2	3	
v (km h^{-1})	A	3.60	5.50	6.60	5.23a	5.10	6.70	7.80	6.53a
	B	3.40	5.20	6.10	4.90b	4.80	5.90	6.70	5.80ab
	C	3.10	4.80	5.50	4.47b	4.30	5.40	6.20	5.30b
Hcut (cm)	A	11.81	12.70	13.82	12.78a	35.18	37.62	39.25	37.35a
	B	11.32	12.35	13.27	12.31b	34.25	36.75	38.37	36.46b
	C	11.05	11.80	12.55	11.80c	33.20	35.84	37.21	35.42c
CP %	A	7.02	7.05	7.11	7.06ab	8.04	8.08	8.11	8.08a
	B	7.09	7.13	7.18	7.13a	8.08	8.12	8.17	8.12a
	C	6.92	6.97	7.03	6.97b	7.97	8.01	8.04	8.01a
CF %	A	24.12	24.03	23.64	23.93ab	22.07	21.95	21.34	22.01a
	B	23.36	23.12	23.03	23.17b	21.72	21.53	21.08	21.44b
	C	24.37	24.28	24.05	24.23a	22.28	22.11	21.63	22.01a
C Ash %	A	5.94	5.86	5.73	5.84ab	5.32	5.24	5.11	5.22ab
	B	5.71	5.62	5.54	5.62b	5.23	5.12	4.87	5.07b
	C	6.14	6.02	5.96	6.04a	5.44	5.30	5.25	5.33a

v - working speed; Hcut – achieved cutting heigh; CP – crude protein; CF – crude fiber; CAsh – crude ash; A - New Holland FX 28; B – Claas Jaguar 850; C - John Deere 6810; The Tukey test was applied for the influence of type of harvester (treatment average), significance level $p \leq 0.05$

With the increase in the movement speed of the silage harvesters, the cutting height of the stalks increased linearly (Figure 1).



A B

Figure 1 – Effect of movement speed (v) on cutting height (Hcut): (A) Treatment I, (B) Treatment II

During Treatment I (Fig. 1A), the increase was the largest for the harvester B with a regression slope coefficient b = 0.7008, and the least for the harvester C (b = 0.5908). The R^2 factor values show a strong dependence (B = 0.9754, A = 0.9529, C = 0.9453). In Treatment II (Figure 1B), a very strong sensitivity is observed for the harvesters B (b = 2.1747) and C (b = 2.1280). In this treatment as well, the value of the R^2 factor shows a strong dependence (B = 0.9990, A = 0.9923, C = 0.9919).
The cutting height of the stems affected the content of crude proteins, as their content increased with the cutting height (Figure 2). The highest crude protein content in Treatment I (Fig. 2C) was achieved by the harvester B (7.18% at a cutting height of 13.27 cm), and the lowest by the harvester C (6.92% at a height of 11.05 cm). In Treatment II (Figure 2D), the maximum crude protein content was also achieved by the harvester B (8.17% at a cutting height of 38.37 cm), and the minimum by the harvester C (7.97% at a height of 33.20 cm). The lowest sensitivity during Treatment I (Figure 2C) was recorded by the harvesters A (regression slope coefficient b = 0.0451) and B (b = 0.0460), and the highest by the harvester C (b = 0.0733). It was found that this dependence is very strong for all types of tested silage harvesters, C (R^2 = 0.9973), B (R^2 = 0.9907), A (R^2 = 0.9847) respectively. During Treatment II (Fig. 2D), the minimum sensitivity was observed in the case of the harvester C (b = 0.0171), and higher in the case of the harvesters A (b = 0.0208) and B (b = 0.0213). A very strong dependence was found in all three cases A (R^2 = 0.9960), B (R^2 = 0.9655) and C (R^2 = 0.9903).

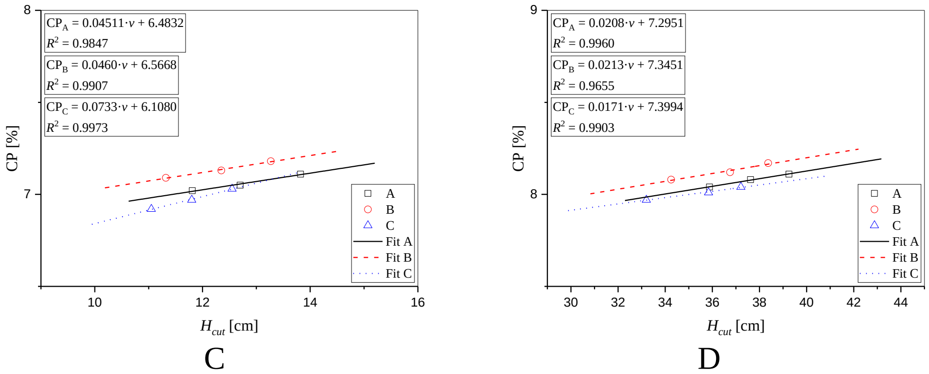
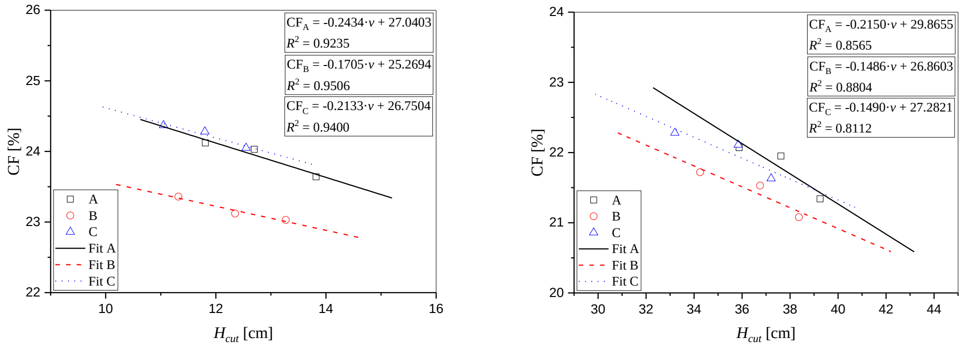


Figure 2 - Effect of cutting height (Hcut) on crude protein content (CP): (C) Treatment I, (D) Treatment II

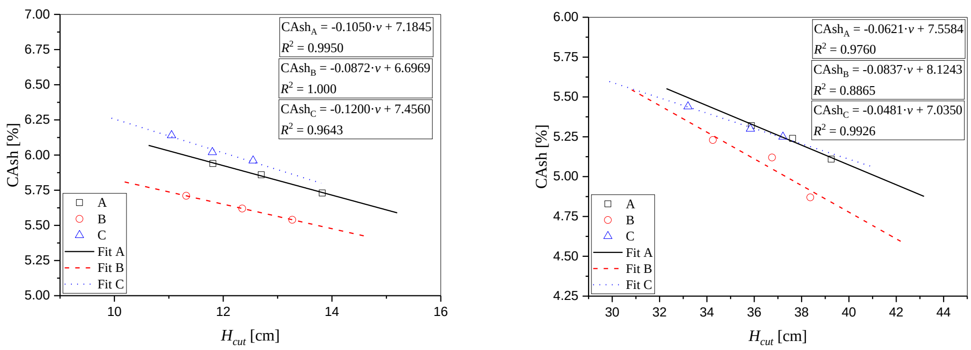
With an increase in the cutting height of the corn stalks, the crude fiber content decreases (Figure 3). The lowest crude fiber content was achieved during the operation of the harvester B (23.03% at a cutting height of 13.27 cm (Treatment I, Figure 3E) and 21.08% at a cutting height of 38.37 cm (Treatment II, Figure 3F).



E F

Figure 3. Effect of cutting height (Hcut) on crude fiber content (CF): (E) Tretman I, (F) Tretman II

The maximum values of crude fiber content were achieved by the harvester C (24.05% at a cutting height of 12.55 cm (Treatment I) and 21.63% at a cutting height of 37.21 cm (Treatment II). The content of crude fiber decreases linearly due to an increase in cutting height. During Treatment I (Figure 3E) the lowest sensitivity was achieved with the harvester B (regression slope coefficient b = -0.1705), and slightly higher with the harvesters A and B (b = 0.2434 and b = 0.2133). The values of the R^2 factor show a strong dependence in all examined silage harvesters A (R^2 = 0.9235), B (R^2 = 0.9506) and C (R^2 = 0.9400). In Treatment II (Figure 3F), harvesters B (with a regression slope of b = -0.1486) and C (b = -1490) recorded the least decline. This relationship was moderately strong for the harvesters A (R^2 = 0.8565) and C (R^2 = 0.8112), and strong for the harvester B (R^2 = 0.8804). The obtained results of crude protein and crude cellulose content are in accordance with previous research (Szymańska et al., 2018; Kung et al., 2008; Kowalik et al., 2013).
The crude ash content did not vary significantly under the influence of the cutting height of the corn stalks (Figure 4). In Treatment I, the concentration ranged from a maximum of 6.14% achieved by the harvester C at a cutting height of 11.05 cm, to a minimum of 5.54% by the harvester B at a cutting height of 13.27 cm (Figure 4G). The decrease is most pronounced during the operation of the harvesters A (regression slope coefficient b = -0.1050) and C (b = -0.1200), and less pronounced with the harvester B (b = -0.0872). The values of the R^2 factor show a very strong dependence in all three cases A (R^2 = 0.9950), B (R^2 = 1.0000) and C (R^2 = 0.9643). In Treatment II (Fig. 4H), we note the lowest sensitivity for the harvester C (b = -0.0481), and the highest for the harvester B (b = -0.0837). A very strong dependence was found for harvesters A (R^2 = 0.9760) and C (R2 = 0.9926) and strong for the harvester B (R^2 = 0.8865).



G H

Figure 4. Effect of cutting height (Hcut) on crude ash content (CAsh): (G) Tretman I, (H) Tretman II

Our research proves that with an increase in the cutting height of the stalks, the crude ash content decreases, which is a consequence of the increase in the share of grains in corn silage and significantly affects the increase in its nutritional value (Diepersloot et al., 2022).

Conclusions

Due to the increase in the cutting height of corn stalks, the crude protein content increases, and thus the nutritional value of corn silage. During Treatment I, the highest crude protein content was achieved by the silage harvester B (7.18%), and the lowest by the harvester C (7.03%). The lowest sensitivity during Treatment I was recorded during the operation of the harvesters A (b = 0.0451) and B (b = 0.0460), and the highest with the harvester C (b = 0.0733). In Treatment II, silage harvester B also achieved the maximum crude protein content of 8.17%, and harvester C the minimum crude protein content (7.97%). The minimum sensitivity during Treatment II was recorded during the testing of the harvester C (b = 0.0171), and higher for the harvesters A (b = 0.0208) and B (b = 0.213). The content of crude fiber decreases with the increase in the cutting height of the stems. Maximum values of crude fiber content were achieved by the harvester C (24.05% in Treatment I and 21.63% in Treatment II). The lowest crude fiber content was achieved during the operation of the harvester B (23.03% in Treatment I and 21.08% in Treatment II). Tukey's test confirmed that a statistically significant difference ($p \leq 0.05$) was found for all investigated traits, except for CP (%) in Treatman II. The results of the comparative test of silage harvesters give an advantage to the harvester B over harvesters A and C.

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APPLICATION OF THE SENSOR IN AGRICULTURE: A REVIEW OF RECENT DEVELOPMENTS

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Abstract

The constant increase in the world population is a trend that also requires the increase in food production, i.e. the increase in crop yields, which represent the main challenge of agricultural production in recent years. This paper aims to provide insight into new trends and technologies necessary for the development of precision agriculture, emphasizing the advantages of integrating smart sensors and innovative technologies. The transition to modern agricultural production enables the increase in yields, a reduction in production costs, and the production of healthier and better quality products. The goal of precision agriculture is to increase yield, efficiency, reduce costs and resources, as well as to minimize the negative impact on the environment. In frames of precision agriculture, there is a trend of introducing sensor detection as a more rational data collection technique. Sensors are devices for detecting, registering and measuring the radiation of electromagnetic energy, its own or reflected. They enable the collection of a larger group of data by applying a simpler technique with minimal costs which can determine the quality of soil and crops. They are also used on a variety of agricultural machinery and robots in order to speed up the data collection process and at the same time prevent the occurrence of malfunctions. The development of precise agricultural production technologies represents a perspective for the improvement of traditional agricultural practices and the transition to modern agricultural production with greater efficiency and sustainability of food provision.

Keywords: *Precision Agriculture, Food production, Data, Innovative technologies.*

Introduction

Agriculture has always been the cornerstone of human civilization, providing a stable source of food and raw materials. However, with a growing world population and limited natural resources, traditional agricultural approaches increasingly show their limitations. In this context, precision agriculture has emerged as an innovative response to the challenges of modern farming, offering sustainable and efficient methods to increase yields and reduce environmental pollution (Yadav & Kumar, 2023).

Precision agriculture relies on advanced technologies to enable accurate and timely management of agricultural processes. One of the key aspects of this practice is the integration of sensors, which collect data on various parameters of the environment and crop conditions. This data allows farmers to make timely decisions, optimize the use of resources such as water, fertilizers, and pesticides, and improve the overall efficiency of agricultural production.

Sensors used in precision agriculture can measure various parameters, including soil moisture, nutrient levels, temperature changes, the presence of pests, and many other factors (Pajić, 2022). These sensors are often linked to systems for remote control and data analysis, allowing real-time monitoring and swift interventions. The use of drones equipped with

cameras and multispectral sensors allows for precise mapping and analysis of large arable land (Dibyajyoti Nath, 2023).
This paper aims to investigate the importance and application of sensors in precision agriculture. It analyzes how these technologies contribute to improving yields, reducing costs, and preserving the environment. The paper will focus on practical examples and case studies of the application of optical sensors, showcasing successful implementations of these technologies in various aspects of agricultural production. Finally, the paper will explore the challenges and future directions of development in this area, which has the potential to transform agricultural production methods.

Sensors

Sensors are devices that can measure various physical quantities such as temperature, humidity, speed, acceleration, position, and light intensity. They provide measurable representations of the measured physical quantity in the form of an electrical signal or changes in the electrical characteristics of an electrical component (Pajić et al., 2022). The purpose of sensors is to detect changes in a given environment and send information to a computer processor. The most commonly used sensors in agriculture are used for measuring meteorological conditions, soil moisture, and plant conditions, as well as for monitoring yield. The sensors are characterized by their ability to register a wider or narrower spectral range, individual spectral lines (rays of one wavelength), or multiple separated spectral lines within one spectral region at once. The fact that sensors are not only used for measurement but also in other areas such as shipbuilding, sports, various sciences, household, medicine, etc. indicates how important sensors are in everyday life.
The sensors can be divided into two types based on the kind of energy they detect: passive or active (Kostić, 2021). Active sensors emit and receive energy, capturing the reflected part from the measured object. They have their light source and can be utilized year-round and throughout the day, regardless of weather conditions and the position of the Sun. Active sensors, unlike passive ones, can send and receive energy. This category includes radars and laser scanners. Active and passive sensor technologies are used in remote sensing to take measurements from a distance or to measure phenomena that are not visible to the naked eye. Passive sensors only detect the energy emitted by the object being observed. Passive multispectral sensors depend on natural light, which can be significantly influenced by factors like sun exposure, cloud cover, architectural structures, and the reflective properties of scanned objects (These variables can impact the consistency of imaging over time, especially when observing a wide area that necessitates prolonged scanning (Fahey et al., 2020; Kostić, 2021). Examples of passive sensor technologies include photographic, thermal, chemical, and infrared sensors. Also, sensors can be classified according to different criteria (Pajić, 2022):

- by the sizes they measure,
- types of signals and interfaces,
- features and characteristics,
- quality class,
- principle of work,
- production technology,
- fields of application,
- values.

The sensor's main features include:
Sensitivity - refers to how the output value changes when the measured value changes, such as in an electrical measuring instrument. If a displacement of 0.1 mm results in a voltage

change of 0.1 V at the output, then the sensor's sensitivity is 1 V/mm. Sensors used to detect small changes must have high sensitivity.
Linearity - ideal sensors are designed to be linear, i.e., the sensor's output signal is linearly proportional to the measured value. Linearity is challenging to achieve, and any deviations from the ideal are called linear tolerances. Linearity is expressed as a percentage of deviation from the linear value. It is the maximum deviation of the output curve from the line that best fits one calibration cycle, and it is related to the accuracy of the sensor.
Accuracy is typically assessed through absolute and relative error. Absolute error is the disparity between the actual value of the measured quantity and the measurement result (sensor output value). Relative error is calculated as the ratio of the absolute error to the actual value. The relative error is often presented as a percentage.
Signal resolution - represents the smallest value change that a sensor can recognize in the measured quantity. Resolution is related to the accuracy of the measurement and represents the sensor's ability to reproduce a certain set of readings within a given accuracy.

Sensors in precision agriculture

The use of sensors in agricultural production has evolved alongside technological advancements. Initially, sensors were complex systems designed to measure temperature or humidity, but today's sensors are modern, precise, and offer a wide range of capabilities. It is expected that sensor applications in agriculture will continue to expand in the future, playing a crucial role in achieving sustainable, efficient, and productive agricultural practices. Modern agricultural systems now integrate various types of sensors, enabling the monitoring and control of numerous parameters, leading to improved efficiency, productivity, and overall sustainability in agricultural production (Saiz-Rubio & Rovira-Más, 2020; Paul et al., 2022).
In precision agriculture, sensors are used on devices to test soil properties, on machines for feeding and protecting crops, and for harvesting crops. These sensors are installed on the machines and connected to the control unit and GPS receiver. The data obtained through these measurements is precise because the measurements are taken during agrotechnical operations. The most well-known system of this kind is OptRx from Ag Leader (https://www.agleader.com/blog/ag-leader-releases-optrx-crop-sensor/). The sensors are calibrated to the machinery (sprayer). What sets it apart is its direct connection to the display, which allows access to sensor readings. The sensor uses a wave that responds even in high-density plant environments. Another widely used approach for assessing soil properties involves the Veris MSP3 sensor system (Jurišić & Plaščak, 2009). These sensors function based on the same principle as the previously mentioned system, offering essential information for soil management. However, unlike the other system, the Veris MSP3 necessitates using processed data alongside a computer program to generate different types of maps that can be applied for agricultural purposes.

Sensory measurement of crop characteristics

Sensory measurement of crop properties is one of the main approaches in modern agricultural production systems. Accurate measurement of plant characteristics is made possible by sensors that have achieved high tracking accuracy through technological progress (Marković et al., 2020). Sensory measurement of crop properties enables optimization of the use of resources such as fertilizers, pesticides and water, thereby reducing production costs. Multispectral sensors used in remote sensing and sensor measurement of crop properties can determine the state of the plant by observing it outside the visible part of the spectrum. Multispectral images have a high resolution and offer the analysis of different wavelengths, which allows identification of plant problems, timely detection of diseases, deficiencies of nutrients, water and other essential elements. The obtained multispectral images enable the calculation of data from different channels based on the absorption and reflection of solar energy. The visible and invisible part of the spectrum of solar electromagnetic radiation is represented by means of bands. So the bands represent parts of the spectrum of different wavelengths and frequencies. Visible spectrum (VIS) refers only to part of solar radiation and represents a wavelength of 400 - 700 nm (Skendžić, 2022). Part of the spectrum with a wavelength of 700 – 2500 nm is called Near infrared (NIR) and it is invisible to the naked eye (Figure 1). The NIR spectrum has two sublevels (I – wavelength range - 1,300 nm, II – wavelength range 1,300 - 2,500), (Wójtowicz et al., 2016; Kostić, 2021).

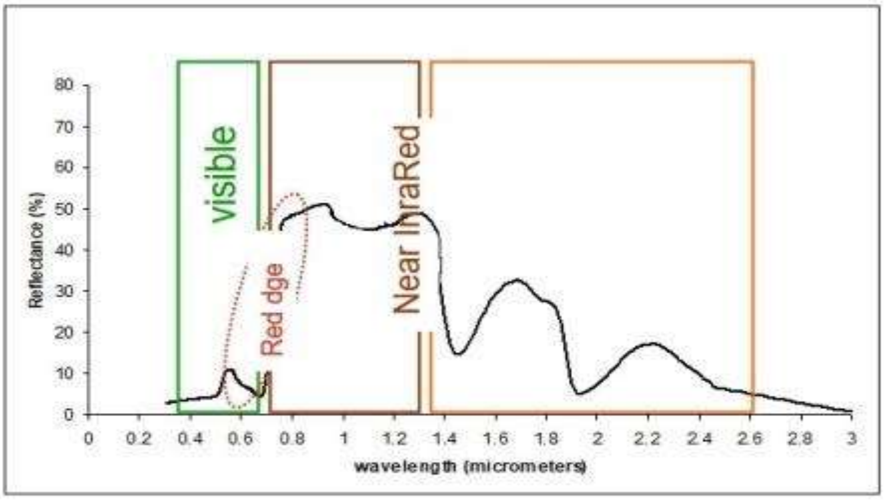

Figure 1. Visible and invisible part of the spectrum

An example of a device for measuring crop characteristics

The Plant-O-Meter device (Figure 2) is a handheld multispectral proximal sensor. This type of sensor belongs to active sensors because it uses its own light source. It has six optical channels: blue (455 nm), green (740 nm), red (657 nm), RedEdge (740 nm), NIR1 (810 nm), NIR2 (940 nm). All information collected in the field can be saved through the application installed on smartphones. Obtaining information from different channels allows the recalculation of over 30 vegetation indices (these indicators make it easier to understand the obtained data).

Figure 2. Plant-O-meter (https://www.plant-o-meter.com/)

Vegetation index values describe crop health in a good way, monitoring growth stages and identifying problems (Xue & Su, 2017). The values obtained by recalculating the amount of solar radiation of different wavelengths make it possible to identify the area and precisely apply the necessary measures in order to respond in a timely manner.

Conclusion

The application of sensors in agriculture has brought many reliefs to agricultural producers. Sustainable and more efficient production using different types of sensors enabled the control of conditions and timely intervention. With further technological progress of the precision agriculture system and the sensors themselves, it is possible to expect improvements in terms of various sensor characteristics and contribute to the creation of a sufficient amount of health-safe food.

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CONTENT OF MINERAL MATTER IN THE GRAIN OF POPCORN IN A SUSTAINABLE CULTIVATION SYSTEM WITH COVER CROPS

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Abstract

The nutritional properties of popcorn (*Zea mays* L. *ssp everta* Sturt.) are very attractive to consumers, making it a highly valuable product with excellent functional properties. The increased percentage of consumption of corn with specific properties is a consequence of its quality, because it belongs to gluten-free food, with a high content of fiber and other important components for human nutrition. In some parts of the world, it occupies a significant part in the diet of the population, while in others it represents a healthy snack. Popcorn is a good source of minerals and is low in calories. The paper examined the content of mineral matter (Ca, Zn) in popcorn (hybrid ZPSC 611*k* FAO 600) grown in a sustainable system with the use of cover crops (CC) and biofertilizers (BF). The experiment was set up at the Maize Research Institute "Zemun Polje" during 2013/14-2014/15. Cover crops included variants: T1-winter fodder pea, T2-winter oat, mixture T3 - winter fodder pea + winter oat, and control variant T4 - bare soil. CC were sown in autumn, plowed at the end of April or May, after which 1/2 of the plot was treated with BF, and the main crop in both years was winter wheat. Popcorn was sown in mid-May at a density of 65,000 plants per ha. Cover crops in combination with biofertilizer had a significant effect on increasing the content of the examined parameters in the grain, especially in the second year with more favorable meteorological conditions. By comparing the examined years, we observe that the highest Ca content was achieved on the variant with winter fodder pea (313.75 mg g^{-1}) with the application of BF, while the highest values of Zn (58.94 mg g^{-1}) were recorded on the variant with a mixture of winter fodder pea and winter oat without BF in the second year. The results show that winter fodder pea is a good choice as a cover crop, it produces a large biomass, protects the soil and, in combination with biofertilizer, increases the biomass of popcorn and the concentration of mineral substances in popcorn grains.

Key words: *mineral matter, popcorn, cover crops, sustainable cultivation system.*

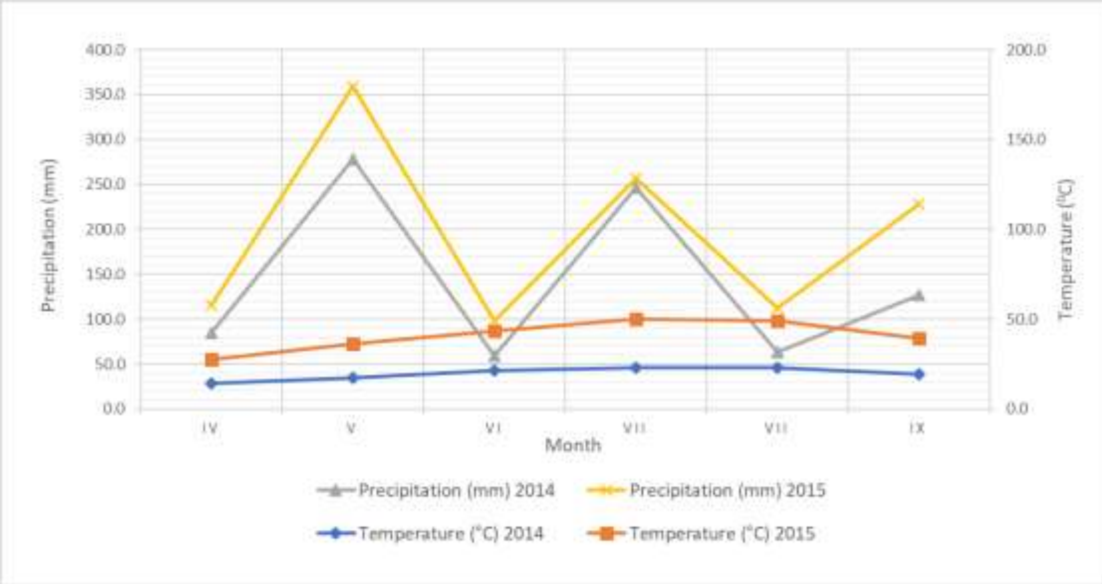
Introduction

Sustainability in agricultural production is largely based on increasing biodiversity and reducing the intake of agrochemicals, especially fertilizers. Revilla et al. (2021) state that a strategy to transition agricultural production with a large investment to a sustainable cropping system is a priority, and some combinations of uses may be more appropriate for sustainable production under different environmental conditions. Cover crops are an important part of sustainable agriculture. This biological measure maintains or increases the level of organic matter in the soil and the main role of the specific crop cultivation system is reflected in the improvement of the physical and mechanical properties of the soil, the water regime, the increase in the content of nutrients, the suppression of weeds and the achievement of higher yields in the main crops (Marcillo and Miguez, 2017. Daryanto et al., 2018; Simic et al., 2020). Microbiological fertilizers play a significant role in maintaining fertility and increasing crop yields (Mahdi et al., 2010). The contribution of microbial fertilizers to the quality of the obtained products has also been confirmed, and a good example is the increase in the content of lycopene and vitamin C in tomato fruit (Verma et al., 2015; Ochoa-Velasco et al., 2016), as well as the increased content of vitamin C and glutathione in maize grain (Dragičević et al., 2013; Dolijanović et al., 2017). To improve the agroecosystem, it is best to grow mixtures of cover crops (Ranaldo et al., 2015). This increases the productivity, stability and efficiency of resource utilization in cultivation systems. The biggest benefit from the use of cover crops in the cultivation of popcorn is their ability to contribute to a higher and better yield, and considering that cover crops contribute to the reduction of weeds, the introduction of cover crops into the cultivation system can represent an alternative use of herbicides (Tardi et al., 2015). The key reason for growing cover crops in a sustainable and organic agricultural production system is to increase soil fertility, reduce the occurrence of weeds, pests and diseases, as well as increase biological diversity (biodiversity) in the agroecosystem (Sturm et al., 2018). The most common crops for green fertilization are mainly plant species from the Fabaceae family (peas, peas, lupins, beans, lentils, clovers and alfalfa), which with the help of nitrogen fixation often provide enough N for their needs, and a significant amount remains on the land and will use it . the next crop (Oljača and Dolijanović, 2013; Blanco-Cankui et al., 2015). The reduction of input and losses of nitrogen in/from the soil by growing legumes due to very efficient mineralization, enables a significant reduction in fossil energy consumption and greenhouse gas emissions (Kocira et al., 2020, White et al., 2017; Abdalla et al., 2019). Leguminous species, which form a large amount of organic matter in a relatively short period of time, are capable of fixing atmospheric nitrogen. For the maize crop, which is a large consumer of nitrogen, legumes are a good choice because they grow quickly in the fall, protect the soil during the winter, and start growing early in the spring, accumulating a large amount of nitrogen-containing organic matter (Cutti et al., 2016; Jabran et al. , 2018). The application of cover crops in the technology of growing plants could achieve direct and indirect economic benefits, which are reflected in the reduction of production costs, primarily of fertilizers, pesticides, etc., as well as the improvement of the agroecosystem, especially the soil (Daryanto et al., 2018; Jacobs et al. al., 2022.). Lesueur et al. (2016) state that biofertilizers must be a priority in such cropping systems, if viewed from the perspective of food security and economic progress. Higher crop productivity and significant improvement of the agroecosystem can be supported by the use of biofertilizers. According to Malusà et al. (2016); Roychowdhury et al. (2017), and Mącik et al. (2020), biofertilizers stand out among the best tools in modern agriculture, which have a low impact on the environment. They represent an indispensable part of the integrated system of nutrient management in the soil and affect the reduction of the intake of mineral fertilizers, thereby ensuring the optimal supply of crops with mineral nutrients (P, Ca, Cu, Zn, etc.). The main feature that distinguishes popcorn from other types of corn is the formation of a large "flake" or "popcorn" after the kernel explodes in response to heating. When the popcorn kernels are heated, the water in the floury endosperm turns into water vapor and the kernel bursts, increasing its volume by 20-30 times. In doing so, the endosperm turns into a white, loose and porous mass. The form and content of mineral substances of popcorn are attractive for consumers and a good choice in nutrition, because it belongs to the group of products with high functional properties. Popcorn is a good source of fiber, Ca, Fe, P, niacin and has low caloric value, especially if it is prepared without oil and other additives, or with a very small amount of oil (Park et al., 2000; Paraginski et al., 2016). Milašinović-Šeremešić et al. (2019) in testing the nutritional quality of corn grains of ten different genotypes observed significant differences between genotypes in the content of starch, protein, oil and cellulose. It was found that corn has a higher content of phenolic acids than many other cereals (Ndolo and Beta,

2014. Fardet et al. (2008) state that corn is the richest source of vitamin E compared to other cereals, including wheat. Dolijanović et al. (2018) state that cover crops, especially leguminous ones, had a significant impact on increasing the protein content of sweet corn grains. Research related to cover crops and the application of biofertilizers, and especially their mutual interaction on popcorn productivity and grain quality when nutritional properties, such as the content of essential elements, are taken into account are few in the literature. The aim of this work is to examine the influence of the application of different cover crops and biofertilizers on the content of mineral substances Ca and Zn in the grain of the tested popcorn hybrid, in order to produce a nutritionally better product for human consumption.

Materials and methods

A two-year trial was set up during 2013/2014. and 2014/2015. in the experimental field of the Maize Institute "Zemun Polje" (44°52′N; 20°20′E), according to the split-plot system in four repetitions. The pre-crop in both years was winter wheat, after which autumn soil preparation was carried out (deep plowing and fine pre-sowing soil preparation), then sowing of cover crops at the end of October/beginning of November. Before sowing, soil samples were taken for agrochemical analyzes at a depth of 0-20 cm and 20-40 cm. Fertilization of cover crops was carried out together with basic tillage. The goal was to provide 120 kg N ha^{-1}, 90 kg P ha^{-1} and 60 kg K ha^{-1} to the main crop. The entire amount of P and K was introduced in the autumn with the fertilizer monopotassium phosphate (0:52:34), while the required amount of N was introduced in the spring, together with the sowing of popcorn, in the form of individual fertilizer Urea, namely 120 kg ha^{-1} N (T2 and T4 variants), 80 kg ha^{-1} N (T1 variant) and 90 kg ha^{-1} of pure feed (T3 variant). The remaining 40 and 30 kg ha^{-1} N, respectively, are considered to be provided by nitrogen fixation. Immediately after plowing the cover crops, the Uniker biofertilizer (mobilizer of nutrient elements) containing strains of proteolytic and cellulolytic bacteria in the amount of 10 l ha^{-1} was applied to the soil on half of the elementary plot, which supported the mineralization of the introduced crop residues and whose application has a favorable effect on increasing soil fertility. Popcorn was sown by hand at the end of May and the crop was grown in a natural water regime. The size of the basic plot was 7x5 m. The sowing density was 65,000 plants per ha, with an inter-row spacing of 70 cm and a distance between plants in a row of 22 cm. Popcorn (*Zea mays* L. *everta* Sturt-fam. Poaceae), hybrid ZPSC 611k (FAO 600) belongs to the mid-late vegetation group. Under optimal conditions, it gives a yield of about 6 t ha^{-1} of dry grain. The bulk volume is from 38 to 40 cm^3 g^{-1}. This hybrid has a yellow-orange pearl type grain. After harvesting, samples were taken for chemical analysis of popcorn kernels in order to determine to what extent the applied sustainable system of cultivation of these crops contributed to the increase in its quality and content of essential elements Ca and Zn, thus recommending the tested technology as useful for growing corn for human consumption. After wet digestion with $HClO_4$ + HNO_3, the concentration of essential mineral elements (Ca and Zn) was determined with inductively coupled plasma-optical emission spectrometry (Spectroflame, 27.12 MHz and 2.5 kW, model P, Spectro Analytical Instruments, Kleve, Germany). The obtained data were processed statistically, using the method of analysis of variance (ANOVA) for three-factor experiments. For individual comparisons, the least significant difference test (LSD test at the significance level 0.05 and 0.01) was used. Meteorological conditions (average monthly air temperature and monthly precipitation) during the vegetation period of the main crop in both years of the study are shown graphically (*Graph* 1).



Graph 1. Climate diagram for the vegetation period of the main crop in the examined years

More favorable meteorological conditions in terms of the amount and distribution of precipitation were measured in the first year of the research. The needs of popcorn for precipitation depend on the stage of development and were less at the beginning of the growing season, and the highest (250-280 mm) in the period of flowering and cob formation. The first year is characterized by the optimal air temperature, which had an impact on grain yield, and which further affected the quality, that is, the content of the tested parameters. Precipitation during the months of June and July in the second examined year was significantly less, while air temperatures recorded higher values, which did not favor popcorn due to the extremely dry period during the summer month.

Results and discussion

The influence of growing cover crops and application of biofertilizers on changes in the chemical composition of popcorn kernels, (Ca and Zn content) , are tabulated for two years of testing (*Tables* 1 and 2). Based on the statistical analysis of the data, it can be seen that the influence of the factors and their interrelation had a statistically significant impact on the examined parameters. The obtained results showed that winter forage pea is an excellent and useful cover crop, especially in combination with biofertilizer, to support the accumulation of popcorn biomass, yield potential and concentration of mineral substances Ca and Zn. The highest Ca values were measured in the second year of the study and amounted to 313.75 mg g^{-1} (*Table* 1). The above results could be attributed to lower air temperatures and optimal amount of precipitation, which had a positive effect on fertilization and grain formation in popcorn. The action of biofertilizers depended to a high degree on meteorological conditions, especially the amount and distribution of precipitation during the corn growing season. Winter fodder peas had the greatest impact as a cover crop, a more robust crop compared to winter oats. In addition, plant residues of winter fodder peas are a good basis in the soil, which encouraged the work of N-fixing bacteria and other soil microorganisms, which had a positive effect on the quality of popcorn kernels and increased content of essential substances. Similar research has shown a good combination for cover crops for the accumulation of essential elements in corn with specific properties, i.e. winter fodder kale and a mixture of vetch + winter oats + BF were achieved in sweet corn grain (Dragicevic et al., 2021). Winter oats and a mixture of legumes and winter oats gave the worst result, especially in the first year of the trial, which was not favorable for the main crop due to the uneven distribution of precipitation and insufficient amounts, followed by elevated temperatures in the summer period. Application of bioferilizers had a positive, but not statistically significant, effect on the content of Zn in popcorn kernels in both investigated years. A mixture of winter fodder peas and winter oats (without the application of biofertilizers) showed a positive effect on the Zn content compared to the control. The mixture variant maintained this trend during both years of testing (*Table* 2), and the highest content was 58.94 mg g^{-1} without BF in the second year.

Table 1. Ca content (mg g-1) in popcorn kernels for the period 2013/14 - 2014/15.

Year (A)	**2013-2014**		**2014-2015**	
Biofertilizator (C)	Without BF	BF	Without BF	BF
Mineral matter/ Treatments (B)	**Ca (mg g^{-1})**		**Ca (mg g^{-1})**	
T1	111.60	39.71	143.75	313.75
T2	91.62	48.22	277.50	176.25
T3	77.89	53.12	146.56	200.63
T4	91.31	52.63	100.00	290.94
Average	**93.10**	**48.42**	**166.95**	**245.39**

Factors	A**	**B****	**C****	**AB****	**AC****	**BC****	**ABC****
LSD 0.05	5.11	7.23	5.11	10.22	7.23	10.22	14.46
LSD 0.01	8.75	12.37	8.75	17.49	12.37	17.49	24.74

p<0.01 very significant (**); p<0.05 significant (*); p>0.05 no significant

Thanks to its nutritional value, popcorn is most used as a "healthy snack" around the world. Popcorn corn grain is an excellent source of essential amino acids and mineral elements, such as K (up to 2456 mg kg^{-1}), Na (up to 148 mg kg^{-1}), Mg (up to 387 mg kg^{-1}), Ca (up to 306 mg kg^{-1}) and P (up to 2486 mg kg^{-1}), with lower concentrations of Fe, Mn, Zn, Cu and Cr (Naji et al., 2013; Abebe et al., 2017). The higher nutritional value of popcorn could represent a good basis for its sustainable cultivation system, which includes cover crops and biofertilizers (Ahmed and Hassan, 2021). It is known that microorganisms, and especially actinomycetes, promote the growth and development of legumes (AbdElgawad et al., 2020), it could be assumed that the prolonged influence of plant residues, such as forage peas in the soil, improved the development and activity of actinomycetes, resulting in an increased availability of nutrients matter, and thus the growth of corn and the quality of the grain yield.

Table 2. Zn content (mg g^{-1}) in popcorn kernels for the period 2013/14 - 2014/15.

Year (A)	2013-2014		2014-2015	
Biofertilizator (C)	Without BF	BF	Without BF	BF
Mineral matter/ Treatments (B)	**Zn (mg g^{-1})**		**Zn (mg g^{-1})**	
T1	5.63	3.93	34.22	47.59
T2	6.08	6.44	44.81	41.81
T3	2.28	7.22	58.94	44.91
T4	3.62	6.60	37.13	39.88
Average	**4.40**	**6.05**	**43.77**	**43.55**

Factors	A**	B**	C^{nz}	AB**	AC^{nz}	BC**	ABC**
LSD 0.05	1.50	2.18	1.50	3.01	2.18	3.01	4.25
LSD 0.01	2.57	3.64	2.57	5.15	3.64	5.15	7.28

$p<0.01$ very significant (**); $p<0.05$ significant (*); $p>0.05$ no significant

Ugrenović et al. (2024) in research came to the results, which show that the increased application of plant residues of the mixture of white lettuce + oats as a cover crop in the soil significantly increases the total number of microorganisms and their activity, which contributes to the increase of organic matter in the soil and improved crop production. Ciampitti and Vyn (2013) emphasized a positive correlation between higher amounts of N in maize plants and increased accumulation of Ca, Mg and trace elements in the grain. Dragicevic et al. (2024) stated that higher protein concentration in popcorn kernels was accompanied by greater accumulation of essential mineral elements, such as Ca, Mg, Fe and Zn, which contributed to the nutritional quality of popcorn kernels. In their research, the highest value of Zn (16.71 mg g^{-1}) was achieved in the treatment with winter fodder peas + BF, while good results in terms of Ca content (876.34 mg g^{-1}, i.e. 759.61 mg g^{-1}) were achieved by winter fodder kale with and without BF. Differences in the content of mineral substances existed in the year in which there was a lack of precipitation and high air temperatures, and if the meteorological conditions were more favorable, the content was much higher, both in the variants without and in the variants with the application of biofertilizers.

Conclusions

The combined application of different cover crops and biofertilizers expressed a variable effect on the accumulation of the main grain components (nutrients), from which Ca and Zn were isolated. The most significant statistical influence was shown by cover crops and biofertilizer, as well as their mutual interaction. The best results in Ca content were achieved by the winter forage pea cover crop in combination with biofertilizer, while the highest Zn content was measured on the mixture variant in the second year of testing. The results of this research could contribute to the development of sustainable production of corn with specific properties, all with the aim of improving the quality of popcorn kernels. Sustainable cultivation systems, in addition to the benefits of increasing soil cover and increasing crop yields, can have a positive impact on the nutritional quality of cultivated grains, such as popcorn.

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VARIATION OF SEEDS NUMBER IN PRIMARY SPIKE OF BREAD WHEAT

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Abstract

The number of seeds in primary spike is a yield characteristic that is related to the degree of pollination and fertilization of spikes. The aim of this work is to investigate the variation of number of seeds in primary spike in wheat grown under different environmental conditions. For this study were used the 10 wheat genotypes which grown in the field experiment on a plot size of 1 m^2 in three replication in two vegetation seasons (2015-2017). The seeds were sown at a distance of 0.10 m in rows of 1.0 m length between which the distance was 0.2 m. At the full maturity stage the 20 plants replication^{-1} were used for the analysis of the number of seeds in primary spike. The analysis of variance was performed by MSTAT C (5.0 version). The results showed significant differences among wheat genotypes in both years, according to number of seeds per primary spike. On average in the first vegetation season the number of seeds per primary spike varied in range from 55.63 in G-3502-5 to 75.10 in G-3501-1 genotype. In the second vegetation season the number of seeds per primary spike varied from the lowest 53.55 in the G-3502-2 genotype to the highest 77.62 in G-3612-4 genotype. The differences in the number of seeds per primary spike were determined due to genotype, environmental factors in vegetation season, and interaction genotype/environment.

Key words: wheat, genotype, number of seed spike^{-1}, environment.

Introduction

Wheat yield is a complex trait which formed as results of interaction of yield components as well as traits of stem, spike and seed. Yield of wheat includes seeds per spike, seed mass and spike per unit square area (Slafer et al., 2014). The number of seeds per spike and the mass of seeds per spike have a large contribution to the formation of the yield, the value of which largely depends on the genetic factors and the environmental factor (Feng et al., 2018; Li et al., 2016; Knežević et al., 2006). Varying the number of seeds per spike depends from developmental stage of spikelets in spike, efficiency of floret fertility (García et al., 2014; Li et al., 2016: Knežević et al., 2024) as well influence of mineral nutrition (Djokić et al, 1998; Sugár et al., 2016). Each wheat spikelet has more than one seed, which indicates their correlation and these represents an essential components in forming of seed yield (Knežević et al., 2021; Zhou et al., 2021; Wolde et al., 2019).The seeds number spike^{-1} is determined by several factors, such as spikelet number per spike, and the fertility of each floret, seed size per

spike (Sreenivasulu and Schnurbusch 2012; Guo et al., 2015; Knežević et al., 2018a) which depends on genotype, environment and interaction of genotype/environment/scientific measures technology growing (Guo and Schnurbusch 2015; Knežević et al., 2018b; Urošević et al., 2023). These traits are determined by genetic factors. Up to nowdays are known genes in wheat genome which associate with increases in number of seed spike^{-1} which contribute to increasing yield. For seeds number per spike identified many candidate genes which located on chromosomes 2A, 2D, 3B, 4A, 5A, and 6D (Mizuno et al., 2021; Glenn et al., 2022). Genotypes flowering earlier tend to have lower spikelets number per spike than those flowering later (Shaw et al., 2013; 2014; González-Navarro et al., 2015). In favorable environments wheat genotypes express their efficiency of floret fertility and seeds survival, better absorption of nutrient, efficient photosynthesis and translocation of photosynthate to seeds (Djokić et al, 1995; Gegas et al., 2010; Guo et al., 2016). During the wheat breeding, the seeds number per spike was changed and increased on the base of increasing translocation of assimilate to developing spikes and seeds, which effects on improving potential of yield (Foulkes et al., 2011; Reynolds et al., 2022).
The aim of this work was to investigate the variability of the number of seeds per primary spike of the divergent winter wheat genotypes growing in different environmental conditions.

Materials and Methods

For this study were used the 10 wheat genotypes which grown in the field experiment on a plot size of 1 m^2 in three replication in two vegetation seasons (2015-2017). The seeds were sown at a distance of 0.10 m in rows of 1.0 m length between which the distance was 0.2 m. At the full maturity stage the 20 plants replication^{-1} (total 60 plants per genotypes) were used for the analysis of the number of seeds in primary spike in each of two vegetation season of experiments. The analysis of variance was performed by MSTAT C (5.0 version) and significant differences were estimated by F-test values and tested by test value of LSD $_{0.05}$ and LSD $_{0.01}$.

Weather conditions

During the both vegetation seasons of experiment were registered different value of temperature variation and dynamics and amount of precipitation. The average temperature 9.96 ^{0}C in the first vegetation season was higher than in the second vegetation season ($\overline{t}$ =8.74 ^{0}C) and than in 10 year periods ($\circ\,\overline{t}$ =8.50). The distribution and average monthly precipitation was different between two vegetation seasons and in the first vegetation season the total amount of precipitation was 651mm and higher than total amount of precipitation 523 mm. In the both vegetation season the total amount of precipitation was higher than in 10 year periods (417 mm). In the both growing season after sowing October-November at the time of seed germination and sprouting plants, the values of temperature and precipitation were similar. However, in period February-April the amount of precipitation in the first vegetation season (250.5 mm) was higher than in the second (174.0 mm), while the distribution of precipitation in second vegetation season was more favorable (Table 1).

Table 1. Average monthly temperature and total monthly precipitation in Kraljevo

	Period	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Xm	Total
Temperature °C	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	
Temperature °C	2016/17	10.6	6.8	0.0	-4.7	5.2	10.8	11.1	16.8	22.1	**8.74**	
2000-2010		11.8	6.4	1.7	-0.1	2.6	5.9	11.6	16.4	20.4	**8.50**	
Precipitation (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6		**651.0**
Precipitation (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0		**523.1**
2000-2010		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3		**417.8**

Results and Discussion

The number of seeds per primary spike varied in analysed wheat genotypes depends of environment in growing season. In the first vegetation season the lowest number of seeds per primary spike was 55.63 in the genotype G-3502-5 and the highest was 75.10 in the genotype G-3501-1, while the average value for all 10 genotypes was 65.66 seeds spike^{-1}. In the second vegetation season, the number of seeds per primary spike varied in range from the lowest 53.55 in the genotype G-3502-2 to the highest 77.62 in the genotype G-3612-4 with average value 68.06 seeds spike^{-1} for all 10 genotypes. The average number of seeds spike^{-1} for all 10 genotypes in the second vegetation season was 68.06 and higher than the average number of seeds spike^{-1} for all 10 genotypes in the first vegetation season (65.66) table 2.

Table 2. Variability of the number of seeds per primary spike in wheat genotypes

Cultivars	First vegetation season	Second vegetation season	Average
G-3501-1	75.10 ^{abcd}	76.62 ^{bc}	75.86 ^{a}
G-3501-2	69.68 ^{de}	63.85 ^{ef}	66.76 ^{c}
G-3502-1	70.34 ^{bcd}	78.56 ^{a}	74.45 ^{ab}
G-3502-2	59.52 ^{fg}	53.55 ^{h}	56.53 ^{d}
G-3502-3	74.22 ^{abcd}	74.10 ^{abcd}	74.16 ^{ab}
G-3502-4	56.54 ^{fgh}	61.78 ^{fg}	59.16 ^{d}
G-3502-5	55.63 ^{gh}	61.25 ^{fg}	58.44 ^{d}
G-3503-1	56.21 ^{gh}	62.32 ^{fg}	59.26 ^{d}
G-3604-3	70.89 ^{bcd}	70.92 ^{cd}	70.90 ^{b}
G-3612-4	68.54 ^{de}	77.62 ^{ab}	73.08 ^{ab}
Average	**65.66**	**68.06**	**66.84**
LSD	Genotype	Year	Genotype x year
LSD $_{0.05}$	4.569	1.344	6.542
LSD $_{0.01}$	6.612	1.782	9.396

On average for two growing seasons, the number of seeds spike^{-1} was the highest 75.86 in G-3501-1 and the lowest 56.53 in the genotype G-3502-2. The large number of seeds can be explained primarily by rare sowing (low density sowing), which provided optimal conditions for the development of a greater number of spikes and flowers, which resulted in higher average values for the number of seeds per spike. In this study were established significant differences between genotypes for the number of seeds spike^{-1} in both vegetation seasons. Also, number of seeds spike^{-1} in each of genotype was different between vegetation seasons, which indicates the effect of genotype, environment and their interaction in each vegetation season of the experiment (table 2 and 3). Based on the established variation in the number of seeds per spike in varieties and in different growing seasons, it can be concluded that the genotype and environmental conditions have a significant influence on the change in the number of seeds per spike, which is in agreement with the results of other researches (Zečević

et al., 2010; Knezevic et al., 2006; 2012; Branković et al., 2016; Bhutto et al., 2016; Kondić et al., 2017).
In another study of Serbian wheat, the number of seeds spike^{-1} in wheat varied in range from56.00 to 75.10 seeds (Zečević et al., 2010), from 54.56 to 77.83 seeds (Knežević et al., 2012), from 33.52 to 63.82 seeds (Jocković et al., 2014), from 27.9 to 52.73 seeds (Mladenov, 2017), from 37.17 to 66.83 seeds (Šumaruna et al., 2022), from 59.07 to 74.68 seeds (Jocković et al., 2022), from 34.170 to 48.1 seeds (Matković Stojšin et al., 2022). The study of number of seeds spike^{-1} in some Indian genotypes reported variation in range from 42.80 to 56.60 (Arya et al., 2018), in Chinese genotypes from 32.13 to 47.15 (Feng et al., 2018), while in world variety from the German Federal *ex situ* Genebank maintained at the Leibniz Institute of Plant Genetics and Crop Plant Research Gatersleben Genetic number of seeds spike^{-1} varied between 32.97 and 71.31 and in the wheat varieties from Europen and Asia and North America countries the number of seeds spike^{-1} varied between 40.23 and 83.71 (Philipp et al., 2018). These differences of number of seeds spike-1 expressed due to origin of different geographical region and different genetic structure created in different breeding program which enables significant phenotypic variations in different environments in the conducted experimental studies.
The number of seeds spike^{-1} is genetically determined traits in which inheritance included system of minor genes which effect modified in interaction with environmental factors, (Knežević et al., 2006; Würschum et al., 2018; Mizuno, et al., 2021; Wittern et al., 2022). The minor genes and genes associated with seeds spike^{-1} allow expression the significant phenotypic variations under the influence of environmental factors, depends of floret fertility, phenological phase of development (Glenn, et al., 2021). The regime of mineral nutrition, soil moisture and air temperature have influence to developing of spike organs (spikelets, florets fertility, seeds floret^{-1}), photosynthesis efficiency, translocation of assimilate to spike and survival of developing seeds (Kondić et al., 2017; Guo et al., 2018; Wittern et al., 2022). Therefore wheat varieties express different level of susceptibility or tolerance to favourable or stress environmental conditions in in flowering and grain-setting.
The analysis of components of variance established that the differences between the genotypes for the number of seeds spike^{-1} were significant and highly significant. The significant differences among wheat genotypes according to the average values of the number of seeds spike^{-1}, indicate genetic divergence of genotypes. The analysis of components of variance indicates that genetic factors had the highest share (68.95%) in the expression of the number of seeds spike^{-1}, and than interaction genotype/environment (14.59%), while the least share had environment (0,89%) table 3.

Table 3. Analysis of variance for the number of seeds per primary spike in wheat genotypes

Source of variance	Degree of freedom (DF)	Sum of squares (SS)	Mean of Sum of squares (MS)	F -test	Probability	Component of variance	
						σ^2	(%)
Repetitions (R)	2	35.623	17.811	1.3892^{ns}	0.2617	-	-
Genotypes (G)	9	3506.124	389.569	30.3832**	0.0000	56.783	68.95
Year (E)	1	26.934	26.934	2.1006^{ns}	0.1554	0.731	0.89
Genotype x Year (G xE)	9	439.823	48.869	3.8114**	0.0017	12.016	14.59
Error	38	487.230	12.822	-	-	12.822	15.57
Total	59	4495.734	-	-	-	82.352	100.00

In study some Serbian and foreign varieties Zečević et al. (2010) by analysis of phenotypic variance established highly significant F values for varieties, years and their interaction, while

the highest share in the total phenotypic variance belongs to the vegetation season (48.71%), less interaction genotypes/environment (28.88%), and the smallest share had the genetic factors (15.48%).
The number of seeds spike^{-1}, is the result of the degree of flower fertility and is directly dependent on the number of spikelets spike^{-1}, the number of flowers spike^{-1}, and the success of pollination/fertility and seed setting, which is positively related to the number of seed m^{-2}, mass of spike, mass of seeds spike^{-1}, and seed weight m^{-2}, which has a significant contribution to increasing yield (Peltonen-Sainio et al., 2007; Foulkes et al., 2011; Würschum et al., 2018). Variation in the number of seeds spike^{-1}, depends on the genotype, environmental conditions and their interaction (Knežević et al., 2006). Sowing time, regime of nutrition with nitrogen fertilizers, temperature, availability of water in the soil and mineral influence the formation of the number of spikes in a spike, the fertility of flowers and the formation and development of seeds (Hawkesford, 2014; Duan et al., 2018).

Conclusions

In this study were determined significant differences among wheat genotypes according to the number of seeds spike^{-1} in both vegetation seasons. On the base of the results were established differences between two vegetation seasons for the value of number of seeds spike^{-1}, in the same genotype. The variation of the number of seeds spike^{-1} in the same genotype in two vegetation season indicates the genotype's response to different environmental conditions. In the first vegetation season in genotypes G-3501-1 was the highest number of seeds spike^{-1} 75.10 and in the G-3502-5 was the lowest number of seeds - 55.63, while in the second vegetation season in G-3612-4 was the highest number of seeds spike^{-1} (77.62) and in the G-3502-2 was the lowest number 53.55 of seeds spike^{-1}. The differences between genotypes were significant and highly significant for the number of seeds per primary spike. Genetic factors, environmental factors and genotype/environment interaction influenced the expression of the number seeds spike^{-1}. The share of genetic components of variance had the highest (68.95%), than the interaction of genotype/environment (14.59%) and the lowest share had environment (0.89%) in the determination of expression of seeds spike^{-1} in studied ten wheat genotypes.

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FORECASTING THE IMPACT OF CLIMATE CHANGE ON WATER AVAILABILITY FOR WINTER WHEAT CROP IN THE TOPLICA DISTRICT OF SERBIA

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Abstract

This study examines the impact of climate change on water availability for winter wheat crops in the Toplica region of Serbia, projecting its consequences up to the year 2100. Simulations of precipitation (P), air temperature (T), potential winter wheat evapotranspiration (ETc) and water deficit/surplus were analyzed. The simulations for P and T utilized results from a multi-model ensemble of seven regional climate models from the EURO-CORDEX project. Climate projections were based on the RCP4.5 and RCP8.5 scenarios. Data from three distinct periods were analyzed: the reference period (1971-2000), the near future (2021-2050), and the distant future (2071-2100). The results indicate an expected increase in P, T and ETc during the winter wheat growing season (October–July). At the same level (October-July), the availability of water for the winter wheat crop is projected to remain similar to that of the reference period. Predicted changes range from a surplus of 10 mm (RCP4.5 - near future) to a maximum deficit of 16 mm (RCP8.5 - distant future) compared to the reference period. During the winter months (October-February) a slight increase in the water surplus is expected (maximum 15 mm/month; RCP8.5 - distant future), while during the spring and summer months (March-June) a slight increase in the water deficit is anticipated (maximum 11 mm/month; RCP8.5 - distant future). The conclusion is that climate changes should not have a negative effect on winter wheat production until the end of this century in terms of water supply. An exception is the far future under the RCP8.5 scenario, when an increase in the water deficit is expected in the spring months, which could cause a decrease in the final yield.

Keywords: *climate change, water deficit, winter wheat, Toplica district, Serbia.*

Introduction

The main risks in managing water resources in Europe due to climate change are in the agricultural sector (Iglesias and Garrote, 2015). Southern Europe is one of the most vulnerable regions, due to projected increases in air temperature of up to 5°C and the occurrence of temperature extremes. For the territory of Serbia, an increase in air temperature and a decrease in precipitation during the summer months are projected, which will lead to more frequent droughts (Lalić et al., 2011; Đurđević et al., 2018; Vuković et al., 2018; Tovjanin et al., 2019). The most significant decrease in effective precipitation of 25% can be expected in the southern and southeastern parts of the country, which, accompanied by increased evapotranspiration, will result in reduced water availability for crops during the growing season (Lalić et al., 2013). Estimates suggest that in the territory of northern Serbia, climate change, due to more frequent and severe summer droughts, will have a negative effect on soil water regime (Gregorić et al., 2020), and thus on the final yield of spring crops (Lalić et al., 2011), but will have positive effects on the production of winter crops (Lalić et al., 2011). Therefore, one of the adaptation measures to climate change in southern Europe is precisely the change in crop planting structure in favor of winter crops. The aim of this study is to illustrate the water availability for winter crops in southern Serbia (Toplica district) by the end of the 21st century. Will winter wheat, as the most common plant species in the Toplica region, experience more or less water scarcity during its growing season compared to the period 1971-2000, and in what period of the year? The results of this research will contribute to the development of strategies to combat the negative effects caused by climate change.

Material and method

Simulations of precipitation (P) and air temperature (T) were conducted for the southern region of Serbia, Toplica district, followed by the calculation of potential wheat evapotranspiration (ETc) and water deficit/surplus in the near (2021-2050) and far (2071-2100) futures. The obtained simulations were compared with simulated values in the reference period (1971-2000). The significance of changes was examined using the Mann-Whitney test (MW) ($p=0.05$) (Mann and Whitney, 1947). Water deficit or surplus during the winter wheat growing season (October to June) was calculated as the difference between ETc and effective precipitation (Peff). Effective precipitation was calculated using the expression $0.8 \cdot P$ (Saadi et al., 2015; Stričević, 2007). ETc was calculated as the product of potential evapotranspiration (ETo) and crop coefficient (kc). The crop coefficient was adjusted for the climatic conditions of the Toplica district, following the methodology of FAO 56 (Allen et al., 1998). The modified Hargreaves method was used to calculate ETo (Trajkovic, 2007). The calculation of ETo was based on simulated air temperature (T). Simulations of T as well as precipitation (P) were the result of a multi-model ensemble of 7 regional climate models with a horizontal resolution of 12.5 km from the EURO-CORDEX project (http://www.euro-cordex.net/). Simulations were based on observed daily data on precipitation and maximum and minimum air temperature (Tmax, Tmin) for the period 1971-2000 from meteorological stations in Prokuplje and Kuršumlija. Observed data were obtained from the Republic Hydrometeorological Service of Serbia.

Results and discussion

Simulations of mean daily air temperature (Tmean) during the winter wheat growing season show increased values in the near and distant futures (Table 1). The increase in T ranges from 1.0°C (near future, RCP4.5) to 3.8°C (distant future, RCP8.5).

Table 1. Simulated values and changes in mean temperature (Tmean), effective rainfall (P_{eff}), and potential winter wheat evapotranspiration (ETc), compared to the reference period (1971-2000). Values refer to the period October-July

		(Oct-Jul) 1971-2000	RCP4.5		RCP8.5	
			2021-2050	2071-2100	2021-2050	2071-2100
Prokuplje	Tmean (°C)	9.2	10.3	11.1	10.5	13.0
	Δ Tmean (°C)		1.1	1.9	1.3	3.8
	P_{eff} (mm)	359	384	393	384	402
	Δ P_{eff} (mm)		25	34	25	43
	ETc (mm)	409	423	434	427	459
	Δ ETc (mm)		14	25	18	50
Kuršumlija	Tmean (°C)	8.5	9.5	10.3	9.7	12.2
	Δ Tmean (°C)		1.0	1.8	1.2	3.7
	P_{eff} (mm)	427	448	460	447	463
	Δ P_{eff} (mm)		21	33	20	36
	ETc (mm)	394	408	418	412	446
	Δ ETc (mm)		14	25	19	52

Regarding effective precipitation (P_{eff}) during the wheat growing season, simulation results indicate an expected increase in precipitation in the Prokuplje area, ranging from 25mm (near and far futures, RCP4.5) to 43mm (distant future, RCP8.5), and in the Kuršumlija area, an increase ranging from 20mm (near future, RCP8.5) to 36mm (distant future, RCP 8.5). It should be noted that the increase in precipitation is "attributed" to the increase in P_{eff} during the winter and spring months (October-May), while less precipitation is expected during the summer months (June and July) compared to the reference period (Table 2) (Idrizović, 2023; Koca et al., 2023)

Table 2. Projected changes in average monthly sum of effective precipitation (ΔPeff) and potential winter wheat evapotranspiration (ΔETc) in the near and distant future compared to the reference period (1971-2000). A statistically significant change is shown in bold (Mann-Whitney p=0.05).

	Prokuplje (mm)								Kursumlija (mm)							
	RCP4.5				RCP8.5				RCP4.5				RCP8.5			
	2021-2050		2071-2100		2021-2050		2071-2100		2021-2050		2071-2100		2021-2050		2071-2100	
	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc	Δ Peff	Δ ETc
Oct	**4.7**	0.2	**6.4**	**0.6**	3.7	**0.5**	4.1	**1.3**	6.4	0.3	**7.5**	**0.6**	3.5	**0.6**	3.5	**1.6**
Nov	**10.3**	**0.2**	**10.0**	**0.6**	8.3	**0.4**	**16.6**	**1.1**	**9.9**	**0.3**	**9.3**	**0.7**	5.9	**0.4**	**13.7**	**1.2**
Dec	1.5	**0.3**	-0.3	**0.5**	2.1	**0.3**	**6.3**	**1.2**	1.8	**0.3**	-0.1	**0.5**	2.2	**0.3**	**5.5**	**1.3**
Jan	3.4	**0.4**	**6.8**	**0.7**	**5.3**	**0.3**	**5.8**	**1.4**	2.4	**0.6**	**4.7**	**0.8**	3.0	**0.4**	**5.0**	**1.6**
Feb	2.7	**0.3**	**4.2**	**0.9**	2.5	**0.4**	3.6	**1.7**	3.2	**0.3**	**4.8**	**0.9**	2.7	**0.4**	3.3	**1.7**
Mar	**5.8**	**2.0**	**5.8**	**3.8**	2.8	**2.9**	**6.2**	**7.5**	**7.3**	**1.9**	**6.7**	**3.8**	4.5	**2.9**	**7.2**	**7.7**
Apr	2.1	2.0	**3.1**	**4.9**	2.7	**4.1**	3.8	**11.0**	3.1	1.9	5.0	**5.0**	1.7	**4.3**	3.0	**11.5**
May	2.2	**3.9**	1.9	**7.4**	0.3	**4.8**	1.6	**12.9**	1.0	**3.7**	4.0	**7.2**	1.3	**4.8**	1.7	**13.3**
Jun	-3.6	**3.1**	-4.3	**3.5**	-4.6	**3.2**	-1.1	**7.7**	-3.1	**3.0**	-3.2	**3.4**	-4.5	**3.2**	-1.1	**7.8**
Jul	**-4.3**	**1.6**	-0.2	**2.0**	2.2	**1.5**	-3.4	**4.6**	**-10.5**	**1.5**	-5.6	**1.9**	0.0	**1.4**	-5.5	**4.5**

The simulated increase in T is reflected in ETc, resulting in an expected increase in winter wheat water demand during the growing season in the future, under both RCP scenarios (Table 1). It is observed that the change in ETc is greater in the distant future compared to the near future under both scenarios. Additionally, the change is greater under the RCP8.5 scenario compared to RCP4.5. The maximum increase in ETc is 50mm (Prokuplje, RCP8.5), and 52mm (Kuršumlija, RCP8.5). The distribution of ETc on a monthly basis shows an increase throughout the growing season in both scenarios and both near and distant futures. The largest increase in ETc is projected in May, reaching 13 mm/month by the end of the century under the RCP8.5 scenario at both locations (Table 2). An increase in potential evapotranspiration in the future has also been observed for maize crops in the same region (Koca et al., 2023).

Mainly due to different rainfall regimes, a water deficit was observed in the reference period in the Prokuplje area (50mm), while Kuršumlija had a surplus (33mm) of water available to winter wheat crops during their growing season. In the future, the projected deficit (Prokuplje) is expected to slightly decrease, while the surplus (Kuršumlija) is expected to slightly increase (Table 3). This pattern is excepted for the distant future period under the RCP8.5 scenario, where Prokuplje is expected to experience a slight increase in water deficit (by 7 mm), and Kuršumlija a decrease in surplus by 16mm.

Table 3. Projected values of winter wheat water surplus/deficit and change (Δ) compared to the reference period (1971-2000). The values refer to the period October-July.

Water deficit		1971-2000	RCP4.5		RCP8.5	
			2021-2050	2071-2100	2021-2050	2071-2100
Prokuplje	(mm)	50	39	41	43	57
	Δ (mm)		-11	-9	-7	7
Kuršumlija	(mm)	-33	-41	-41	-34	-17
	Δ (mm)		-8	-8	-1	16

Intra-seasonal analysis indicates that during the winter wheat growing season in the observed areas, the occurrence of water deficits in the spring and summer months (March-June) is expected, as it was observed in the reference period. Surpluses of water are characteristic of the autumn and winter months (October-February) (Figure 1 (a) and (b)). The maximum values of monthly water deficits are projected in May and range from 84 mm (near future, RCP4.5) to 93mm (distant future, RCP8.5) (Figure 1 (a) and (b)).

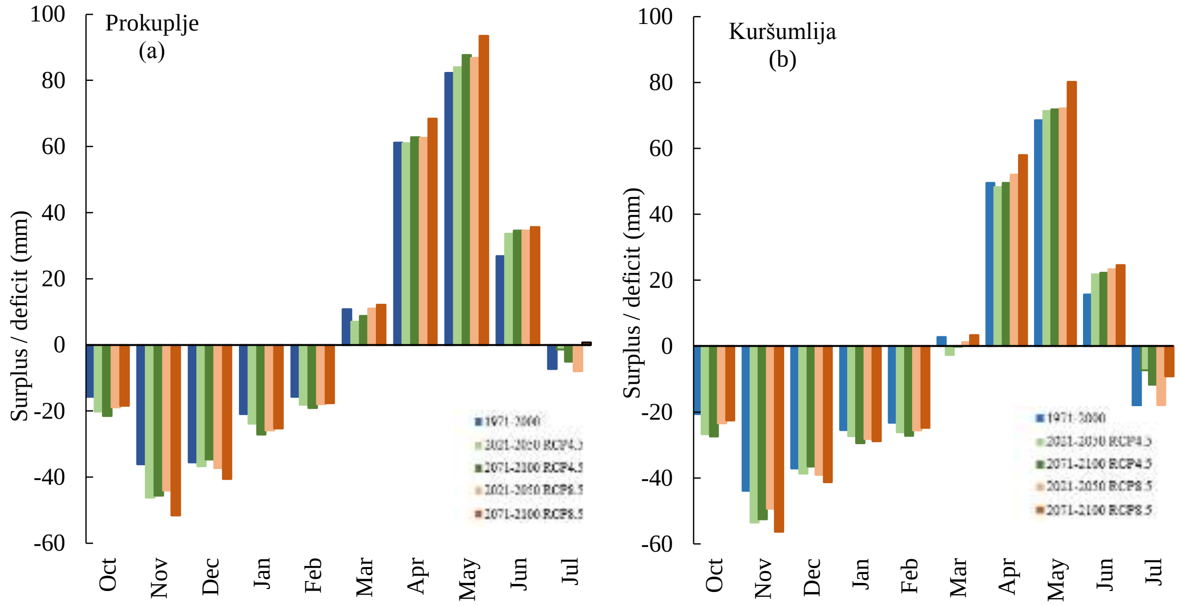

Figure 1. Projected water surplus/deficit for winter wheat in the reference and future periods, for the Prokuplje (a) and Kuršumlija (b) areas. Positive values represent water deficit, while negative values represent water surplus.

In comparison to the reference period, it can be concluded that there won't be significant changes in the near future (2021-2050) under both scenarios. In the winter months, a slight increase in water surplus is expected (maximum 10mm in November - RCP4.5), while in the spring months of April-May, the situation is expected to remain nearly unchanged (changes ranging from -1mm to 4mm) (Table 4). In the distant future period (2071-2100) under the RCP4.5 scenario, significant changes are also not expected. A slight increase in water surplus is anticipated during the winter months (maximum 9mm in November), and almost no change is expected during the spring months (April-May) (changes ranging from 0 to 5mm).

Table 4. Projected change in available water (Δ) of winter wheat compared to the reference period by month. Positive values represent a decrease and negative values an increase in the amount of water. A statistically significant change is shown in bold (Mann-Whitney p=0.05).

Month	Prokuplje (mm)				Kursumlija (mm)			
	RCP4.5		RCP8.5		RCP4.5		RCP8.5	
	2021-2050	2071-2100	2021-2050	2071-2100	2021-2050	2071-2100	2021-2050	2071-2100
Oct	-4.5	-5.8	-3.2	-2.7	-6.1	**-6.8**	-2.9	-1.9
Nov	**-10.1**	**-9.4**	-7.9	**-15.4**	**-9.6**	**-8.7**	-5.5	**-12.4**
Dec	-1.2	0.8	-1.8	**-5.1**	-1.5	0.6	-1.9	-4.2
Jan	-2.9	**-6.2**	**-5**	**-4.4**	-1.8	**-3.9**	-2.7	-3.4
Feb	-2.4	**-3.3**	-2.2	-1.9	-2.9	**-4**	-2.3	-1.6
Mar	-3.8	-2	0.2	1.4	-5.4	**-3**	-1.6	0.5
Apr	-0.1	1.7	1.5	**7.3**	-1.3	0	2.5	**8.5**
May	1.8	5.5	4.5	**11.2**	2.7	3.2	3.6	**11.6**
Jun	6.8	**7.8**	**7.8**	**8.8**	6.2	6.6	7.7	9
Jul	5.9	2.2	-0.7	8	**11.9**	7.5	1.4	**10**

The least favorable prognosis in terms of water availability is provided by the RCP8.5 scenario in the distant future. In the winter months (RCP8.5, distant future), an increase in existing water surplus is predicted (maximum 15mm more water in November), while during April and May, a decrease in water amount is expected (by 8mm in April and by 12mm in May). The period April - May is particularly important from the aspect of winter wheat water supply, because then wheat is in the phase of intensive growth and preparation for the heading phase. Water and nutrient demands are highest during this period, and water shortage during this period could significantly reduce final yields (Jovanović and Stikić, 2012).

Based on the presented results, it can be concluded that in the near future, climate change will not have a negative effect on winter wheat production in terms of water availability. Only in the distant future, under the RCP8.5 scenario, a larger deficit of accessible water is expected in the spring and summer months (compared to the reference period). Similar conclusions were reached by Matović et al. (2021) simulating the parameters of water regime of Zemun chernozem under winter wheat cultivation, based on climate model projection according to the RCP8.5 scenario. They concluded that in the near future (2021/2022-2050/2051), more favorable water regime conditions for wheat production are expected (reduced need for irrigation and lower yield reductions than in the reference period (1970/1971-1999/2000), while in the last three decades of the 21st century, worse water regime conditions, higher irrigation needs, and higher yield reductions are expected. Stričević et al. (2021), based on ensemble projections of climate and AquaCrop models, concluded that in Serbia, high winter wheat yields will be achievable in the future even without irrigation. Similar research conducted in different regions of the world (Central Europe, Eastern England, Azerbaijan, Iran, Iraq) indicates that under climate change conditions in the future, water deficits are expected, and the amount of water needed to replenish wheat and barley will increase, requiring irrigation (Thaler et al., 2012; El Chami and Daccache, 2015; Ashofteh et al., 2015; Mirgol et al., 2020; Salman et al., 2020).

Conclusion

If climate changes in the south of Serbia (Toplica district) occur according to the RCP4.5 scenario, we should not expect a deterioration of the water conditions for winter wheat production until the end of the 21st century. In the winter months, in this case, a small increase in water surplus is expected, while in the spring and summer months, the situation is expected to remain almost unchanged (negligibly small increase in the water deficit). If the CO_2 emissions are higher, leading to climate changes following the RCP8.5 scenario, then in the near future, no changes in winter wheat crop water supply should be expected, but in the distant future, an increase in water deficit is anticipated during the spring months, which could affect the reduction of the final yield.

Acknowledgement

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INFLUENCE OF GENOTYPE AND IRRIGATION ON YIELD COMPONENTS OF SWEET CORN (ZEA MAYS L. VAR. SACCHARATA STURT.)

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Abstract

Sweet corn (Zea mays L. var. saccharata Sturt.) is a thermophilic plant species that is sensitive to lack of moisture during the growing season. The experiment was set during 2022 and 2023 in the Velika Plana area on anthropogenic land. Sowing was done in mid-May with an inter-row spacing of 70x22 cm. Three sweet corn hybrids (Enterprise F1, Union F1 and Sweet nougat F1) were included in the experiment. Irrigation was carried out by a drip system with tapes with a capacity of 10 l•m-1•h and three irrigation norms were applied: I – full watering norm (100%), II – 50% of the full norm and III – control treatment under the conditions of a natural wetting regime. This work aimed to examine the effect of watering rate and different hybrids on cob length, cob diameter, total cob weight and grain weight. The length and diameter of the piston were parameters that in the Enterprise F1 hybrid had significantly higher values compared to the other two hybrids. The mass of the piston had values from 174.32 to 322.35 g. The highest values were achieved with the Enterprise F1 hybrid and the differences were statistically significant compared to other variants. Variants with watering showed the same trends, as well as the grain mass parameter per cob, which ranged from 97.60 to 214.50 g. For production practice, the Enterprise F1 hybrid is recommended with a minimum watering rate of 50% compared to the full watering rate.

Key words: *sweet corn, genotype, irrigation rate, production characteristics.*

Introduction

Sweet corn (*Zea mays* L. var. *saccharata* Sturt.) is an extremely important vegetable crop in the world. From a crop grown in temperate regions in the USA and Canada, sweet corn has become a crop produced worldwide (Revilla et al., 2021). In recent years, there has been a great interest in the production of sweet corn in the Republic of Serbia and neighbouring countries. More and more agricultural farms include sweet corn in their production to increase the profitability of their farms (Nastić et al., 2022). The reason for the increase in the area under sweet corn is its agrotechnics, which do not differ to a great extent from the agrotechnics of mercantile corn. The differences are in the specific mechanization for harvesting, while the income per unit of area with the production of sweet corn is significantly increased. Due to its diverse use in fresh or processed forms, more and more significant areas belong to sweet corn. By processing it into syrup, it can be used as a sweetener in soft drinks or as an ingredient in the preparation of salad, as a baked or boiled cob (Ozlem et al., 2014; Uwah & Ogar, 2014). Revilla et al. (2021) state that in the production of sweet corn, it is necessary to apply irrigation and intensive pest control, and its advantage is that it can be produced in different cultivation systems (as a main, secondary or intercrop). As a thermophilic plant species, it is sensitive to low temperatures and lack of moisture during the growing season, but it does not tolerate high soil moisture either (Bekavac, 2012). The same author states that the availability of sufficient amounts of water during the development stages significantly affects the yield and quality of sweet corn. Lack of water negatively affects plant growth, ear diameter and mass and total carotenoid content in grain and leaf surface, Nemeskeri and Helyes (2019) point out in their research. A large number of different sweet corn hybrids present on the market require testing under different production conditions (Soare et al., 2019). The main problem in the production of certain hybrids is the adaptability of crops to the production conditions of the environment, especially to air temperatures and soil moisture. According to that, it is necessary to include hybrids with good production characteristics in research to determine their adaptability to certain climatic conditions (Matei, 2016). This work aimed to examine the effect of watering rate and different hybrids on cob length, cob diameter, total cob weight and grain weight.

Material and Methods

The research was conducted set 2022 and 2023 in the area of the village of Bresje near Velika Plana on anthropogenic land. The experiment was set up according to a randomized block system, in four repetitions. Sowing was done in mid-May with an inter-row spacing of 70x22 cm. Three hybrids of sweet corn (*Enterprise* F_1, *Union* F_1 and *Sweet nougat* F_1) were included in the experiment. Irrigation was carried out by a drip system with tapes with a capacity of 10 l•m-1•h and three irrigation norms were applied: I – full watering norm (100%), II – 50% of the full norm and III – control treatment under the conditions of a natural wetting regime. To maintain the moisture content in the soil at 60% PVK, that is, to determine the full and reduced watering rate, the moisture content in the soil was monitored by placing tensiometers in all variants. The watering rate is calculated using the formula:

$$\mathbf{Nz = 10 \bullet D \bullet (PVK\%\ vol - \theta z) = mm \bullet m^{-2}}$$

Nz - represents the watering rate (mm•m-2), D – the depth of the soil layer (m), PVK% vol – the hydrolimit of the field water capacity in percent by volume, θz - is the soil moisture read from the tensiometer curve at the water holding force read on the tensiometer. The watering duration was calculated from the ratio of the watering rate and the flow rate of the drip irrigation system (10 l•h-1 or 10 mm•h-1). Dividing this watering duration of irrigation in half, we get the duration of irrigation for the reduced amount of watering (50% of the full norm). During the growing season, standard agrotechnical measures were applied in the production of sweet corn. The obtained research results were statistically processed by analysis of variance and tested with the LSD test (Least Significant Difference Test) in the IBM SPSS Statistics program, version 26.0. and are presented tabularly and graphically.

Results and Discussion

Based on the data shown in *Table 1*, we can see that the piston length parameter differed statistically very significantly between the tested hybrids. Variants with irrigation in the *Enterprise* F_1 and *Sweet nougat* F_1 hybrids compared to the natural wetting regime showed statistically significant differences. There was no statistically significant difference between the different irrigation rates. The average value of the length of the piston during the examined period ranged from 19.13 to 22.53 cm. The highest average value for the length of the piston was recorded in the hybrid *Enterprise* F_1 and was 21.94 cm, while the smallest average length of the piston was measured in the hybrid *Sweet nougat* F_1 19.88 cm.

Table 1. Average values of cob length (cm), cob diameter (cm), total cob weight (g) and grain weight (g) of tested sweet corn hybrids

Hybrid (A)	Irrigation (B)	Cob length (cm)	Cob diameter (cm)	Total mass cob (g)	Grain weight (g)
Enterprise F_1	Control	20.91	4.45	255.63	160.70
	Irrigation 50%	22.39	4.84	321.63	212.63
	Irrigation 100%	22.53	4.90	322.35	214.50
	Average	**21.94**	**4.73**	**299.87**	**195.94**
Union F_1	Control	20.50	4.46	231.45	136.57
	Irrigation 50%	20.58	4.47	255.25	161.73
	Irrigation 100%	20.66	4.88	276.28	172.20
	Average	**20.58**	**4.60**	**254.33**	**156.83**
Sweet nugat F_1	Control	19.13	4.16	174.32	97.60
	Irrigation 50%	19.98	4.34	210.30	126.35
	Irrigation 100%	20.51	4.25	206.82	121.12
	Average	**19.88**	**4.25**	**197.15**	**115.02**
	Average/Average	**20.80**	**4.53**	**250.45**	**155.93**

Factors	**A****	**B****	**AB^{nz}**	**A****	**B****	**AB****	**A****	**B****	**AB***	**A****	**B****	**AB^{nz}**
LSD 0.05	0.50	0.50	0.87	0.07	0.07	1.13	11.82	11.82	20.50	9.63	9.63	16.67
LSD 0.01	0.66	0.66	1.14	0.10	0.10	0.17	15.60	15.60	26.93	12.67	12.67	21.94

$p<0.01$ very significant (**); $p<0.05$ significant (*); $p>0.05$ no significant

The tested factors, hybrid and irrigation, as well as their mutual interaction, had a statistically significant effect on the diameter of the piston. The difference between irrigation and natural wetting regimes was statistically very significant. The average value of the piston diameter ranged from 4.25 to 4.73 cm. The highest value was recorded with the *Enterprise F_1* hybrid and was 4.90 cm. There was no statistically significant difference between the different irrigation norms (*Figure 1*). Hybrid and irrigation had a statistically very significant effect on total cob mass. The average values of the total mass of the piston ranged from 197.15 to 299.87 g. The highest value was recorded in the *Enterprise F_1* hybrid and is 322.35 g in the full watering norm. The interaction between hybrid and irrigation had a statistically significant effect, while there were no significant differences between different irrigation norms.

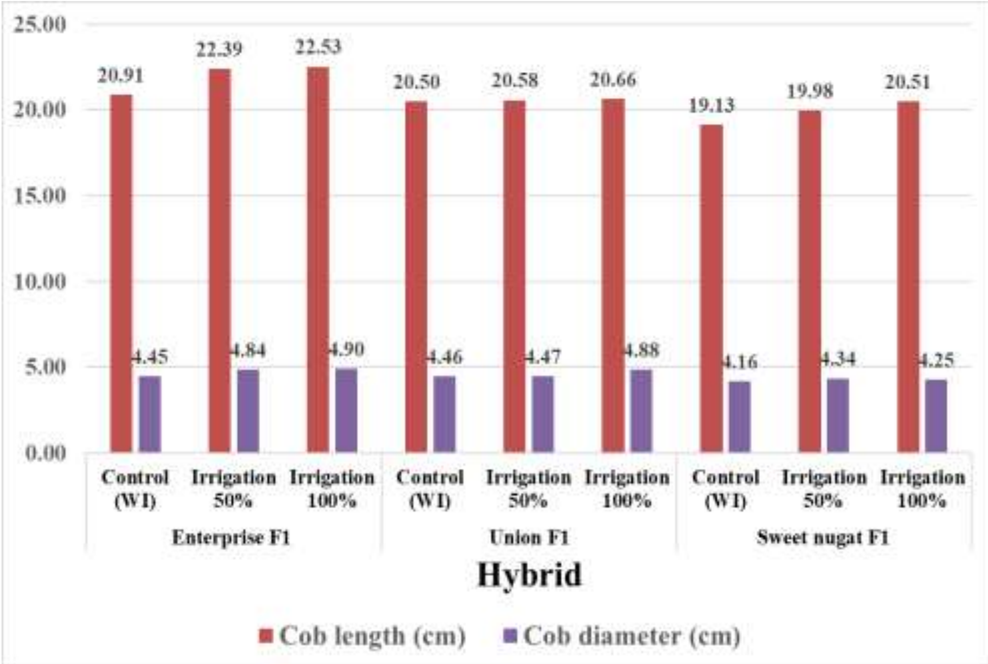

Figure 1. Average values of cob length (cm) and cob diameter (cm) of tested sweet corn hybrids

For the grain weight parameter, the hybrid and irrigation had a statistically significant influence. The grain mass values ranged from 97.60 to 214.50 g. The highest value for the examined trait, total grain mass, was measured in the *Enterprise F_1* hybrid and was 214.50 g in the full watering norm and had no statistically significant difference compared to 50% of the full watering norm, where 212.63 g was measured (*Figure 2*). The highest average value was recorded for the *Enterprise F_1* hybrid and was 195.94 g, while the lowest average value was measured for the *Sweet nougat F_1* hybrid and was 115.02 g. The interaction of hybrids and irrigation was not statistically significant, and there were no significant differences between different irrigation norms. The results of the two-year research are in agreement with the results of the research by Muslimah et al. (2023) who state that drip irrigation is a very effective and economical agrotechnical measure in the production process of sweet corn. They determined that irrigation had an effect on increasing the length, diameter and mass of the cob, and the genotype also had a very significant effect on the examined traits. Siafrudina et al. (2012) state that the manifestation of certain traits in sweet corn is largely influenced by genetic factors in interaction with production conditions. Zainudin (2005) states that different genotypes of sweet corn affect the length and diameter of the cob. Siafrudina et al. (2012) state that the manifestation of certain traits in sweet corn is largely influenced by genetic factors in interaction with production conditions. Zainudin (2005) states that different genotypes of sweet corn affect the length and diameter of the cob.

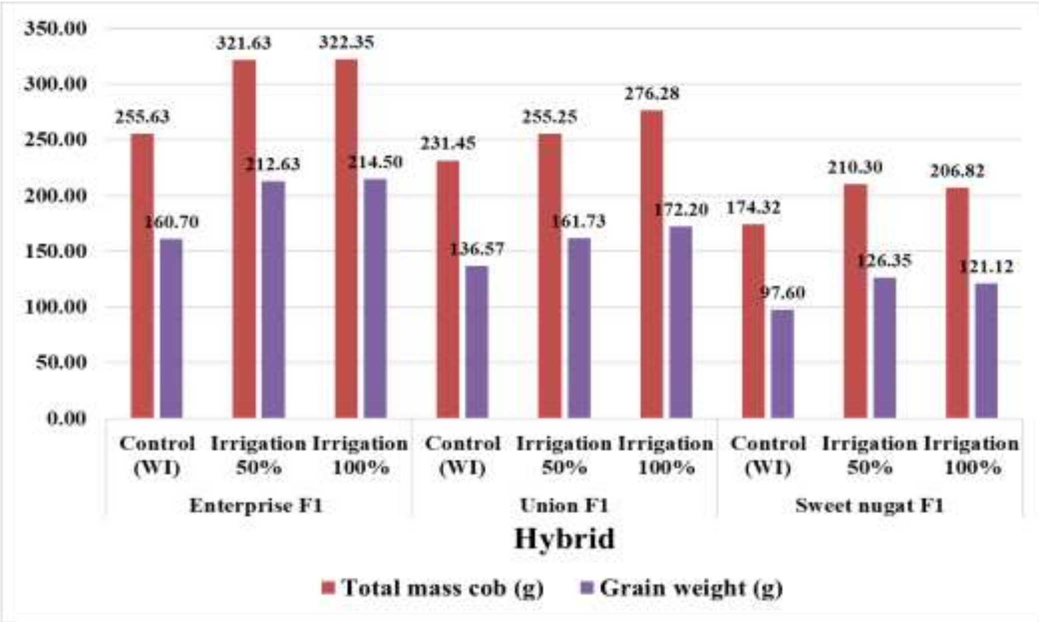

Figure 2. Average values of total cob weight (g) and grain weight (g) of tested sweet corn hybrids

Conclusions

Based on the two-year results of the study on the influence of genotype and irrigation on cob length, cob diameter, total cob weight and sweet corn grain weight, it is concluded that genotype had a statistically significant influence on the tested parameters of sweet corn. In the irrigation conditions, significantly higher parameter values were achieved compared to the control variant. Between the full watering norm and 50% of the full watering norm, no statistical significance was determined for the tested properties. For production practice, the Enterprise F1 hybrid is recommended with a minimum watering rate of 50% compared to the full watering norm.

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MARKER ASSISTED SELECTION FOR β-CAROTENE RICH MAIZE: VALIDATION OF MULTIPLEX-PCR ASSAY FOR *crtRB1* AND *lcyE* FAVOURABLE ALLELES

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Abstract

The improvement of β-carotene content (BC) using gene-specific molecular markers is one of the successful examples of marker assisted selection (MAS). Maize genotypes with favourable alleles of *crtRB1* and *lcyE* genes that enhance BC can be used in the biofortification programmes. The *crtRB1* has been predominantly introgressed to develop BC enriched maize. However, given that *crtRB1* and *lcyE* act additively, pyramiding of these genes is desirable to achieve the highest nutritional benefit. This study is a part of the marker assisted breeding program conducted at the Maize Research Institute Zemun Polje with an aim to convert the standard maize to β-carotene rich genotypes adapted to temperate regions. Validation of the multiplex polymerase chain reaction (PCR) assay was done in the backcross-derived population and both donor and recipient parental lines to simultaneously discover the allelic combinations among the segregants. Multiplex-PCR analysis confirmed the results of uniplex assays for both genes. The donor parent carried the favorable allele of *crtRB1* (543 bp) and *lcyE* gene (650 bp) band, while the recurrent parent exhibited the unfavorable *crtRB1* (296 bp) and *lcyE* allele (300 bp). Heterozygous individuals were clearly distinguishable with the fragments of the both favourable alleles, while unfavorable alleles showed a single band due to non-separation of 296 bp (*crtRB1*) and 300 bp (*lcyE*) fragments. Multiplex-PCR assay saved time, money and labor resources and therefore showed the advantage over uniplex assays. Being time and cost effective, multiplex assay confirmed potential to enhance the efficiency of marker assisted selection.

Keywords: *β-carotene, crtRB1 gene, lcyE gene, maize, marker assisted selection.*

Introduction

The World Health Organization has classified vitamin A deficiency (VAD) as a public health problem, the leading cause of preventable night blindness, anaemia, weakened immune system, increased morbidity and mortality (WHO, 2009). Vitamin A cannot be synthesized inside the human body and therefore it must be provided through diet. It can be found in plants as a provitamin β-carotene, the most abundant carotenoid, which can be converted to vitamin A by an oxygenase present in the intestine. Maize is one of the major sources of provitamin A (ProVA) for humans. Thus, the improvement of provitamin A carotenoids in maize varieties through breeding or biofortification is a promising strategy to alleviate VAD (Natesan *et al*., 2020).

The improvement of β-carotene content using gene-specific molecular markers is one of the successful examples of marker assisted selection (MAS). Two genes, *lycopene epsilon cyclase (lcyE)* on chromosome 8 and *beta-carotene hydroxylase1 (crtRB1)* on chromosome 10, have been shown to regulate the accumulation of proA compounds (Babu *et al*., 2013). Downregulation of *lcyE* reduces the ratio of the α-carotene branch to the β-carotene branch (Harjes *et al*., 2008). *CrtRB1* alleles were found to be associated with reduced transcript expression of the gene which correlates with higher β-carotene concentrations in the kernel composition (Yan *et al*., 2010). Maize genes encoding key enzymes in the carotenoid biosynthetic pathway are presented in Figure 1.

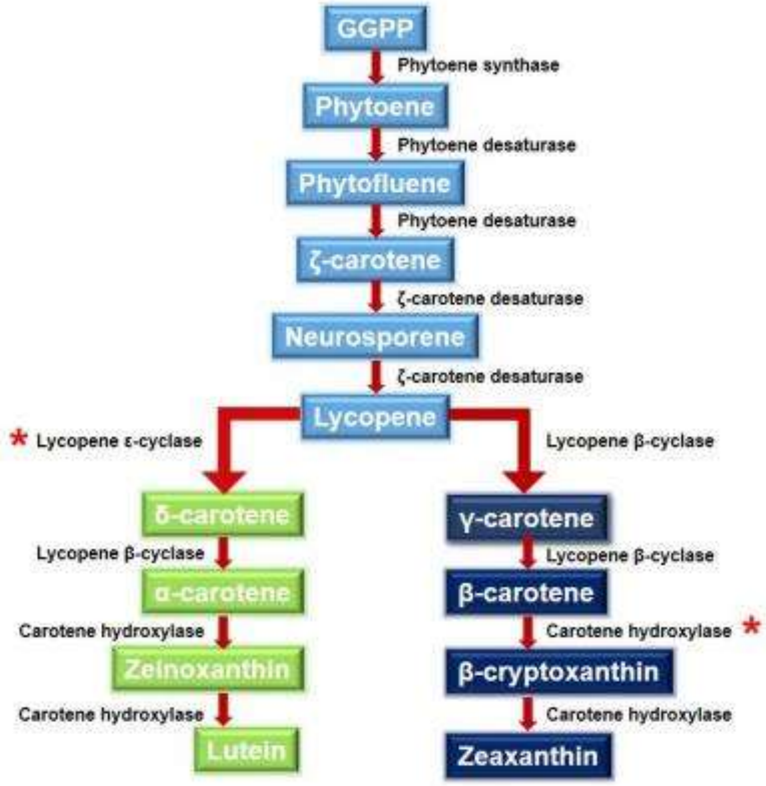

Figure 1. Maize genes encoding key enzymes in the carotenoid biosynthetic pathway

With a much larger effect on proVA concentration, *crtRB1* gene has been predominantly introgressed to develop β-carotene enriched maize (Babu *et al*., 2013). However, given that *crtRB1* and *lcyE* act additively, pyramiding of these genes is desirable to achieve the highest nutritional benefit (Zunjare *et al*., 2018). Regarding the traits controlled by multiple genes, individual genes can be identified and selected at the same time and in the same individuals, and thus marker assisted backcross breeding is particularly suitable for gene pyramiding (Begna, 2020). The process of stacking of two or more genes responsible for traits of interest from single or multiple parents can be accelerated using molecular markers to identify the progeny with homozygous favorable alleles at each generation (Nayak *et al.*, 2017).
This paper is a part of the marker assisted breeding program conducted at the Maize Research Institute Zemun Polje aimed at converting the standard maize to β-carotene rich genotypes adapted to temperate regions. The aim of this research was to validate the multiplex polymerase chain reaction (PCR) assay for simultaneous identification of favorable alleles of *crtRB1* and *lcyE* genes and confirm its effectiveness in the marker assisted breeding program.

Material and methods

The progenies derived by backcrossing in 2021, with both donor and recipient parental maize inbred lines, were used to simultaneously discover the allelic combinations among the segregants. The DNA was extracted from the leaves collected from the four-weeks-old plants applying modified Dorokhov and Klocke protocol (1997). The DNA quantity and quality were analyzed with the bio-spectrometer BioSpetrometer kinetic (Eppendorf, Germany) and diluted to a working concentration of 20 ng/µL for the PCR assay. The set of primers used in this analysis is presented in Table 1. Polymerase chain reaction was carried out in 20 µL reaction volume containing: DreamTaq™ Green PCR Master Mix (2X) (Thermo Scientific™, USA), 0.5 µM primers each and 1 µL DNA template.

Table 1.The set of primers used for multiplex-PCR assay

Gene	Primer	Sequence
crtRB1	*crtRB1*-3′TE-F	5′-ACACCACATGGACAAGTTCG-3′
	crtRB1-3′TE-R1	5′-ACACTCTGGCCCATGAACAC-3′
	crtRB1-3′TE-R2	5′-ACAGCAATACAGGGGACCAG-3′
lcyE	*lcyE*-5′TE-F	5′-AAGCAGGGAAGACATTCCAG-3′
	lcyE-5′TE-R	5′-GAGAGGGAGACGACGAGACAC-3′

Amplifications were performed in thermocycler Biometra TProfessional Standard 96 (Biometra, Germany) with the following program: an initial denaturation at 95 ºC/10 min, followed by 19 cycles each of denaturation at 95 ºC/1 min, annealing at 60 ºC/1 min (-0.5 ºC/cycle) and extension at 72 ºC/1 min; another 19 cycles of 95 ºC/1 min, 55 ºC/ 1min and 72 ºC/1 min, with final extension at 72 °C for 10 min. The amplified fragments were resolved by 8% polyacrylamide gel electrophoresis (Mini Protean Tetra-Cell, BioRad, USA) at 80 V for 1.5 h. After staining with 0.5 µg/µl ethidium bromide, they were visualized under UV transilluminator and documented in gel documentation system BioDocAnalyze (Biometra, Germany). The size of the PCR amplicons was determined relative to the 100 bp molecular weight ladder.

Results and discussion

Maize Research Institute Zemun Polje has a breeding program aimed to create maize enriched with β-carotene, using the integrated conventional and molecular breeding approach. This refers to conversion of commercial inbred lines to their β-carotene enriched counterparts and development of the β-carotene enriched hybrids adapted to temperate climate. The codominant nature of *crtRB1*-3′TE marker has already been confirmed in our previously published papers (Kostadinović *et al*., 2018; Kostadinović *et al*., 2020), enabling its utility in marker assisted introgression of the favorable allele of *crtRB1* into the local maize genotypes. The favorable allele *crtRB1*-3′TE has been proven to increase the β-carotene concentration two to ten times (Babu *et al.*, 2013; Muthusamy *et al.*, 2014). However, *lcyE* and *crtRB1* genes act additively and hence, further enhancement in the proA could be achieved by pyramiding favorable alleles of these genes (Zunjare *et al*., 2018).
Co-dominant and gene-specific molecular markers for both *crtRB1* and *lcyE* genes have been used in marker assisted selection programmes reducing the requirement of evaluation at phenotypic level (Babu et al. 2013). For foreground selection of these two genes, uniplex-PCR assays are being used. Our previous results of the uniplex-PCR analysis (Kostadinović *et al*., 2018; Kostadinović *et al*., 2020) correspond with the findings in several studies (Muthusamy *et al*., 2015; Pitambara and Nagarajan, 2016; Natesan *et al*., 2020). Applying the protocol modified from Zunjare *et al*. (2018), multiplex-PCR assay was used in this research for simultaneous detection of *crtRB1* and *lcyE* favourable alleles. Multiplex-PCR analysis confirmed the results of uniplex assays for both genes, i.e. all the bands detected by uniplex

assays were also present in products of multiplex-PCR analysis. The donor parent (lane 1) carried the favorable allele of *crtRB1* (543 bp) and *lcyE* gene (650 bp) band, while the recurrent parent (lane 2) exhibited the unfavorable *crtRB1* (296 bp) and *lcyE* allele (300 bp). Heterozygous individuals were clearly distinguishable with the fragments of the both favourable alleles, while unfavorable alleles showed a single band due to non-separation of 296 bp (*crtRB1*) and 300 bp (*lcyE*) fragments (lanes 3, 4, 5, 7, 12 and 13). These results are in accordance with Zunjare *et al*. (2018). The gel profile of multiplex-PCR in BC_2F_2 generation is presented in Figure 2.

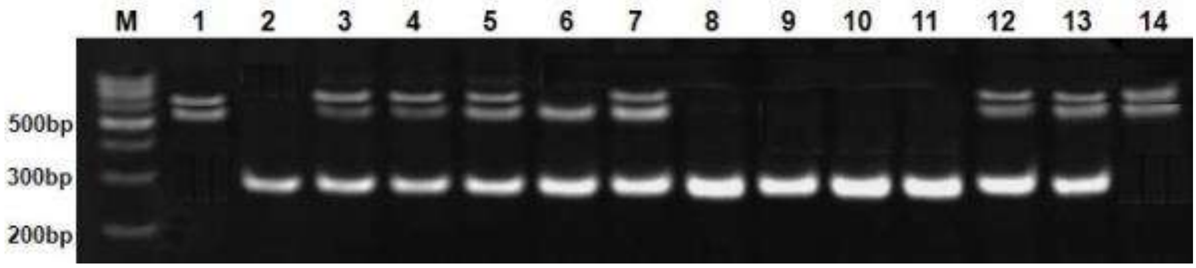

Figure 2. Multiplex PCR assay for *crtRB1* and *lcyE* genes. M:100 bp DNA ladder, 1-donor parent, 2: recipient parent, 3-14: BC_2F_2 individuals.

As the main advantages of multiplex-PCR assay, saving valuable time and money should be emphasized. Zunjare *et al*. (2018) reported a fund savings of 41.09%, as well as a time savings of 50.00%, with one multiplex-PCR for *crtRB1* and *lcyE* genes compared to two uniplex assays. These authors also pointed out the electricity savings, reduced depreciation of thermal cycler and half of the manpower requirements to perform the assay. It could be concluded that multiplex-PCR assay can be effective for marker assisted gene pyramiding of both favorable alleles in a single genetic background.

Conclusions

Multiplex-PCR assay could be effective for pyramiding of *crtRB1* and *lcyE* favorable alleles for maize β-carotene enrichment. Being time and cost effective, multiplex assay confirmed potential to enhance the efficiency of marker assisted selection. Maize genotypes with favorable alleles of *crtRB1* and *lcyE* genes that enhance β-carotene can be used in the biofortification programs alleviating VAD worldwide.

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INFLUENCE OF THE CULTIVAR, FERTILISER, AND IRRIGATION ON ICEBERG LETTUCE MORPHOLOGY - SINGLE FACTOR VS INTERACTION EFFECTS

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Abstract

This study aimed to examine the effect of cultivar, fertiliser and irrigation, and their combined effect, on different lettuce agronomic parameters and the possible occurrence of tipburn. Two iceberg lettuce cultivars ('Umbrinas' and 'Kavir') were cultivated during summer in the open field, in the black marsh soil containing a wide calcium-to-magnesium ratio. Plants were grown at a density 32×32 cm with regular agricultural practices. Three different levels of magnesium sulfate fertiliser (100 ml, 300 ml, 500 ml solution) were added twice during the growing cycle. Plants were irrigated using a drip irrigation system and sprinklers. After harvest morphological parameters were estimated (head weight, height, diameter, number of leaves, fresh leaf weight, stem height, diameter, and fresh weight). Cultivar 'Umbrinas' showed the highest value of all parameters: head weight (557.15 g), head height (13.91 cm), head diameter (16.90 cm), number of leaves (18.73), fresh leaf weight (527.50 g), stem height (65.73 mm), stem diameter (20.32 mm), and stem fresh weight (29.65 g). Application of all levels of magnesium sulfate fertiliser did not affect observed parameters compared to the control. Drip irrigation led to significantly higher head height, diameter and number of leaves, while sprinkler irrigation significantly enhanced the values of all stem parameters. Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler irrigation treatment, whereas drip-irrigated plants showed no signs of tipburn. Interaction of all three factors showed a statistically significant impact on head weight, number of leaves, fresh leaf weight, and stem height.

Keywords: *Lactuca sativa*, *Magnesium sulfate fertiliser*, *Drip irrigation*, *Sprinkler irrigation*, *Morphology traits.*

Introduction

Lettuce (*Lactuca sativa*, L.) is an annual leafy vegetable crop from the Asteraceae family. It has wide morphological viability, classified into several types (butterhead, iceberg, leaf, romaine, Batavia, and stem). It is rich in health-beneficial secondary metabolites, mainly phenolic compounds, carotenoids, and folate, even though it has low nutritional value (Ćavar Zeljković et al., 2023). Iceberg lettuce is usually used as a fresh-cut or minimally processed leafy vegetable, with thick leaves, a specific crisp texture, and neutral to a slightly bitter flavour. The advantage of growing lettuce is quick and all-year-round production in different systems (open field, covered systems or hydroponics) with the possibility of multiple growing cycles. It is a cool season crop with optimal temperatures of 21-23 °C (day) and 15-18 °C

(night). During the summer, daily temperatures usually exceed 30 °C which can raise the risk of bolting (Ilić et al., 2017). Early bolting leads to a shortened vegetative phase, decreased biomass, poor head formation, bitter taste, and consequently plants lose marketable value (Lafta et al., 2021). Magnesium (Mg) as a secondary nutrient is important for a wide range of processes in plants such as photosynthesis, nutrient metabolism, cell membrane stability, enzyme activation, and resilience against various stressors (Ahmed et al., 2023). The BCSR (Basic cation saturation ratio) concept is based on the existence of an optimum saturation of the soil cation exchange capacity, particularly cations Ca, Mg, and K, and that optimum ratio will lead to maximum yields (Bear et al., 1945). On the contrary, research by McLean et al. (1983) suggested that for maximum crop yields emphasis should be undertaken on providing adequate levels of each basic cation rather than trying to modulate the optimal ratio. Antagonism between Ca and Mg can occur both on a soil and plant tissue level, and high concentrations of each of the elements can interrupt the acquisition of the other. Calcium (Ca) is considered a relatively immobile element within plant tissue. The transpiration rate usually regulates the uptake and movement of water and Ca where rapidly transpiring outer leaves gain more water and Ca compared to inner leaves. Environmental factors that promote rapid growth, modify the transpiration rate and change the balance of Ca distribution favour the occurrence of tipburn (Sago, 2016). Tipburn is mainly recognised as a calcium-associated physiological disorder visually seen as the browning of leaf margins which reduces the quality and shelf life of fresh and processed products. Proper irrigation management can have an impact on lettuce yield, head size and uniformity, chemical content, and browning-related enzymes (Luna et al., 2012). Drip irrigation has several advantages compared to sprinkler irrigation in a manner that it reduces water usage and loss caused by evaporation, decreases soil erosion, leakage of fertilisers and pesticides, and availability of water for the weeds.
Therefore, this study aimed to examine the effect of cultivar, fertiliser and irrigation, and their combined effect, on different iceberg lettuce agronomic parameters, and the possible occurrence of tipburn during summer production.

Materials and methods

Two iceberg lettuce cultivars were studied 'Umbrinas' (Rijk Zwaan, the Netherlands) and 'Kavir' (Meridiem Seeds, Spain). Lettuce seeds were sown on July 23 in peat cubes size 4 cm in the substrate Potgrond H (Klasmann-Deilmann, Germany) in a glasshouse condition in the company Grow Rasad (Serbia). Plants were cultivated in the summer open-field experiment from August to October 2018 in the company Iceberg Salat Centar (Surčin, Serbia). Seedlings were transplanted on August 11, manually in the black marsh soil. Before the experiment, randomised soil samples were taken at 0-30 cm depth. A pH was determined potentiometrically (SRPS EN ISO 10390, 2022), Ca and Mg using extraction with molar ammonium acetate solution (ISO/TS 22171, 2023), total nitrogen was measured with dry combustion (SRPS ISO 13878, 2005), readily available phosphorus and potassium were determined using AL method (Egnér et al., 1960), and volumetric method for $CaCO_3$ (SRPS ISO 10693, 2005). Regular agricultural measures were applied (weed hoeing, irrigation, and preventive protection against diseases and pests). The experiment was arranged in a complete block design using Mg sulfate fertiliser (Yara Tera KRISTA, MgS, Norway) where 30 g was dissolved in 15 l of water and added twice during the growing cycle. Fertiliser was applied in three different levels (100 ml, 300 ml, 500 ml), and control (without fertilisation). Plants were irrigated using a drip irrigation system (T-Tape 506-10-1350, Rivulis, Israel) and sprinklers (type 501-U, NaanDan Jain, Israel) 11 times during the vegetation period. After transplanting, firstly we used sprinklers, and after successful rooting, plants were irrigated using two systems with the same quantity of water. The dimension of the plots was 1.3 × 2 m, in three replications, the distance between plants was 32 × 32 cm, 50 cm among repetitions, and 100 cm among treatments. Lettuce plants were harvested 55 days after transplanting when heads were fully matured and reached commercial size. Agronomic parameters were measured by taking 6 plants per treatment using a scale, digital caliper, and ruler. The presence of the leaves affected by the tipburn was analysed during the morphological measurements. Statistical analysis was performed using a three-way ANOVA with a Tukey's test for post hoc comparison. All results were calculated at a significance level α of 0.05 using the software SPSS Statistics (Version 25.0.; Armonk, NY, USA: IBM Corp) and Microsoft Office Excel 2019 (Microsoft Corp., USA). During the growing cycle air temperature, air relative humidity, and precipitation were collected using RC-4HC Data Logger (Elitech Technology Inc., USA) and rain gauge (TFA Dostmann GmbH & Co. KG, Germany**)** (Table 1). Plants were exposed to different photoperiods from 14 h 16 min to 11 h 35 min.

Table 1. Climatic data during the iceberg lettuce growing cycle

	Average air temperature (˚C)	**Maximum air temperature (˚C)**	**Minimum air temperature (˚C)**	**Average air humidity (%)**	**Total precipitation (mm)**
August 2018	23.7	34.7	14.2	66.0	19.0
September 2018	19.2	33.5	2.9	62.9	12.1
October 2018	12.4	23.3	3.0	71.2	1.0

Results and discussion

Cultivar showed significant influence on all tested morphological parameters except head diameter (Table 2).

Table 2. Main and interaction factors effects affecting morphological traits in iceberg

	Head height (cm)	**Head diameter (cm)**	**Head weight (g)**	**Number of leaves**	**Fresh leaf weight (g)**	**Stem height (mm)**	**Stem diameter (mm)**	**Fresh stem weight (g)**
Main factors								
Cultivar								
Umbrinas	13.91±0.38 **b**	16.90±0.37	557.15±18.99 **b**	18.73±0.78 **b**	527.50±18.14 **b**	65.73±4.81 **b**	20.32±0.80 **b**	29.65±2.62 **b**
Kavir	12.07±0.36 **a**	16.76±0.42	510.83±16.68 **a**	14.38±0.66 **a**	493.00±16.31 **a**	37.79±3.47 **a**	18.60±0.68 **a**	17.83±2.25 **a**
Fertiliser								
Control	12.77±0.32	16.72±0.42	542.71±13.07	16.50±0.91	519.96±12.12	48.90±3.31	20.03±1.10	22.75±1.79
100 ml	13.15±0.39	16.96±0.45	543.88±23.08	15.79±0.68	519.17±22.41	53.47±3.95	19.12±0.73	24.71±2.67
300 ml	12.95±0.34	16.74±0.32	521.33±15.10	17.04±0.66	498.96±15.21	48.88±3.99	19.24±0.57	22.38±2.38
500 ml	13.09±0.41	16.90±0.39	528.04±20.09	16.88±0.65	502.92±19.16	55.79±5.32	19.45±0.55	25.13±2.91
Irrigation								
Sprinklers	12.73±0.40 **a**	16.49±0.38 **a**	538.27±17.32	12.63±0.62 **a**	510.19±17.37	55.03±4.16 **b**	20.20±0.89 **b**	28.08±2.86 **b**
Drip irrigation	13.25±0.34 **b**	17.17±0.41 **b**	529.71±18.35	20.48±0.82 **b**	510.31±17.08	48.49±4.13 **a**	18.72±0.59 **a**	19.40±2.02 **a**
Significance								
Cultivar (C)	***	ns	***	***	***	***	**	***
Fertiliser (F)	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation (I)	**	**	ns	***	ns	**	**	***
Interaction factors								
C × F	ns	ns	***	ns	***	**	***	*
C × I	***	ns	*	*	*	ns	ns	ns
F × I	*	*	**	***	**	ns	ns	ns
C × F × I	ns	ns	*	***	*	**	ns	ns

The data show the means (n = 6) ± SE. Values followed by the same letter are not significantly different at the 0.05% level of probability according to Tukey's test. Groups of data within the same parameter and factor with no letters are not different from each other. Asterisks indicate significant differences at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, non-significant.

The results showed the highest value of head weight (557.15 g), head diameter (16.90 cm), head height (13.91 cm), number of leaves (18.73), fresh leaf weight (527.50 g), stem height (65.73 mm), stem diameter (20.32 mm), and stem fresh weight (29.65 g) in cultivar 'Umbrinas'. Head weight is one of the most important traits impacting the lettuce yield. Although 'Umbrinas' was suggested as a cultivar for spring cultivation, during our trial this cultivar exhibited the highest head fresh weight compared to the summer cultivar 'Kavir'. On the other side, 'Kavir' showed lighter and smaller stems, which is a crucial point for the processing industry, by obtaining a higher proportion of edible parts and less waste. Research by Rouphael et al. (2017) showed a significant genotypic influence of different iceberg cultivars on the head fresh mass. Furthermore, in the same study cultivar 'Umbrinas' obtained a lower head fresh weight (485 g) compared to our trial (557.15 g). The head size is a considerable parameter for the consumer's choice since cultivars with longer and larger heads are preferred and recognised as good marketable heads of high quality (Maboko and Du Plooy, 2008). Our results for head diameter are in line with or even higher compared to 25 crisphead lettuce cultivars examined by Lafta et al. (2021). Number of leaves obtained in our experiment was lower compared to trial with iceberg lettuce in hydroponics (Maia et al., 2024).

Stem fresh mass is determined by stem length and diameter in the sense that longer and larger stems are heavier. According to Yuri et al. (2004) stems up to 6 cm are the most favorable for the processing industry, while values up to 9 cm are still acceptable, whereas values higher than that are usually not recommended. Our results for both cultivars showed acceptable values of stem height (3.78; 6.57 cm, Table 2). Cultivar 'Umbrinas' showed 1.7 times longer stem than 'Kavir' which could mean that this cultivar is susceptible to high temperatures during the growing cycle. Likewise, our results for stem diameter are in line with the results of Maia et al. (2024) under hydroponic cultivation in a conventional greenhouse in the summer.

Application of all levels of Mg sulfate fertiliser did not affect any of the observed morphological parameters (Table 2). Nevertheless, the application of 100 ml solution of fertiliser obtained the highest head weight, height and diameter, while 500 ml solution contributed to the highest stem fresh weight and height, and number of leaves but without statistically significant difference. Preciado-Mongui et al. (2023) found an optimal fertilisation formula for lettuce using nitrogen and Mg fertiliser to optimise yield and balanced nutrition. Soil analysis in our experiment showed a neutral to slightly alkaline reaction (pH (H_2O)- 7.6, pH (KCl)- 7.1), very high concentration of Ca 12.375 mg/kg, and high concentration of Mg 726 mg/kg, wide range ratio Ca/Mg (10.23), and adequate levels of macronutrients (total nitrogen-0.19%; readily available phosphorus-26.67 mg/100 g; readily available potassium-18.13 mg/100 g, and 2.82% $CaCO_3$). Results of Ca and Mg, and their ratio, which means Mg ion deficiency, while pH was in the acceptable range for availability of various nutrients (Hazelton and Murphy, 2016). Mg fertiliser was applied twice but possibly with a low dosage of Mg. There is a possibility that with an increased quantity, applied during basic tillage and the vegetation period, continuously, it can be expected to lower the current Ca/Mg ratio to a more balanced level. Literature data are not consistent about the significance of the Ca/Mg ratio. Regression analysis showed no relationship between soybean and corn yields and soil Ca/Mg ratio (Leiva Soto et al., 2023). Research by Zhang (1999) showed that both soil Ca/Mg ratio and plant Ca/Mg ratio affected lettuce yield. The lower critical level of the soil Ca/Mg ratio was 0.5 and within a range from 0.50 to 7.70, no reduction of the yield was observed.

Drip irrigation system led to significantly higher head height and diameter, number of leaves, while irrigation with sprinklers led to significantly higher stem parameters. Type of the irrigation did not influence the head weight. According to Kaniszewski et al. (2017) drip irrigation system gave better results compared to sprinkler irrigation.
Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler's irrigation treatment, whereas drip-irrigated plants showed no signs of tipburn (Table 3).

Table 3. Number of lettuce plants affected with tipburn after harvest

Cultivar	Umbrinas		Kavir	
Irrigation	Sprinklers	Drip irrigation	Sprinklers	Drip irrigation
Fertiliser	**Number of plants affected by tipburn**			
Control	2	0	0	0
100 ml	1	0	0	0
300 ml	1	0	0	0
500 ml	0	0	1	0

Application of 500 ml solution resulted in no tipburn in the 'Umbrinas', while in the 'Kavir', the same level of solution led to the appearance of tipburn in 1/6 analysed plants. Results of Kuronuma et al. (2022) with tipburn incidence and calcium acquisition in lisianthus showed that tipburn can be significantly decreased with increasing Mg level in two of three cultivars.
Even though the influence of fertilisation was not statistically significant on particular morphology traits, three main factors conjointly affected head weight, number of leaves, fresh leaf weight, and stem height, in a way that cannot be predicted by testing just a single factor.

Conclusion

The presented study showed that the cultivar influenced all tested parameters except head diameter. Cultivar 'Umbrinas' showed the highest value of all morphological parameters. On the other side, 'Kavir' showed more favourable values of the stem measurements compared to the head parameters relation which is important for the processing industry, especially during early and mid-summer cultivation to avoid raw material and economic losses. The application of magnesium sulfate fertiliser did not affect the tested parameters. Drip irrigation led to significantly higher head height, diameter and number of leaves, while sprinkler irrigation significantly enhanced the values of all stem parameters. Tipburn was noticed mainly in the 'Umbrinas' cultivar in the sprinkler's irrigation treatment.The application of 500 ml Mg solution resulted in no tipburn in the 'Umbrinas', while in the 'Kavir', led to the appearance in 1/6 analysed plants. Nevertheless, the interaction of all three factors showed a statistically significant impact on head weight, number of leaves, fresh leaf weight, and stem height.

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NOVEL BATAVIA LETTUCE CULTIVARS-CHOOSING THE OPTIMAL CULTIVAR FOR FRESH AND PROCESSED PRODUCTS USING WASPAS METHOD

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Abstract

Six Batavia lettuce cultivars ('Tourbillon', 'Champollion', 'Impression', 'Voltron', 'Bataille', and 'Batsun') were grown in the black marsh soil in March-May 2020. 'Tourbillon' is a standard Batavia cultivar, while five others are not registered on the Variety list in Serbia. This study aimed to select the optimal Batavia cultivar according to agronomic parameters, suitable for the fresh market and processed products by applying the WASPAS (Weighted Aggregates Sum Product Assessment) method. The correlation analysis was carried out to reduce the number of criteria having high coefficient values above 0.8 to exclude double influence on the ranking results. The study involved six alternatives (cultivars) and six different criteria regarding agronomic parameters (rosette height, rosette fresh weight, number of leaves, stem diameter, stem fresh weight, and core ratio). According to the expert’s opinion, we depicted morphological traits of interest significant for fresh and processed lettuce products. Thus, according to the selected criteria and both scenarios, the obtained results showed that cultivar A2-'Voltron' was the best-ranked for fresh and processed products, while cultivar A3-'Champollion' showed the least favorable results. A SAW (Simple Additive Weighting) method was applied to verify and confirm the obtained results by the WASPAS method. Therefore, this study showed that the WASPAS method could present an initial step toward decision-making in the selection of novel lettuce cultivars for the fresh market and ready-to-eat salads and highlighted the importance of choosing the adequate cultivar for different purposes to avoid quality and economic losses.

Keywords: *Lettuce, Morphology traits, MCDM methods, WASPAS, SAW.*

Introduction

Lettuce (*Lactuca sativa*, L.) is an annual leafy vegetable crop from the Asteraceae family. Different groups such as butterhead, iceberg, leaf, romaine, Batavia, and stem include numerous cultivars. Batavia lettuces are like iceberg but with loose-filled rosettes containing relatively crisp-textured leaves in light green color. Wide-range viability has been observed among cultivars in morphological traits, fresh mass production and nutritional value. Unlike other cooked or thermally processed vegetables lettuce, provides nutrients since it is eaten raw (Kim et al., 2016). It can be consumed as a fresh vegetable or processed in ready-to-eat products. Leafy vegetable retail has changed in recent decades as a result of changes in living style, consumers' preferences and needs (Sucheta et al., 2020).
From the breeding point of view, genetic diversity is important in selecting the most favorable genotypes to achieve higher yields. Agronomic parameters such as fresh weight, number of leaves, stem length, plant diameter can be important parameters to farmers, producers, and breeders. A decrease in the stem height and the number of old and yellowish leaves enhances

the proportion of edible fresh weight used in processing and avoids losses (Simko et al., 2014). The morphological traits can affect the market price and advanced marketing influence (Mampholo et al., 2016). The decision-making process corresponds to the existence of several criteria, which causes more complexity during the making of final and optimal choices by using different multi-criteria decision-making methods (MCDM). By setting the criteria experts are called to assess the alternatives based on the decision criteria (Gokasar et al., 2022). One of the numerous MCDM methods is the WASPAS method which combines two methods: the weighted sum model (WSM) and the weighted product model (WPM). According to Sorooshian et al. (2022) WASPAS method was widely applied in about 20 different fields, mainly in engineering, computer science, mathematics, business, management, accounting, environmental science, and energy, while in agricultural and biological sciences just several papers. The same authors highlighted the ability of the WASPAS method to be used in combination with other MCDM methods such as AHP, TOPSIS, MOORA, COPRAS, SWARA, Entropy, fuzzy methods, and sensitivity analysis.
Considering several different criteria (quantity, quality, economic) can affect farmer acceptance and consumer choice of the cultivar in the production, the experts in decision-making methods use the appropriate method in the cultivar selection process and make a final decision between several different alternatives (cultivars). This study aimed to choose the most suitable novel Batavia cultivar for the fresh market and processing industry for ready-to-eat products by applying the MCDM methods.

Materials and methods

Six green open-type Batavia lettuce cultivars 'Tourbillon', 'Champollion', 'Impression', 'Voltron' (Rijk Zwaan, the Netherlands), 'Bataille', and 'Batsun' (Nunhems, The Netherlands) were grown in the spring open-field experiment in 2020. Seeds were sown on February 15 in peat cubes in the substrate Potgrond H (Klasmann-Deilmann, Germany) in a glasshouse condition in the company Grow Rasad (Serbia), and seedlings production lasted 25 days. After several days of acclimatisation, plants were transplanted manually in the black marsh soil on March 15. Soil analysis showed adequate levels of macronutrients (total nitrogen-0.21%; readily available phosphorus-25.42 mg/100 g; readily available potassium-22.34 mg/100 g) and humus 4.1%. Regular cultural practices for the lettuce outdoor production were applied (irrigation, weed hoeing, and preventive protection against diseases and pests). The experiment was arranged in a complete block design, the dimension of the plots was 1.3 × 2 m, with three replications, and the distance between plants was 27 × 27 cm. Lettuce plants were harvested on May 28, 74 days after transplanting, when rosettes reached commercial size and fully matured. Morphology parameters were measured by using a scale, ruler, and digital caliper. A one-way ANOVA was used to test the effect of different Batavia cultivars on the morphology traits by using a Tukey test for post hoc comparison. All results were calculated at a significance level α of 0.05. Statistical analysis was performed with the software SPSS Statistics (Version 25.0.; Armonk, NY, USA: IBM Corp) and Microsoft Office Excel 2019 (Microsoft Corp., USA). Data for air temperature, air relative humidity, and total precipitation were collected using RC-4HC Data Logger (Elitech Technology Inc., USA) and rain gauge (TFA Dostmann GmbH & Co. KG, Germany**)** (Table 1). Plants were exposed to different photoperiods, ranging from short to long days, from 11 h 53 min to 15 h 15 min.

Table 1. Climatic data during the lettuce vegetation period

	Average air temperature (°C)	Maximum air temperature (°C)	Minimum air temperature (°C)	Average air humidity (%)	Total precipitation (mm)
March 2020	7.3	21.2	-4.0	59.4	21.2
April 2020	12.1	27.5	-3.1	52.5	9.4
May 2020	15.5	28.2	6.3	66.5	69.3

For the evaluation and selection of the most favorable cultivar for fresh market and food processing, we used the MCDM method-WASPAS (Weighted Aggregates Sum Product Assessment) method (Zavadskas et al., 2012), which is a combination of two previously established multi-criteria decision-making methods: the Weighted Sum method (WS) and the Weighted Product method-(WP). Correlation analysis was applied to test possible linear correlation using the Pearson coefficient among morphological parameters (criteria) to eliminate their double influence on the ranking results. To verify the results obtained by the WASPAS method we used another MCDM method- SAW (Simple Additive Weighting) (MacCrimmon, 1968).

Results and discussion

One-way ANOVA was used to test the effect of the cultivar on different morphological parameters with Tukey’s test for post hoc comparison. Cultivar showed a significant influence on all tested morphological parameters except the number of leaves (Table 2).

Table 2. Effect of Batavia lettuce cultivars on agronomic parameters

	Rosette height (cm)	Rosette diameter (cm)	Rosette fresh weight (g)	Number of leaves	Fresh leaf weight (g)	Stem height (cm)	Stem diameter (cm)	Stem fresh weight (g)	Core ratio
Cultivar									
Tourbillon	21.27±0.60 **c**	34.47±1.08 **b**	579.00±44.80 **b**	44.33±1.20	542.00±46.86 **b**	4.07±0.03 **abc**	2.03±0.03 **ab**	22.33±0.67 **ab**	0.19±0.01 **ab**
Voltron	17.43±0.18 **a**	30.53±0.73 **a**	455.67±19.81 **ab**	43.33±0.88	433.67±16.47 **ab**	3.60±0.31 **ab**	1.90±0.06 **a**	14.00±2.00 **a**	0.21±0.02 **ab**
Champollion	19.50±0.12 **abc**	34.90±0.59 **b**	575.33±55.00 **b**	48.33±3.48	532.67±58.76 **ab**	4.47±0.09 **bc**	2.40±0.20 **b**	27.67±2.41 **b**	0.23±0.01 **b**
Impression	20.03±0.29 **bc**	32.67±0.60 **ab**	547.33±10.85 **b**	41.00±1.16	511.00±21.39 **ab**	4.63±0.23 **c**	1.83±0.03 **a**	20.67±0.88 **ab**	0.23±0.01 **b**
Bataille	18.00±0.82 **ab**	30.77±0.62 **a**	388.33±18.70 **a**	46.00±1.73	371.00±17.37 **a**	3.10±0.25 **a**	1.73±0.03 **a**	15.33±3.39 **a**	0.17±0.01 **a**
Batsun	18.37±0.26 **ab**	29.73±0.87 **a**	468.33±24.80 **ab**	47.33±2.03	448.33±22.70 **ab**	3.73±0.18 **abc**	1.90±0.06 **a**	17.67±2.19 **ab**	0.20±0.01 **ab**
Significance									
Cultivar	***	**	**	ns	*	**	**	**	*

The data show the means (n = 3) ± SE. Values followed by the same letter are not significantly different at the 0.05% level of probability according to Tukey’s test. Groups of data within the same parameter with no letters are not different from each other. Asterisks indicate significant differences at * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ns, non-significant.

Results for a single parameter using ANOVA showed that the cultivar 'Tourbillon' had the highest value of rosette height (21.27 cm), rosette fresh weight (579.00 g), and leaf fresh weight (542.00 g). Cultivar 'Champollion' showed the highest values of rosette (34.90 cm) and stem diameter (2.40 cm), stem fresh weight (27.67 g), core ratio (0.23) as well as 'Impression' core ratio (0.23) and stem height (4.63 cm). Fresh matter is usually considered the most valuable trait due to its contribution to lettuce yield. Additionally, the number of leaves is an important parameter also contributing to the yield. Both consumers and the food industry are interested in cultivars with a larger number of leaves, especially the consumer’s attention is concentrated on the appearance, volume and secondarily the number of leaves (Graciano et al., 2020). Rosette diameter shows the horizontal expansion of the rosette and the progress of its morphology and shape formation. Despite the consumer’s preference for larger and heavier plants, this is not usually a beneficial trait because larger plants can be easily damaged during packaging and transport, and with reduced quality of the final product (Suinaga et al., 2013). Stem length is also a relevant trait in lettuce breeding due to its connection with bolting. According to Yuri et al. (2004) stems between 6-9 cm are favorable for the processing industry. Our results are in line with the desirable stem length (3.10-4,63 cm, Table 2) and all cultivars were suitable for the processing industry, with the highest obtained in cultivar 'Impression'. According to Maboko and Du Plooy (2008) core ratio (stem height to rosette height) should not exceed 0.5. In our experiment, all cultivars showed core ratios between 0.17-0.23 (Table 2) which can be considered as growing within favourable environmental conditions, even though maximum air temperatures in April and May were above 27 °C in the phase of intensive leaf growth (Table 1). A strong positive correlation was found between rosette fresh weight and stem height (0.75^{**}, Table 3) pointing out the importance of the stem height in rosette weight. Our results were in line with the similar linear correlation between these two parameters (0.719) (Madar and Hájos, 2021).

Table 3. Correlation analysis of tested morphological criteria

Criteria	Rosette height	Rosette height	Rosette fresh weight	Number of leaves	Leaf fresh weight	Stem height	Stem diameter	Stem fresh weight	Core ratio
Rosette height	1								
Rosette diameter	0.71^{**}	1							
Rosette fresh weight	0.72^{**}	0.80^{**}	1						
Number of leaves	-0.08	0.21	0.23	1					
Leaf fresh weight	0.67^{**}	0.75^{**}	0.99^{**}	0.28	1				
Stem height	0.60^{**}	0.61^{**}	0.75^{**}	-0.06	0.68^{**}	1			
Stem diameter	0.29	0.70^{**}	0.71^{**}	0.58^{*}	0.70^{**}	0.47	1		
Stem fresh weight	0.63^{**}	0.81^{**}	0.71^{**}	0.39	0.64^{**}	0.71^{**}	0.72^{**}	1	
Core ratio	0.15	0.36	0.52^{*}	0.003	0.46	0.87^{**}	0.42	0.51^{*}	1

Asterisks indicate significant differences at * $p \leq 0.05$; ** $p \leq 0.01$.

One-way ANOVA showed differences between cultivars when one parameter was taken into account, yet in situations when it is needed to include several parameters, we employ MCDM methods. Before starting the WASPAS method the correlation analysis was applied to test possible linear correlation using the Pearson coefficient among morphological parameters (criteria). The criteria rosette diameter, leaf fresh weight and stem height, showed a strong linear relationship with coefficient values (greater than 0.8) and were excluded from the multi-criteria decision-making procedure to eliminate their double influence on the ranking results. Thus, the ranking procedure of the 6 cultivars using the WASPAS method was carried out taking into account 6 different criteria: rosette height (C1), rosette fresh weight (C2), number of leaves (C3), stem diameter (C4), stem fresh weight (C5), and core ratio (C6). The initial 6 × 6 matrix formed in the first step is normalised in the second step in such a way that the benefit criteria (C2 and C3) are normalised by bringing the values of the initial matrix criteria into relation with their optimal value, which is their maximum value, while the cost criteria are normalised by bringing in relation to the optimal value of each criterion with their individual values (Table 4).

Table 4. Normalised matrix

Cultivar (Alternatives)	C1	C2	C3	C4	C5	C6
Tourbillon (A1)	0.82	1.00	0.92	0.85	0.63	0.90
Voltron (A2)	1.00	0.79	0.90	0.91	1.00	0.83
Champollion (A3)	0.89	0.99	1.00	0.72	0.51	0.75
Impression (A4)	0.87	0.95	0.85	0.95	0.68	0.74
Bataille (A5)	0.97	0.67	0.95	1.00	0.91	1.00
Batsun (A6)	0.95	0.81	0.98	0.91	0.79	0.85

Alternatives: A1- 'Tourbillon'; A2- 'Voltron'; A3- 'Champollion'; A4- 'Impression'; A5- 'Bataille'; A6- 'Batsun'; Criteria: C1- rosette height; C2- rosette fresh weight; C3- number of leaves; C4- stem diameter; C5- stem fresh weight; C6- core ratio.

In the next step, based on the data from the normalised matrix, weighted matrices are formed by WS (the product of the normalised values of each criterion and the weighting coefficients for each) and by the WP method (by scaling the normalised values of the criteria with the values of the weighting coefficients for the same). The weight coefficients and the ranking list of cultivars were determined through two scenarios. In the first scenario, the ranking was performed in case cultivars are used for fresh consumption, and the criteria of rosette fresh weight, number of leaves and stem fresh weight were given greater importance compared to the other observed criteria. In the second scenario, the assumption is that the cultivars are for processing and producing ready-to-eat products, and in that case, greater importance is given to the criteria of rosette fresh weight, stem fresh weight and core ratio. The values of the weight coefficients according to the scenarios are given in Table 5.

Table 5. Weight coefficients (w_i) according to the two scenarios

Scenario	C1	C2	C3	C4	C5	C6
S1 w_i	0.056	0.278	0.278	0.056	0.278	0.056
S2 w_i	0.056	0.278	0.056	0.056	0.278	0.278

S1-scenario 1 (for fresh market); S2-scenario 2 (for processed products); W_i- weight coefficients

The relative importance of each alternative is determined by summing the value of the weighted matrix for the same (WS method) as well as by multiplying the value of the weighted matrix for each alternative (WP method). The relative importance of the alternatives in the WS and WP methods, as well as the total relative importance of individual alternatives, under the condition of equal influence of both methods on the obtained solution (λ=0.5), for the first scenario, is given in Table 6.

Table 6. Relative importance, the total relative significance for each alternative and ranking list for scenario 1

Alternatives	WS Q_i	WP Q_i	Q_i	Rank
A1	0.85	0.84	0.842	4
A2	0.90	0.89	0.896	1
A3	0.83	0.79	0.810	6
A4	0.83	0.82	0.825	5
A5	0.87	0.86	0.864	3
A6	0.87	0.86	0.865	2

Q_i-the relative importance; WS- Weighted Sum method; WP-Weighted Product method

According to scenario 1 and fresh lettuce consumption, the best-ranked cultivar is 'Voltron', followed by 'Batsun', while 'Champollion' was the worst-ranked. The ranking list of analysed cultivars for lettuce processing (scenario 2) is given in Table 7.

Table 7. Ranking list of analysed alternatives for scenario 2

Alternative	A1	A2	A3	A4	A5	A6
Rank	3	1	6	5	2	4

Results for scenario 2 showed that the 'Voltron' remained the best-ranked cultivar for processing, as well as the 'Champollion', which remained the worst-ranked in the ranking list. The stability of the obtained ranking list was checked using the SAW method. Ranking results for both scenarios are shown in Table 8.

Table 8. Ranking list of the alternatives for both observed scenarios using the SAW method

Alternatives	A1	A2	A3	A4	A5	A6
Rank (S1)	4	1	6	5	2	3
Rank (S2)	3	1	6	5	2	4

According to the SAW method the best-ranked cultivar 'Voltron'-A2 and the worst-ranked cultivar 'Champollion'-A3 stayed in the same positions for both scenarios which can confirm that the WASPAS method was suitable for solving this kind of decision-making problem in lettuce production. Also, the ranking positions of other cultivars stayed at the same position except for scenario 1 where alternatives 5 and 6 in the SAW method changed their positions. WASPAS method in agriculture included research on the efficiency of agricultural enterprises in Serbia (Lukić et al., 2021), the selection of pear (Nedeljković et al., 2022) and apple varieties in raising orchards (Nedeljković et al., 2023), assessment of nutrient deficiencies in kinnow (Pathania et al., 2023). This research showed that WASPAS and SAW methods can be used as decision-making tools for the selection of lettuce cultivars for different purposes. To the best of our knowledge this is the first experiment regarding the selection of lettuce cultivars using the WASPAS and SAW method and its contribution is to make optimal solution in cases when there are different criteria (traits) that should be taken into account.

Conclusion

Choosing an adequate cultivar is one of the most important steps in lettuce production. This research was conducted on five new cultivars that at the moment of experiments have not been registered in Serbia. Therefore, before involving them in production for the fresh market and/or processed industry, it is necessary to consider more criteria before making a final decision. For the fresh market, experts' opinions depicted several criteria (rosette fresh weight, number of leaves, stem fresh weight), being different for the processing (rosette fresh weight, stem fresh weight, core ratio). Application of WASPAS method derived cultivar 'Voltron' as the best-ranked alternative, while the lowest-ranked was 'Champollion', for both scenarios. To obtain more accurate results, a SAW method was applied. This method confirmed the ranking list for both scenarios obtained by the WASPAS method. Therefore, the presented results suggest that the WASPAS method provided an initial step toward decision-making in the selection of novel lettuce cultivars for the fresh market and ready-to-eat salads and that the cultivar 'Voltron' should be taken into consideration for registering on the variety list in Serbia.

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YIELD RESPONSE OF MAIZE TO IRRIGATION AND PLANTING DENSITY IN THE VOJVODINA ENVIRONMENT IN SERBIA

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Abstract

The aim of this study was to compare irrigation scheduling and planting density in maize and to evaluate yield, water use efficiency (WUE), irrigation water use efficiency (IWUE) and financial efficiency of water use for irrigation (F_{IWUE}). A two-year field experiment was conducted in Vojvodina region (Serbia), and maize was subjected to three irrigation levels (rainfed and supply at 60 and 100% of crop evapotranspiration, ETc) interacting with three planting density (54,900, 64,900 and 75,200 plants ha^{-1}). The results indicate a large year-to-year variability, mainly due to a rainfall event in the first year during the silking stage. A significant irrigation and planting density effect was observed for all variables studied. The results showed that the reduction of irrigation decreased yield and WUE, but improved the IWUE. Increasing the planting density increased yield, IWUE and F_{IWUE}. In addition, increasing planting density increased the WUE, but only in the irrigated treatments. Treatment 100% ETc and 75,200 plants ha^{-1} achieved the highest grain yield (13.098–16.125 Mg ha^{-1}) and a relatively high WUE (2.525–2.958 kg m^{-3}). A significant “D × I” interaction was observed for grain yield and WUE and IWUE. The effect of planting density on grain yield and WUE was strongest at 100% ETc and the highest planting density. The treatment 60% ETc and 75,200 plants ha^{-1} obtained the highest IWUE (2.356 kg m^{-3}) and F_{IWUE} (\$2.543 m^{-3}). Maize can be grown in the Vojvodina environment with acceptable yields while saving irrigation water and increasing planting density. By taking advantage of the positive interaction between these two factors, resource utilization can be maximized.

Keywords: *Sprinkler irrigation, Planting density, Water use efficiency, Economic water productivity.*

Introduction

Maize (*Zea mays* L.) is widely cultivated in Serbia. The area under cultivation is 996,527 hectares and the average grain yield is about 790 g m^{-2}, while in Vojvodina, which is characterized by a temperate continental climate, cultivation covers 569,556 hectares and the average grain yield is about 840 g m^{-2} (Statistical Yearbook of the Republic of Serbia, 2021). However, due to the high variability in precipitation, maize yields in Serbia vary considerably from year to year (Dugalić *et al.*, 2024). According to Pejić *et al*. (2011), most maize crops in Vojvodina are produced under rainfed conditions, however, some areas with this crop are produced under irrigation to stabilize production. Under such water-limited conditions, maize productivity is highly dependent on irrigation, but in many intensive agricultural areas the availability of irrigation water is steadily declining as a result of climate change and increasing consumption. In many areas of southern Europe, irrigation water is becoming an increasingly limited resource (García-Tejero and Durán-Zuazo, 2022), so appropriate irrigation scheduling is required to maximize water use efficiency and profit. Appropriate use of irrigation water and plant density is critical to increase yield and water productivity (Zhang *et al*., 2022).

Evaluating crop response to irrigation in combination with planting density could help determine the best allocation of available resources to crops on the farm to maximize profit and reduce groundwater pollution. Literature on the effects of water and planting density on yield and related parameters such asWUE and IWUE can be found in Zhang *et al*. (2022), Wang *et al*. (2021), Xu *et al*. (2017) and Assefa *et al*. (2016). Irrigation affects maize grain yield, and the highest yield is obtained when crop evapotranspiration is fully restored, although WUE and IWUE generally decrease with an increase in irrigation water.

Djaman and Irmak (2012) found that the IWUE of maize decreased with irrigation amount in the first year of the experiment, which was conducted in a silt loam soil, while they showed the opposite trend in the second year. In a two-year study, Payero *et al*. (2006) evaluated the response of maize to deficit irrigation in the semi-arid environment of the US Great Plains. They found that WUE in this environment did not increase favourably with deficit irrigation, as WUE increased linearly with the ratio of actual crop evapotranspiration to potential plant evapotranspiration. An increase in plant density generally increases the grain yield of maize until an optimum number of plants per unit area is reached (El-Hendawy *et al*., 2008). Hou *et al*. (2020) also reported that higher plant densities in maize lead to higher grain yields. Norwood (2000) concluded that for maize, deficit irrigation combined with adequate plant population is a viable alternative to dryland farming in Kansas, where groundwater resources are declining.

The objectives of this study were: (i) to evaluate the impact of sprinkler irrigation scheduling in interaction with planting density of maize grown in a Vojvodina environment, and (ii) to analyze water use efficiency for maize.

Materials and methods

The field experiments were carried out in 2018 and 2019 in Vojvodina province, Serbia (44°52’ north latitude; 20°20’ east longitude, 81 m above sea level) in the experimental farm of the Maize Research Institute “Zemun Polje”. The soil was a Chernozem on loess (Calcic Chernozems), silty-clay-loam with the following average properties at 0–50 cm depth: clay 31.2%, silt 50.4%, sand 18.4%, pH (water) 7.8, total nitrogen 0.20%, organic matter 3.2%, plant available P_2O_5 13.1 mg per 100 g soil, plant available K_2O, 30.6 mg per 100 g soil, bulk density 1.32 kg dm^{-3}, field capacity water content 0.36 m^3 m^{-3}; permanent wilting point water content 0.17 m^3 m^{-3}, available soil water 190 mm m^{-1}.

Comparisons were made between three irrigation regimes with the restitution of 100 % (I_{100}) and 50 % (I_{60}) of the crop’s evapotranspiration (ETc) and rainfed (I_0) and three planting density (D_1 – 54,900, D_2 – 64,900 and D_3 – 75,200 plants ha^{-1}).

Irrigation was planned on the basis of a water balance calculated as the sum of estimated daily crop evapotranspiration (ETc), subtracting the portion of rainfall and stored soil water. ETc was calculated as suggested by the FAO, where the reference evapotranspiration (ETo) was calculated using the Penman–Monteith model before being modified by a crop coefficient (Allen *et al*., 1998). Irrigation was carried out every 7 days throughout the growing season to cover crop water requirements at a depth of 90 cm. A hand move sprinkler irrigation system was used.

A four replicate split-plot design was used, with irrigation “I” in the main plot and planting density “D” in the subplot. The experimental plots were located within an area planted with maize, and each experimental plot was 5.6 m × 8.0 m, corresponding to eight rows of maize. The individual plots were separated by bufferstrips. The maize hybrid ZP 677 from the Maize Research Institute “Zemun Polje”, FAO class 600, was sown by hand on May 5, 2018 and

April 26, 2019 with a row spacing of 0.7 m. At the time of physiological maturity, a sample area of 11.2 m^2 (the two middle rows of each plot, 8 m long) was harvested by hand and the mass of grains was determined. The grain samples were oven-dried at 65 °C to constant weight. The main details of the experiments are given in Table 1.

Table 1. Maize management during the 2 years of the experiment

Agronomic practices	2018	2019
Sowing date (day of year – DOY)	106	104
Irrigation supply (I_{60} and I_{100})	8–13	7–13
Seasonal irrigation rate (mm) treatments (I_{60} and I_{100})	90–239	94–291
Harvest date (DOY)	270	255
Length of growing season (days)	164	151

Soil water content was measured weekly in all experimental plots at sowing and harvest using the gravimetric method based on conventional dry weight multiplied by bulk density. Soil samples were taken in three points of the central zone of each plot in 10 cm soil layers (0–120 cm depth). Soil bulk density was also measured at the beginning of the growing season.
Seasonal crop water use (WU) was estimated using the following water balance equation (Kresović *et al*., 2016):

$$WU = P + I \pm SWC - D \quad (1)$$

where P is precipitation, I is irrigation,“ΔSWC” is the change in volumetric soil water content in the 0–1.2 m depth layer between sowing and harvest and D is water loss due to deep percolation, all in mm. The term D was calculated according to a water balance approach using the daily values of ETc, precipitation and irrigation and based on the gravimetrically measured soil water content at sowing. In this water balance model, losses due to surface runoff and capillary return flow are considered negligible.
Water use efficiency (WUE, kg m^{-3}) was calculated as the ratio between dry grain yield (in kg ha^{-1}) and seasonal water use (in mm). Irrigation water use efficiency (IWUE, kg m^{-3}) was also calculated as the ratio of the increased yield compared to the rainfed treatment with seasonal irrigation amount. The financial efficiency of water use for irrigation (F_{IWUE}, \$ m^{-3}) was calculated as the ratio of income generated by the application of irrigation and the cost of using irrigation.
Analysis of variance was performed for the 2-year period, considering year as a random effect. Least significant difference (LSD) was used as a test to separate means. Statistical procedures were performed using SAS statistical software (SAS version 9.4, 2016).

Results and discussion

Climate. In both years, the average temperatures (with the exception of September 2018) were always above the long-term average temperatures. In both years, these conditions were generally suitable for the heat requirements of maize, with daily temperatures in the range of 12–25 °C and slightly lower summer temperatures in the first year. The ETc also fluctuated between the two years and was 370–535 mm in 2018 and 304–501 mm in 2019 during the sowing-harvest period.

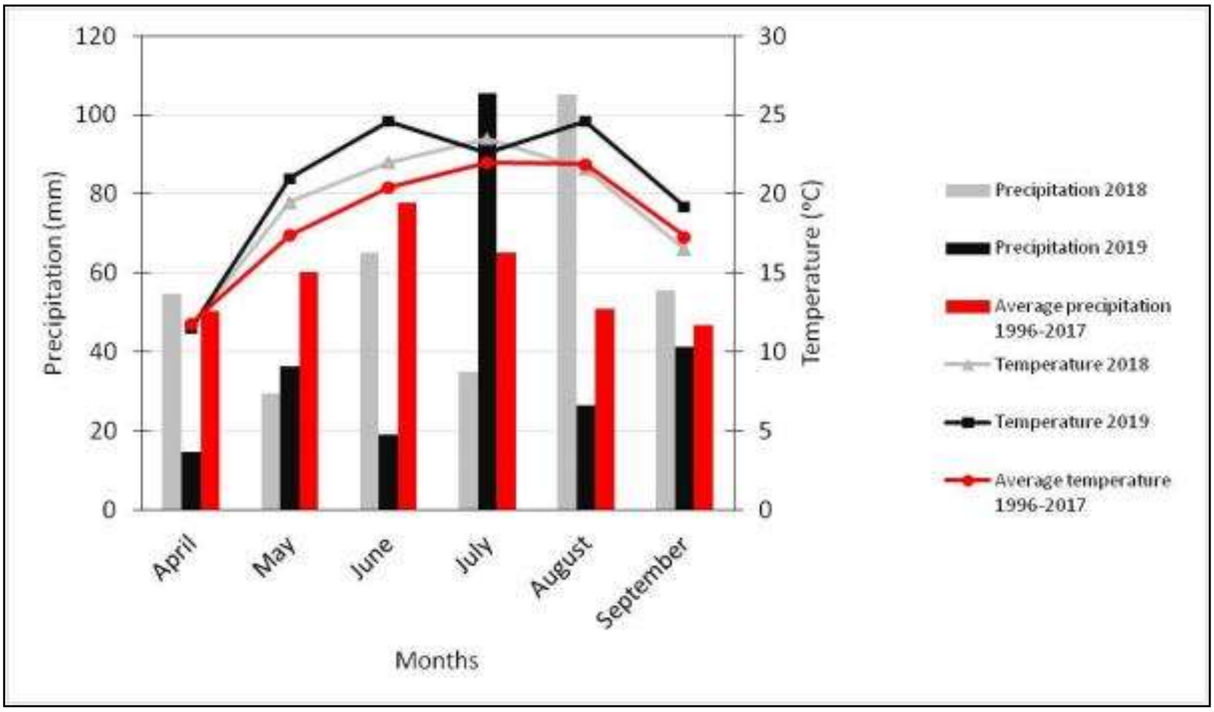

Fig. 1. Monthly climate data in Zemun Polje during the growing season of maize in 2018 and 2019 and the 20-year average.

Total precipitation during the maize cycle amounted to 345 mm in 2018 and 243 mm in 2019, which is below the long-term average (351 mm). In 2018, 18 mm of precipitation fell in the first 10 days of July during the silking maize stage, which was important for both grain yield formation and response to irrigation. In contrast, 40 mm of precipitation fell in the first 10 days of July 2019.

Grain yield production. The grain yield differed significantly between the two years (Table 2). In 2018 the average grain yield was 13.144, in 2019 9.561 Mg ha^{-1}. This can be explained by the climatic conditions, which were more favourable for ear fertility in 2018 (lower temperatures and humid days in the period after anthesis). Maize grain yields differed between irrigation and planting density treatments, but the “D × I” interaction was significant. In fact, irrigation enhanced the effect of plant density and vice versa (Fig. 2).
The differences between planting density treatments for each irrigation regime were small at I_{60} (10.519, 11.218 and 10.833 Mg ha^{-1} for D_1, D_2 and D_3, respectively), while they were considerably larger at I_{100} (13.323, 14.054 and 14.612 Mg ha^{-1}, respectively). This confirms the positive effect of adequate soil irrigation on water availability and the plant’s ability to take up water and plant nutrients simultaneously.
El-Hendawy *et al*. (2008) reported a greater response of maize yield to planting density when soil water was sufficient and a lesser response when water was deficient. They also reported an average yield increase of 63.5 % with sufficient (100 % ET) versus insufficient water supply (60 % ET) and significant yield decreases at 95,000 plants ha^{-1}, averaging 51 and 40 % of the values at 48,000 and 71,00 plants ha^{-1}, respectively, in Ismailia region, Egypt. In our study, yield increases with irrigation water were 66% at I_{100} versus I_0 and 36% at I_{100} versus I_{60}. The response to planting density was less effective; grain yield increases were 8.4% at D3 versus D1 and 0.2% at D3 versus D2. They also reported an average yield increase of 63.5% with sufficient (100% ET) versus deficient water supply (60% ET) and whereas yields at

95,000 plants ha^{-1} showed significant decreases average 51 and 40% of values at 48,000 and 71,00 plants ha^{-1}, respectively.

Relationship between water use and yield. In 2018, WUE was considerably higher than in 2019 (+23%), due to better climatic conditions favouring photosynthesis and carbon assimilation (Table 2). During the study period, the WUE ranged from 2.090 kg m^{-3} (I_0-D3) to 3.085 kg m^{-3} (I_{60}-D2). In this study, increasing the planting density and irrigation amount increases the WUE of maize. This agrees with the results of Zhang *et al*. (2022).

Table 2. Dry grain yield, water use efficiency (WUE), irrigation water use efficiency (IWUE) and financial efficiency of water use for irrigation (F_{IWUE}) of maize as a function of year, irrigation and planting density.

	Grain yield, Mg ha^{-1}	WUE, kg m^{-3}	IWUE, kg m^{-3}	F_{IWUE}, \$ m^{-3}
Year				
2018	13.143	2.878	1.570	1.393
2019	9.560	2.331	2.060	2.429
$LSD_{(0.05)}$	0.342	0.319	0.413	0.336
Irrigation				
I_0	9.186	2.653	–	–
I_{60}	10.873	2.535	1.822	1.943
I_{100}	13.996	2.627	1.808	1.879
$LSD_{(0.05)}$	0.217	0.051	0.279	0.275
Planting density				
D1	11.052	2.546	1.401	1.470
D2	11.606	2.673	1.753	1.838
D3	11.397	2.595	2.291	2.425
$LSD_{(0.05)}$	0.200	0.052	0.389	0.397

Averaged over all planting density, the yield of rainfed maize was very low: 11.342 and 7.032 Mg ha^{-1} in 2018 and 2019, respectively. This is consistent with the results of Kresović *et al*. (2016) for rainfed maize in an area with similar climatic characteristics. In rainfed crop, increasing planting density from 64,900 to 75,200 plants ha^{-1} resulted in significantly lower yields in bout years. These results show how difficult it is to achieve a satisfactory maize yield in these areas without irrigation and confirm that water is crucial and will become increasingly important in the future

Al-Kaisi and Yin (2003) obtained similar results under the agroecological conditions of the Great Plains in the USA. They reported that the highest grain yield was obtained in the maize treatment with full water supply at a density of 69,000 plants ha^{-1}. Increasing the plant density had no significant effect on yield, while significantly lower yields were obtained at lower densities than those mentioned. Under conditions of more or less pronounced irrigation, the maize achieved higher yields at lower sowing densities.

The significant "D × I" interaction of WUE, the relationship between grain yield and water use (ETc), is shown in Fig. 2. Higher values were obtained for D2 at I_0 and I_{60} irrigation. In this case, the yield-limiting factor was water availability in rainfed treatment (I_0) and at an irrigation supply of 60% of ETc, which proved to be sufficient to achieve the maximum WUE value at D2 in rainfed cultivation. On the contrary, the WUE values in I_{100} were better in the D3 treatment than in the D1 and D2 treatments.

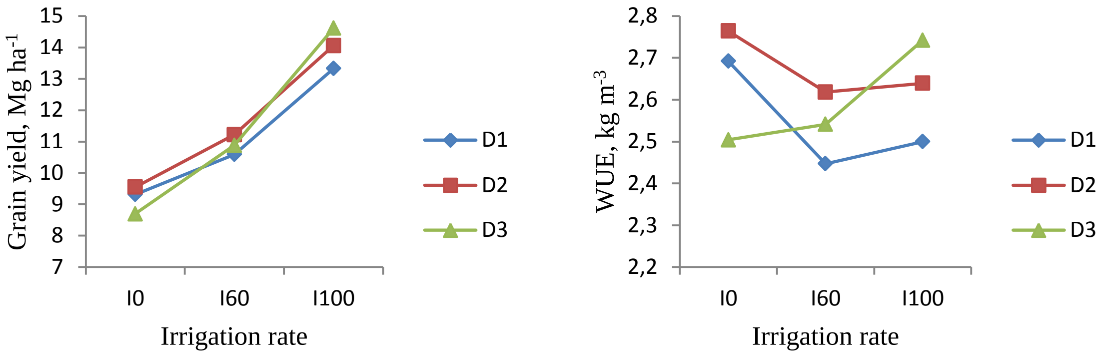

Fig. 2. Significant interaction “planting density” × irrigation for maize grain yield (left) and water use efficiency (right)

The combined effects of irrigation rate and planting density on irrigation water use efficiency (IWUE) were significant in 2019 (dry season) but not significant in 2018 (wet season) (data not shown). In 2018 treatment I_{100}-D3 (2.160 kg m^{-3}) and in 2019 treatment I_{60}-D3 (3.085 kg m^{-3}) had the highest IWUE. On the contrary, in D1, IWUE values were better in I_{100} than in deficit irrigation (I_{60}). On average, the lowest IWUE (1.301 kg m^{-3}) was recorded in I_{60} with 54,900 plants ha^{-1} and the highest IWUE (3.085 kg m^{-3}) in I_{60} with 75,200 plants ha^{-1}. Pejić *et al*. (2011) and Kresović *et al*. (2016) determined similar IWUE values of maize. In contrast to our results, El-Hendawy *et al*. (2008) reported the highest IWUE values for the highest irrigation rate (100% ET) for medium to small densities (48,000 or 71,000 plants ha^{-1}).
In the wet year 2018, no statistically significant differences in financial efficiency of water use for irrigation were found between deficit irrigation (I_{60} – F_{IWUE} = \$1,249 m^{-3}) and full irrigation (I_{100} – F_{IWUE} = \$1,536 m^{-3}). In addition to the water regime, the increased planting density also had a positive effect on the increase in F_{IWUE} values. The interaction between water regime and planting density showed no statistically significant differences. Irrigation resulted in a better F_{IWUE} in the dry year 2019 (Table 2) than in 2018. On average, for all densities, the I_0 treatment had a significantly higher financial efficiency of water use for irrigation (F_{IWUE} = \$2.635 m^{-3}) than the I_{100} treatment (F_{IWUE} = \$2.223 m^{-3}). Also in 2019, the highest F_{IWUE} (\$2.455 m^{-3}) from the application of irrigation was obtained by sowing maize in the highest tested density (D3). Analyzing the interaction between the two factors, it is concluded that the highest F_{IWUE} (\$3.640 m^{-3}) was achieved in the I_{60}-D3 treatment.

Conclusions

Based on these experimental results, an irrigation schedule that provides for partial restoration of crop ETc on a 60% basis allows for water savings (65% – when I_{60} is compared to I_{100}) and ensures a satisfactory maize grain yield level (10.873 Mg ha^{-1}, –22%) in a Vojvodina environment. Irrigation was more effective than planting density in increasing grain yield in both years. Irrigation increases the WUE value at I_{60}. Irrigation water productivity was higher in the I_{60} irrigation regime at higher planting densities, as shown by IWUE values. The maximum F_{IWUE} (\$3.640 m^{-3}) was achieved at I_{60}-D3.
In conclusion, high maize yields and high water use efficiency can be achieved in the Vojvodina region through the use of irrigation and higher planting density, while improving water production efficiency and economic returns.

Acknowledgements

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STRESS RESISTANCE INDICATORS AS THE TOOL FOR SELECTING DROUGHT-TOLERANT WHEAT GENOTYPES

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Abstract

Climate change and the resulting increase in the frequency and severity of drought can have significant impacts on plant production. The use of drought-tolerant crop varieties can significantly improve plant production under drought conditions. Therefore, the goal of this research was to evaluate the drought tolerance of different wheat genotypes using stress resistance indicators. An experiment was conducted with sixteen wheat genotypes in Novi Bečej (Vojvodina) in different growing seasons. The spike weight was used as a phenotypic marker of the effect of drought stress on the plant. Selection based on resistance indicators such as stress tolerance index (STI), mean productivity (MP), and geometric mean productivity (GMP) favors the selection of genotypes Dunavka, Skopjanka, and Fundulea 4, which were characterized by the highest average values of spike weight, especially in favorable growing conditions. However, genotype Fundulea 4 is characterized by the highest stress susceptibility index (SSI) and the lowest yield stability index (YSI), which makes this genotype undesirable for growing under drought conditions. The parameters SSI and YSI favor the selection of the genotype Pitoma, which showed the highest value of spike weight in drought conditions as well as the least reduction in value caused by stress. Genotypes Pitoma, Dunavka, and Skopjanka, characterized by a high yield index (YI), are suitable for cultivation in drought conditions, where they achieved above-average trait values. The most suitable stress resistance indicators for selecting drought-tolerant wheat genotypes are SSI, YSI, and YI.

Key words: *drought stress, stress susceptibility index, stress tolerance index, wheat.*

Introduction

The greatest threats to the availability of food in the present and future are population growth and climate change. Drought is one of the most significant consequences of climate change and can which have a severe impact on agricultural production, especially in arid and semi-arid areas (Cheng et al., 2021). Wheat is the primary food source for around 40% of the world's population and a main source of daily protein and calories for about 2.5 billion people in developing countries (Braun et al., 2010). According to predictions made by Alexandratos et al. (2012), the demand for wheat in developing countries could rise by as much as 60% by 2050. Some of the causes influencing this projected rise in demand include urbanisation, rapid population expansion, and changes in dietary patterns.

According to Darvanto et al. (2016) drought conditions can reduce wheat productivity by 50 to 90% of the crop potential. Drought affects wheat at all growth stages, but it is more severe during the flowering and grain-filling stages, leading to significant yield losses (Sareen et al.,

2023). A worldwide effort to reduce the severity of droughts involves the development of drought-tolerant cultivars. However, progress has been significantly delayed by the complex structure of the drought-tolerance characteristics, which is controlled by a number of genes and greatly influenced by the environment (Pandey et al., 2022). Accordingly, identifying genotypes with tolerant genes is a difficult process (Anwaar et al., 2020). One of the approaches in the identification of drought-tolerant genotypes is the calculation of stress resistance indicators, which compare the value of the yield achieved in drought conditions with the yield in normal conditions (Anwaar et al., 2020, Aksić et al., 2020).
The aim of this study is to identify the wheat genotypes that exhibit the highest drought tolerance in the agro-ecological conditions of the semi-arid climate. Also, the goal is to select the best drought resistance indicator.

Material and Methods

A field experiment was established in Novi Bečej (Banat, AP Vojvodina, Serbia), which includes 16 wheat genotypes (Dukat, Dunavka, Fundulea 4, Iskra, Jedina, Jugoslavija, Kavkaz, Mačvanka 1, Marija, NS 58-04, Pitoma, Poljana, Skopjanka, Tamiš, Vali PKA-7114, and Zvezda). The analysed genotypes were sown according to the randomized block system in three replications with an inter-row spacing of 12 cm, where the size of the basic plot was 2 m^2. The soil type was humogley, which is characterised by a high content of clay. The usual agrotechnics for wheat production were implemented, where monoammonium phosphate (MAP) was used as the basic fertilizer in the amount of 250 kg ha^{-1}, while urea was used in the amount of 250 kg ha^{-1} for crop feeding. In both vegetation seasons, the harvest was performed at the optimal time (the first week of July in 2015/2016 and the last week of June in 2016/2017), when the grain moisture was below 14%. The spike weight was measured in 30 plants for each analysed genotype.
The stress resistance indicators were calculated based on the value of the spike weight under stress conditions (Yd), which characterized the 2016/2017 growing season, and the value of the spike weight in conditions favorable for plant development (Yp), which characterized the 2015/2016 season. The following stress resistance indicators are expressed in this paper:

Stress susceptibility index – SSI (Fisher and Maurer, 1978):

$$SSI = 1-(Yd/Yp)/1-(\bar{Y}d/\bar{Y}p)$$

Mean productivity – MP (Rosielle and Hamblin, 1981)

$$MP = (Yd + Yp)/2$$

Stress tolerance index – STI (Fernandez, 1992)

$$STI = (Yd + Yp)/\bar{Y}^2p$$

Geometric mean productivity – GMP (Fernandez, 1992):

$$GMP = \sqrt{Yd \times Yp}$$

Yield stability index – YSI (Bouslama and Schapaugh Jr, 1984):

$$YSI = Yd/Yp$$

Yield index – YI (Gavuzzi et al., 1997):

$$YI = Yd/\bar{Y}d$$

A cluster analysis was applied according to Ward's method for grain yield per ear and indicators of stress tolerance using the programme IBM SPSS Statistics, Trial Version 22.0

(https://www.ibm.com/). Distances between clusters are expressed as squared Euclidean distances, and the significance of distances was tested by the t-test. The number of cluster groups was identified using a dendrogram, after which a K-means analysis was performed with a predetermined number of cluster groups. After the analyses were carried out, the cluster groups were ranked according to the mean values of the analysed parameters.
During the experiment, large differences were noted regarding the amount of precipitation between the analysed growing seasons. Twice as much precipitation was recorded in the 2015/2016 growing season) compared to 2016/2017 (612 or 300 mm). Because of this, the 2016/2017 season is regarded as dry. In 2015/2016, during the growing season, average monthly temperatures were within the multi-year average, and the amount of precipitation was significantly higher than the multi-year average in almost all months. Heavy rainfall in June (164.0 mm) extended the grain filling period. On the other hand, 2016/2017 growing season was characterised by significantly higher temperatures than the multi-year average and a pronounced deficit of precipitation, especially in the grain-filling phenophase, which caused an earlier harvest of crops in the mentioned season (http://www.hidmet.gov.rs/).

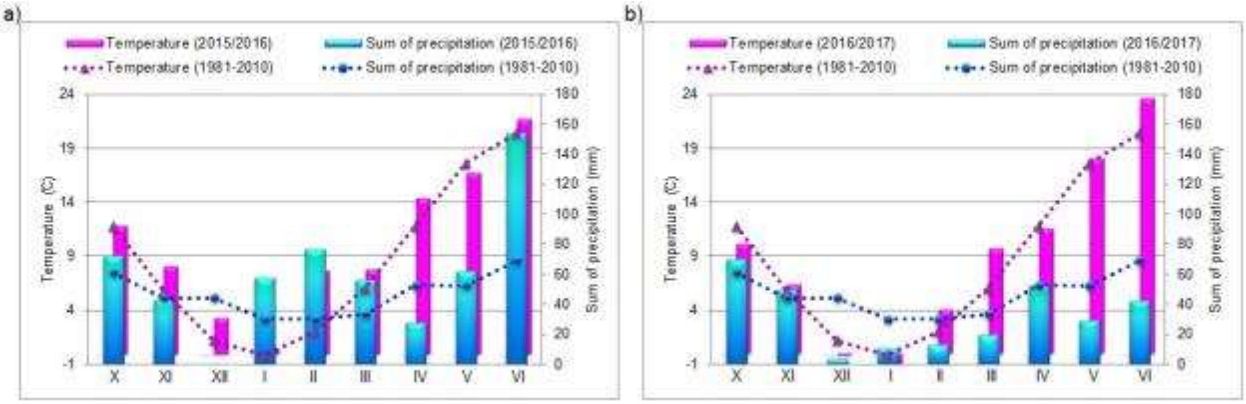

Figure 1. Mean monthly temperatures and sum of precipitation in Novi Bečej locality in 2015/2016 (a) and 2016/2017 (b) vegetation season

Results and Discussion

In this study, it was established that the drought stress conditions in the 2016/2017 vegetation season affected the reduction of the spike weight value in all analysed wheat genotypes, compared to the values achieved in the favourable 2015/2016 vegetation season (Figure 2, a).
The greatest decrease in the spike weight was recorded in the Fundulea 4 genotype (58.0%), while the smallest decrease was observed in the Pitoma genotype (20.2%) (Figure 2, 2). In accordance with the above, spike weight is considered a good phenotypic marker of the impact of drought stress on wheat. Wasaya et al. (2021) also found a decrease in the wheat spike weight under drought stress conditions, which they explained by a reduction in photosynthetic parameters.

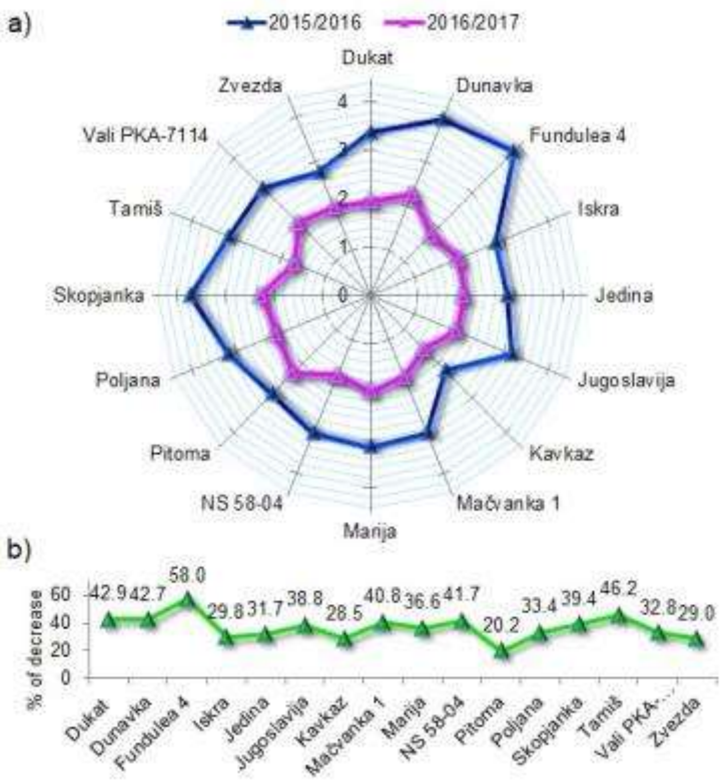

Figure 2. Radar graph of spike weight in examined wheat genotypes grown in drought stress and favorable conditions (a) and decrease (%) in spike weight due to drought stress (b)

The stress resistance indices were calculated, according to the values of spike weight of the analyzed wheat genotypes (Table 1). Indicators of resistance such as mean productivity (MP), stress tolerance index (STI) geometric mean productivity (GMP) take into account the spike weight achieved both in drought and in favorable environmental conditions. Golbashy et al. (2010) noted that STI and GMP are effective in identifying genotypes with high grain yield values in both, non-stress and stress conditions. According to the mentioned stress resistance indicators, the first-ranked cluster group includes the genotypes Dunavka, Skopjanka, and Fundulea 4, which were characterized by the highest average values of spike weight, especially in favorable growing conditions (Table 1, Figure 1,a). Similarly, Aksić et al. (2020) observed that STI, MP, and GMP indicators are positively correlated with grain yield achieved under irrigated conditions. Among the mentioned genotypes, Dunavka and Skopjanka are distinguished by the highest value of the yield index (YI) indicator (1.15), which relates the spike weight of certain genotypes in drought conditions to the average spike weight of all genotypes in drought stress conditions. Although genotype Fundulea 4 has a high value of MP (2.99), STI (0.59), and GMP (2.73), it is characterized by the highest stress susceptibility index (SSI) (1.53), the lowest yield stability index (YSI) (0.42), as well as a low value of the yield index (YI) (0.90). This genotype showed a very high value of spike weight in non-stress conditions, but a low value in drought stress conditions. According to Awnaar et al. (2020), such genotypes are not suitable for growing in wider areas, due to large yield losses under stress conditions. Stress resistance parameters SSI, YSI, and YI favor the selection of the genotype Pitoma, which showed the highest value of spike weight under drought stress conditions, as well as a smaller decrease in value under the influence of drought. Therefore, this genotype is considered the most drought-tolerant. Also, according to the SSI, YSI and YI parameters, the Kavkaz genotype is classified among drought-tolerant genotypes, exhibiting a small decrease in the spike weight under drought. However, according to the MP, STI, GMP and YI indicators, it is classified in the lowest ranked cluster group (4 and 5), characterized by lowest value of spike weight, which makes it an undesirable genotype in breeding programs and for cultivation in semi-arid climate conditions.

Table 1. AMMI analysis of variance for spike weight in 27 wheat genotypes grown in different agro-ecological conditions

Genotypes	SSI	MP	STI	GMP	YSI	YI
Dukat	1.13 (2)	2.66 (2)	0.53 (2)	2.55 (3)	0.57 (4)	0.98 (3)
Dunavka	1.12 (2)	3.12 (1)	0.62 (1)	3.00 (1)	0.57 (4)	1.15 (1)
Fundulea 4	1.53 (1)	2.99 (1)	0.59 (1)	2.73 (2)	0.42 (5)	0.90 (4)
Iskra	0.78 (4)	2.40 (3)	0.48 (3)	2.36 (4)	0.70 (2)	1.01 (3)
Jedina	0.83 (4)	2.39 (3)	0.47 (3)	2.35 (4)	0.68 (2)	0.99 (3)
Jugoslavija	1.02 (3)	2.56 (2)	0.51 (2)	2.48 (3)	0.61 (3)	0.99 (3)
Kavkaz	0.75 (4)	1.90 (4)	0.38 (4)	1.87 (5)	0.71 (2)	0.80 (5)
Mačvanka 1	1.07 (2)	2.48 (3)	0.49 (3)	2.39 (4)	0.59 (3)	0.94 (4)
Marija	0.96 (3)	2.57 (2)	0.51 (2)	2.50 (3)	0.63 (3)	1.01 (3)
NS 58-04	1.10 (2)	2.45 (3)	0.49 (3)	2.36 (4)	0.58 (4)	0.92 (4)
Pitoma	0.53 (5)	2.58 (2)	0.51 (2)	2.56 (3)	0.80 (1)	1.16 (1)
Poljana	0.88 (4)	2.64 (2)	0.52 (2)	2.59 (3)	0.67 (2)	1.07 (2)
Skopjanka	1.04 (3)	3.00 (1)	0.60 (1)	2.90 (1)	0.61 (3)	1.15 (1)
Tamiš	1.22 (2)	2.43 (3)	0.48 (3)	2.32 (4)	0.54 (4)	0.86 (4)
Vali PKA-7114	0.86 (4)	2.63 (2)	0.52 (2)	2.57 (3)	0.67 (2)	1.07 (2)
Zvezda	0.76 (4)	2.36 (3)	0.47 (3)	2.33 (4)	0.71 (2)	1.00 (3)

Note: The numbers in parentheses represent the rank of the cluster group for each resistance indicator, where number 1 is the cluster group with the highest, and number 5 with the lowest mean value of the resistance indicator.

Conclusion

Drought stress affected the reduction of spike weight in all analyzed wheat genotypes, which makes this trait a good phenotypic marker of the impact of drought stress on wheat. Therefore, stress resistance indicators were calculated based on the value of this trait. High values of the MP, STI, and GMP resistance indicators favor the selection of genotypes Dunavka, Fundulea 4, and Skopjanka, characterized by a high average spike weight, especially in non-stressful environmental conditions. Genotype Pitoma characterized by high values of MP, STI, GMP, YSI, YI and low value of SSI is rated as highly drought-tolerant genotype. Also, Dunavka, and Skopjanka are characterised by high YI values and are considered drought-tolerant genotypes. Genotype Fundulea 4, characterised by high values of MP, STI, and GMP and low values of SSI, YSI, and YI, are preferred for cultivation in non-stressful, but not in stressful environmental conditions. The most suitable stress resistance indicators for selecting drought-tolerant wheat genotypes are SSI, YSI, and YI.

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SOCIAL NETWORKING IN STRESS-EXPOSED PLANTS

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Abstract

Throughout the life cycle, plants are exposed to a wide range of environmental factors, some of which may be harmful to their capacity to grow and develop to the fullest extent possible. The ability of a plant to respond rapidly and effectively to stress is one of the most critical aspects in determining its optimal output and survival. Plants have developed a mechanism for communication and signaling throughout their evolutionary history. Plants emit volatile organic compounds (VOCs) into the atmosphere to warn other plants of impending danger. Neighboring plants exposed to volatile organic chemicals develop their own defense systems in response to the stress. The basil plant (Ocimum basilicum) has been subjected to three different salinized water treatments (0, 40, and 80 mM NaCl) in order to investigate plant communication under stress. The main volatile organic compounds (VOCs) found in basil leaves were detected using analytical headspace-gas chromatography/mass spectrometry (HS-GC/MS). In basil, six signaling terpenes and one monoterpenoid were found. Under saline stress, eucalyptol synthesis and emission are intensified. The monoterpenes beta-phellandrene showed a tendency to decline under stress. Interestingly, the concentration of 4-carene is enhanced, while emission of its isomere 3-carene is reduced. In contrast to this, when exposed to stress, the concentrations of pinene, beta-ocimene, and alpha-camphene increased in moderately salinized solutions and decreased in substantially salinized solutions.

Key words: *plants communication, VOC, basil, terpenes, GCMS.*

Introduction

A critical aspect of contemporary agriculture is soil salinization. The production of plants is adversely affected by salinization of agricultural land, which further intensifies soil degradation. Retention of soluble salts in the soil leads to salinization of the soil. It occurs either naturally or as a result of inappropriate human activity, especially farming methods, intense industrialization pressure, and climate changes. In addition to reducing the yield of most crops, salinity affects the physical and chemical characteristics of the soil. There is a noticeable acceleration of the pace of soil salinization. It is anticipated that 50% of agricultural soil will become holomorphic by 2050 (Nachshon, 2018).

During their evolution plants developed a specific mechanism of communication as response to stress. When exposed plants experience stress, stress triggers set off a series of events that include gene expression, signaling cascade reactions, and morphological changes. For stress reactions to be successful or unsuccessful, signal processing time is crucial. Plants can communicate through sophisticated systems of mycorrhizal networks, electrical impulses, and volatile organic molecules released into the atmosphere or soil (Karban, 2008; Sharifi and Ryu, 2021). Volatile organic compounds, or VOCs, emitted by plants exposed to stress, have a particular composition and ratio of compounds. Plants react to messages containing volatile organic compounds (VOCs) by initiating a specific defense mechanism. For instance, the plant may produce specific secondary metabolites to protect itself from a particular kind of stress (Abbas et al., 2021, Rozenkranz et al., 2021).

With nearly sixty varieties, basil is an aromatic member of the *Lamiacea* family. Because of its essential oil concentration and aromatic flavor, basil is utilized extensively in both the culinary and pharmaceutical industry. The predominant aromatic profiles found in each of the species can be used to classify them into chemotypes, plant subspecies that share the same morphological traits but differ in the amounts of chemical compounds they produce in their essential oils (Muráriková et al., 2017). Monoterpenoids, methyleugenol, sesquiterpenoids, and phenylpropanoids fall within this category. Furthermore, there have also been reports of phenolic profiles. The differences in the content of essential oils are caused by factors such as cultivation conditions (temperature, light quality, and quantity), species and cultivar, growth season, leaf position and age, harvesting techniques, and extraction methods (Tangpao et al., 2022). The objective of the present study was to examine the relationship between plant communication and volatile organic compounds (VOCs) emission in limiting settings of saline stress.

Material and methods

The experiment constituted growing plants from seeds in the ecological-climatic conditions of Novi Sad, Serbia (45.2396° N, 19.8227° E). The basil seeds were sown at the beginning of March 2024 into a commercial substrate. When the basil seedling reached 5 cm in plant height, they were transferred to the experimental pots. Without causing any water stress, the seedlings were irrigated by ponding water in each pot until they had grown to an average height of 10-15 cm and had become acclimated to the soil. Following the original harvest of the basil plants in order to establish consistency among the plants, treatments were applied at regular basis. The irrigation interval and saline solution concentration were set at three days and 0, 40, and 80 mM, respectively. After 30 days of exposure to saline stress, basil leaves were harvested and analyzed for qualitative and descriptive composition of VOCs.

Basil varieties grown under stress and under control were analyzed for volatile chemicals using gas chromatography–mass spectrometry (GC/MS, 7890A/ 7694E, Agilent Technologies, USA), in scan mode. The instrument used a 30-m Agilent J&W DB-5MS Ultra Inert column (0.25 mm × 0.25 µm film thickness). For GCMS headspace analysis was used 0.3g of basil samples.

Results and Discussion

In terms of descriptive results, a total of six signaling VOCs were identified in this study (table 1), among which carene exists in isomer forms of 4- and 3-carene. Detailed analysis of the complete volatile compounds profile confirmed that terpenes are essential for stress-related communication. This finding is in line with recent studies. Namely, a thorough examination of the entire profile of volatile molecules highlighted terpenes and terpenoids, the most diverse class of plant secondary metabolites, as critical for stress-related communication (Rozenkranz et al., 2021). Terpenes, which include isoprene, monoterpenes, and sesquiterpenes, make up the greatest group of volatile organic compounds (VOCs) generated by plants under suboptimal growing conditions. Terpenes have the potential to serve as a chemical language for plant communication. Different classes of terpenes are used for warning under specific stress. While in plant-insect interactions, mono-and sesquiterpenes act as communication molecules, isoprene, the smallest and most often released terpene, has a role in abiotic stress resistance (Karban, 2008; Boncan et al., 2021, Abbas et al., 2022;).

In comparison to control plants, there was an increase in the levels of total eucalyptol and 4-carene following exposure to moderate and severe (40 mM and 80 mM NaCl treatment, respectively) saline stress. Specifically, under mild saline stress, the ratio of the cyclic monoterpenoid eucalyptol is raised by 60%. After further exposure to higher concentration of

NaCl, the initial rise declined, however, it remained higher than in the group of reference plants (Table 1). Full gas-mass chromatogram was shown in figure 1.

Table 1. Signaling terpenes emitted by basil leaves exposed to saline stress

Terpene	Ratio T1*/C**	Ratio T2***/C**
Pinene	1.18	0.87
Alpha-camphene	1.19	0.88
Beta-phellandrene	0.90	0.76
Eucalyptol	1.60	1.29
Beta-ocimene	1.11	0.81
Gamma-terpinene	1.41	0.76
4-carene	1.13	1.09
3-carene	0.95	0.58

*T1: treatment of mild salinization of 40mM NaCl solution
**C: control group
***T2: treatment of severe salinization of 80mM NaCl solution

Bicyclic monoterpenes 4-carene showed a similar pattern, with concentrations rising by 13% and 9% under moderate and severe salt stress, respectively. However, during salinization, the levels of the monoterpenes beta-phellandrene and 3-carene decreased in a corresponding manner in comparison to plants cultivated under optimal conditions and not exposed to stress (0.90 and 0.76 and 0.95 and 0.58, respectively, figure 1).

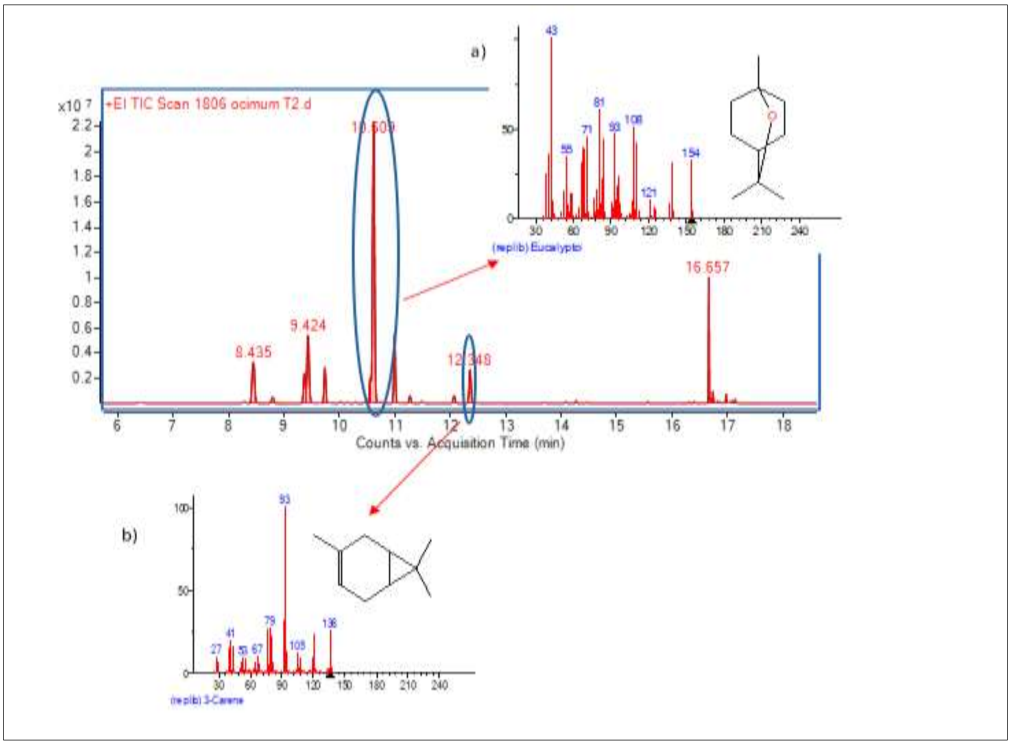

Fig.1 Full GCMS chromatogram in scan mode with mass spectra for a) Eucalyptol and b) 3-Carene

In contrast, the salinization caused decrease of monoterpene beta-phellandrene and 3-carene levels (0.90 and 0.76 and 0.95 and 0.58 compared to plants grown under optimal conditions respectively). Alpha-camphene, beta-ocimene, and pinene release are intensified under mild stress circumstances, however their production is suppressed at 80 mM NaCl concentration.

Compared to abiotic factors, effects of biotic-induced plant-to-plant communication have been studied far more. While the emission of volatile monoterpenoids is triggered by all types of stress, the production of aromatic volatiles appears to be promoted mostly by insect attack.
Additionally, in line with our findings, the volatile monoterpenes, such pinenes and ocimene, are activated by both biotic and abiotic stress (Abbas et al., 2022; Rosenkranz et al., 2021). Moreover, alpha-camphene may serve as a marker for biochemical alterations occurring at infection, whereas pinene, linalool, and ocimene are induced by biotic and abiotic stress (Arimura et al., 2011; Lee et al., 2014, Kleiber et al., 2017, Boncan et al., 2021).
Similar to our findings, several studies have linked the variation in 3-carene concentration to the plant's response to abiotic stress (Lee et al., 2014; Kleiber et al., 2017). Our findings are consistent with Lee et al. (2014), who found terpenine emission in rice under abiotic stress. According to Zebelo et al. (2012) and Kleiber et al. (2017), exposure to biotic and abiotic stress has been shown to cause the emission of gamma-phellandrene in the atmosphere.
Until now, research has not determined a change in beta-ocimene synthesis due to exposure to abiotic factors, but only due to insect invasion (Arimura et al., 2011, Lee et al., 2014). This finding gives room for beta-ocimene to be considered as a candidate for VOCs involved in communication in stressful circumstances, particularly in salinity-related situations.
The alternations in betta-phellandrene synthesis noticed between control and stressed plants can be explained with prior outcomes at gene expression level. Conditions of drought or excessive salinity alter the volatile emission in plants (Fernández-Martínez et al., 2018, Penuelas et al., 2005). A study that cross-referenced the volatile ingredients indicated many alterations in gene expression under constant salt stress. The plant can be protected from abiotic stresses by changes in VOC composition, such as the production of monoterpenes and isoprene. Most of the genes involved in volatile emission were suppressed in tomatoes exposed to salt (Benjamin et al., 2012) and betta-phellandrene synthase was one of these.
Our results demonstrate that concentrations of alpha-camphene, beta-ocimene, and pinene increase after initial exposure to moderate saline stress. Continued exposure to high salt concentrations causes a drop in their levels. This can be explained by the fact that increased salt concentrations are toxic to plants and that metabolic activities of stressed plants are reduced to minimum (Xu and Wu, 2021).

Conclusions

Detailed study of the entire profile of volatile chemicals verified that terpenes are necessary for communication linked to saline stress. For warning under particular stress conditions, different classes of terpenes and their mode/level of synthesis are employed. Upon exposure to moderate and severe salinity stress, there were variations in the amounts of six signaling volatile chemicals compared to control plants. These compounds include alpha-camphene, beta-ocimene, carene (which represents 4-carene 3-carene in two isomer forms), eucalyptol, and beta-phellandrene. The novel aspect is that, up until now, studies have only found that insect invasion causes changes in beta-ocimene production. This result allows beta-ocimene to be taken into consideration as a potential volatile organic compound (VOCs) implicated in stressful situations, especially those including salt. Our findings indicate that following an initial exposure to moderate saline stress, concentrations of alpha-camphene, beta-ocimene, and pinene rise. Their levels decrease with continued exposure to high salt concentrations. This makes sense given that plants are poisonous to higher salt concentrations and that stressed plants have minimal metabolic activity. As a result of initial exposure to moderate saline stress, our data show that concentrations of alpha-camphene, beta-ocimene, and pinene rise. They decrease as a result of prolonged exposure to high salt concentrations. This implies that stressed plants exhibit minimal metabolic activity and higher salt concentrations are toxic to plants.

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THE NORMALIZED DIFFERENCE RED EDGE INDEX (NDRE) IN GRAIN YIELD AND BIOMASS ESTIMATION IN MAIZE (Zea mays L.)

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Abstract

Maize (*Zea mays* L.) is considered one of the most important crops in the world and plays an important role in human food and animal feed. Considering the importance of maize production, as well as significant variability in productivity, biomass and early grain yield evaluation may be significantly important. Crop growth monitoring by remote and proximal sensing based on spectral information could provide promising results in its prediction. In the present study, an active multispectral proximate sensor, namely the Plant-O-Meter (POM) was used in the field trail to provide early yield and biomass estimation in maize crops. The maize crop was grown under field trial conditions with various levels of nitrogen application. This study investigated the contribution of the Normalized Difference Red Edge Index (NDRE) and Normalized Difference Vegetation Index (NDVI) on maize grain yield and biomass estimation during the season. The canopy reflectance was measured between the 4-leaf growth stages (V4) and the end of the tasseling and silking stage (VT/R1) in maize. Pearson's correlation coefficient (r) was applied to determine the correlations between grain yield and biomass with the NDRE and NDVI indices throughout the growing season. Maize grain yield showed a more significant positive relationship with both indices across the V4 stage, while biomass estimation showed the highest correlation at the VT/R1 growth stages. These results imply that NDRE and NDVI indices may be suitable for early yield and biomass estimation, while their accuracy depends on the growth stage of maize.

Key words: *maize, yield, biomass, proximal sensors.*

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops and has the most notable global production, providing human food, animal feed and feedstock for numerous industrial products and biofuel production (Cardador-Martínez et al., 2011). Despite the genetic improvement and high progress in the selection of new maize genotypes, the grain yield potential of maize is still unpredictable and largely affected by abiotic and biotic factors, as well as by a certain combination of different factors (Ljubičić et al., 2023). In maize cultivation, nitrogen (N) management has a significant impact on yield quantity and quality, and it is required for optimal maize development, photosynthesis, and grain production during the growing season. Since the maize crop requires more nitrogen than the soil can supply, it is common practice to apply nitrogen fertilizer. Still, soil nitrogen availability is not persistent, and its amount depends on various elements, such as water status, soil type and meteorological conditions. Therefore, forecasting yield and yield related traits of maize before harvest is becoming increasingly important in areas where climate uncertainty has become prevalent, as well as to recognize environmental stress and mitigate its negative impact on plant growth. Recent studies showed that remote sensing has been applied quite successfully to monitoring open fields and large areas, which provides rapid data collection over wide field areas (Kostić et al., 2016; Ljubičić et al., 2018). The main characteristic of remote and proximal sensing technology in agriculture is that it presents non-destructive measurements and real-time plant monitoring based on canopy reflectance measurements. Depending on the surface's optical characteristics, light is absorbed, transmitted, or reflected. Plants reflect light emitted during different physiological processes or morphological components, while different sensors can detect the fraction of light that a surface reflects. Advancements in canopy reflectance detection accuracy have led to linking specific wavelengths with specific plant traits, physiological processes, or morphological parameters, as well as yield and biomass estimation (Sarlikioti, 2011). Several studies have been conducted to identify the combinations of reflectance at various wavelengths to properly account of the effect of impacting elements on the relationship between plant reflectance and plant traits. The combinations of the reflectance at different wavelength bands have become known as vegetation indices (VI’s). Vegetation indices, defined as mathematical combinations of spectral bands, commonly assess land surfaces in the visible and near-infrared ranges of the electromagnetic spectrum (Lambin, 2001). Vegetation indices represent quantitative measures used to estimate vegetation activity, the fraction of photosynthetically active radiation absorbed by the vegetation canopy, plant health, growth dynamics, vegetation cover or plant vigor (Wang et al., 2005; Xue and Su, 2017). Over the past years, scientists have developed a number of VIs for qualitatively and quantitatively evaluating vegetative covers using spectral measurements. Among numerous indices, the Normalized Difference Vegetation Index (NDVI), developed by Rouse et al. (1974), is the oldest and most well-known vegetation index used as an indicator of vegetation greenness (Ihuoma and Madramootoo, 2017; Zhao et al., 2015). However, NDVI has certain disadvantages and can be sensitive to certain atmospheric effects and has the tendency to saturate over dense vegetation (Agapiou et al., 2011; Gu et al., 2013). By substituting NDVI’s red band with NDRE’s (The normalized difference red edge index) red edge band it is possible to mitigate the issue of saturation. It has been confirmed that NDRE’s red edge band provides better insight into plants in their later stages and is less prone to saturation in the presence of dense vegetation. Therefore, the objectives of this study were to evaluate the potential of using NDVI and NDRE indices to get accurate results in grain yield of maize and biomass estimation. The second objective was to determine the specific growth stage where the correlation between yield, biomass and NDRE and NDVI indices acquired by a multispectral sensor is highest and therefore most suitable for sensing.

Material and Methods

The present research was conducted in a field trial at Ravno Selo, near Novi Sad, Serbia, on the Chernozem type of soil, throughout the 2021 growing season. In order to examine the variation of maize grain yield traits, maize hybrids (*Zea mays* L.) of different maturity were chosen under different nitrogen applications as pre-plant applications. The field experiment was conducted according to randomized block design with three replications. An active, multispectral optical sensor, namely Plant-O-Meter (POM), recently developed at the Biosense Institute in Serbia, was used to measure plants during vegetation. The Plant-O-Meter device possesses a multispectral source, which integrates six light sources into one optical module. Based on six band combinations (Blue: 455 nm, Green: 528 nm, Red: 657 nm, Red Edge: 740 nm, NIR1: 810 nm and NIR2: 940 nm) it provides reflectance for each band separately, allowing the calculation of more than 30 different VIs. As the sensor provides its own light source, it was designed to block the influence of sunlight in any environment. Sensor readings were recorded throughout the growing season, from V4 (four completely developed leaves) growth stage to VT/R1 (tasselling and silking) growth stage of maize

development. The collection of data with the POM was carried out manually by passing over the canopy 0.5 m above and screening the two centre rows in continuous mode at a frequency of 1 Hz. After processing the data with GIS software, the central part of each measured row, or two middle rows per plot, was selected. Based on the six-band combination obtained by multispectral measurements the vegetation indices NDVI and NDRE were calculated.
The normalized difference red edge index (NDRE) was calculated as follows:
NDRE = (NIR−RED EDGE) / (NIR+RED EDGE), where NIR present near-infra red band (810 nm) and RED EDGE present red edge spectral band (740 nm).
The normalized difference vegetation index (NDVI) was calculated as follows:
NDVI = (NIR – RED) / (NIR + RED), where NIR present near-infra red band (810 nm) and RED present red spectral band (657 nm).
Pearson correlation coefficients were used to determine the relationship between NDRE and NDVI indices, grain yield and biomass of maize. All statistical analyses were carried out with the STATISTICA software version 13 (Stat Soft Inc., Tulsa, OK, USA).

Results and Discussion

In this research, the relationship between two vegetation indices, biomass and overall grain yield of maize has been estimated. Average grain yields, biomass and vegetation indices of the examined maize crop are presented in Table 1. In respect of the mean values of NDRE and NDVI values, it was observed that both indices showed the greatest values at the V4 (four developed leaves) leaf stage of maize. The obtained data indicated that a significantly better condition of the plants was observed at the beginning of the growing season, which is confirmed by the data from the NDVI and NDRE vegetation indices. The lowest mean values were observed in the V8/V9 growth stage for the NDRE with a mean value of 0,088, while the NDVI showed the lowest value at the VT/R1 stage of maize, with a mean value of 0,615 (Table 1).

Table 1. The mean values of biomass, grain yield, NDRE and NDVI throughout the growing season

			Growth stage of maize		
GY[a]	AB[b]	V4	V7	V8/V9	VT/R1
					NDRE[c]
7,460	75,302	0,166	0,164	0,088	0,116
				Growth stage of maize	
		V4	V7	V8/V9	VT/R1
					NDVI[d]
		0,765	0,652	0,738	0,615

[a]GY: Mean grain yield (t ha^{-1}), [b]AB: Mean biomass (t ha^{-1}), [c] NDRE: The normalized difference red edge index, [d]NDVI: The normalized difference vegetation index

The maximum values of NDVI observed during the V4 and V8 stages were expected when photosynthesis was at its highest point and the leaf area was the largest. The NDVI and NDRE of maize crop decreased with the growth process, and through the later growth stages, both NDVI and NDRE values gradually declined, reaching the minimum values at VT/VR1. The obtained results indicated that through the growing season and towards the end of the season, reflectance of visible wavelengths increased, while reflectance of NIR decreased because of senescence and less absorption of visible light in the maize leaves. These results are in accordance with previous findings reported by Reynolds et al. (2012) and Zhao et al.

(2023). In respect to the association between NDRE, NDVI indices and grain yield and biomass of maize the calculated ratios showed that different correlations were found between NDRE, NDVI, grain yield and biomass of maize estimation. Regarding the association between grain yield of maize and NDRE, the result revealed that a highly significant and positive association was observed at the V4 (r=0,348**) and VT/R1 (r=0,318**) growth stages. In respect to the NDVI, highly significant and positive association was observed at V4 (r=0,362**) and VT/VR (r=0,321**) growth stages of maize. The significant and positive association between NDVI and maize biomass was also observed at V7 (r=0,209*), Table 2.

Table 2. Relationship between NDRE and NDVI indices with grain yield of maize throughout the growing season

	Growth stage of maize			
GY[a]	V4	V7	V8/V9	VT/R1
	NDRE[b]			
8,460	0,348**	-0,008	0,031	0,318 **
	Growth stage of maize			
	V4	V7	V8/V9	VT/R1
	NDVI[c]			
	0,362**	0,209**	0,177	0,321**

[a]GY: Grain yield t ha^{-1}, [b]NDRE: The normalized difference red edge index, [c]NDVI: The normalized difference vegetation index, **Correlation significant at the 0,01 level, *Correlation significant at the 0,05 level.

In respect to the association between NDRE, NDVI indices and the biomass of maize, the calculated ratios showed similar correlations. Regarding the association between the biomass of maize and NDRE, the result revealed that a highly significant and positive association was observed at the VT/R1 (r=0,309**) and V4 (r=0,288**) growth stages. In respect to the NDVI a highly significant and positive association was observed at VT/VR (r=0,362**) and V4 growth stage (r=0,334**). The significant and positive association was also observed at the V8/V9 (eight to nine developed leaves) growth stage (r=0,209*), Table 3. The obtained results are expected since, during the tasselling and silking stages of maize development, the plants showed the least uniformity. This is a consequence of the different appearance of plants in the reproductive stage, when they express higher variability in leaf color, varying from dark green to pale brown. Similar findings were observed by Vian et al. (2018), while different results and a significant positive correlation between vegetation indices and maize yield in the filling stage were shown by Zhao et al. (2023).

Table 3. Relationship between NDRE and NDVI indices with grain yield of maize throughout the growing season

			Growth stage of maize	
AB[a]	V4	V7	V8/V9	VT/R1
			NDRE[b]	
75,302	0,288**	0,016	0,052	0,309**
			Growth stage of ma ize	
	V4	V7	V8/V9	VT/R1
			NDVI[c]	
	0,334**	0,177	0,209*	0,362**

[a]AB: Aboveground biomass (t ha^{-1}), [b] NDRE: The normalized difference red edge index, [c]NDVI: The normalized difference vegetation index, **Correlation significant at the 0,01 level, *Correlation significant at the 0,05 level.

Although methods of correction of the NDVI index using the NDRE index should provide more accurate data and a better understanding of the obtained data (Hassan et al., 2019; Voitik et al., 2023), the present results indicated the need to consider more parameters for a better assessment of maize plant states and forecasting their grain yield and biomass. Since several investigations were conducted on wheat plants, there is a need for further investigation and clarification for other crops.

Conclusion

Based on the present findings, it can be concluded that the grain yield and biomass of maize may be estimated with high accuracy using both the NDVI and NDRE indices. The estimation of maize grain yield presents a higher level of accuracy at the appearance stage of the V4 growth stage, while the best estimation for biomass was observed at the V6 growth stage of maize. Despite certain advantages of the NDRE index, the NDVI showed higher accuracy than the NDRE in the estimation of maize biomass and grain yield. In light of the present findings, additional research on various cultivars and fertilisation management techniques has to be done in the future, which will provide more information and clarification for other crops.

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MONITORING STRESS IN ARUGULA (*Eruca sativa*) USING A PORTABLE MULTISPECTRAL DEVICE

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Abstract

One of the factors that most severely limits plant production is a rise in salinized soil, which is brought on by a combination of pollution and contemporary farming methods. Arugula (*Eruca sativa*), a green leafy vegetable belonging to the Brassicaceae family, is rich in proteins, fibers, and vitamins A, C, and K. Arugula is a powerful antioxidant and anti-inflammatory because of its biochemical composition. Arugula plants were subjected to 0 mM NaCl (C), 40 mM NaCl (T2), and 80 mM NaCl (T3) solutions for 60 days throughout the 2024 growing season to determine the impact of soil salinity. Indoor growing conditions were provided for the plants in pots. Each treatment was run through three replicates. Temperature was used to estimate the early stress response of the arugula plants. The leaf's progressive reduction in temperature when exposed to salt solution is an indication of a stress reaction. Plant spectral reflectance was measured using the handheld multispectral sensor Plant-O-Meter. The Normalized Difference Vegetation Index (NDVI) and the Enhanced Vegetation Index (EVI) were the two vegetational indices (VI) that were utilized to evaluate the plant response. Significant variations between treatments for both calculated indices are revealed by the calculation of f-ratio values. When ANOVA is used for multiple comparison analysis testing, the NDVI values of T1 and T3, as well as T2 and T3, differ significantly. There are notable variations in EVI values between T1 and T2, as well as T1 and T3 treatments. These findings suggest the possible use of remote sensing tools to forecast stress in arugula by utilizing NDVI and EVI.

Key words: *arugula, NDVI, EVI, salinization, leaf temperature.*

Introduction

In contemporary agriculture, there is a rising gap between the expanding need for food and the corresponding escalating land degradation. Projections of human population show steady growth, while soil degration, of which soil salinization is a major issue, will continue to trend negatively. Contemporary agriculture production, especially irrigation and fertilization management, are one of the major factors which lead to soil salinization. Although anthropogenic influence has a remarkable effect, there are other elements that contribute to soil degradation and subsequent salinization. Salinization is predicted to get worse due to climate change, which will cause more frequent and severe droughts as well as sea level rise (Singh, 2021, Nogacz et al., 2022).

Optimization of agricultural production under salinization is necessary and can be properly implemented if the stress is detected in initial phase and mechanism of action of salt stress on plant as well as its reaction is known. According to recent studies (Nogacz et al., 2022, Vellinga et al., 2021), salinity interferes with nitrogen intake, causing growth inhibition. As a consequence, plant reproduction is halted and normal plant development and productivity are inhibited. Additionally, oxygen exchange is altered during stress. Plants eventually die when the concentration of certain ions, especially chloride, rises because of their toxicity.

Arugula (Eruca sativa), also reffered to as rocket, is green leafy vegetable from Brassicaceae family. Originating in the Mediterranean area, it is very well-liked salad ingredient (Garg & Sharma, 2014). This is supported by the fact that raw arugula has been used in salads in Italy since the Roman era. Usually, the species grows on arid, rugged soil. Traditionally, the plant was gathered from the wild or planted in household gardens alongside other herbs like basil and parsley. Nowadays, arugula is grown commercially in many locations all over the world. Apart from the leaves, edible parts include the blooms, immature seed pods, and mature seeds. Currently, thanks to its content, arugula is used as a garnish for mixed salads, where it lends a pleasant sharpness (Gulfraz et al., 2011, Komeroski et al., 2023).

With only a small quantity of fat, raw arugula reach in proteins, vitamins (A, C, and K), and fibers. It is an abundant source of vitamin folate, while magnesium, calcium, manganese, and vitamin C. Next to the role in nutrition, arugula is recognized as valuable source in medicine and pharmaceutical industry. When used as medicine, the arugula stimulates hair growth and has antibacterial, antidiabetic, antihypertensive, antiplatelet, and antioxidant properties (Gulfraz et al., 2011, Greg and Sharma, 2014).

Vegetation indices (VIs) derived from remote sensing of canopies are straightforward and effective algorithms for quantitative and qualitative assessments of vegetation cover, plant vigor, growth dynamics, and other relevant parameters. More than one hundred vegetation indices have been derived from multispectral imagery (Xue and Su, 2017). These indices facilitate the identification of nutrient deficiencies, water stress, extreme temperatures, soil coverage, diagnosis of biological parameters, and yield estimation (Andrade et al., 2022).

The Normalized Difference Vegetation Index (NDVI) is one of the earliest and most widely used indices for vegetation assessment due to its simplicity and compatibility with any multispectral sensor equipped with visible and near-IR bands (Huang et al., 2021). Mathematically, NDVI is expressed as follows:

$$NDVI = \frac{NIR - RED}{NIR + RED}$$

However, the interaction between soil and atmosphere can complicate the interpretation of NDVI, as reducing the influence of one can amplify the effects of the other. To address this issue, a feedback mechanism was introduced through the development of the Enhanced Vegetation Index (EVI), which simultaneously corrects for both soil and atmospheric effects (Xue and Su, 2017). The EVI is expressed as follows:

$$EVI = 2.5\left(\frac{NIR - RED}{NIR + 2.4RED + 1}\right)$$

The aim of this study was to investigate the early salt stress-induced response in arugula. We summarized the effect of salinization through NDVI and EVI analysis using the portable multispectral optical device Plant-O-Meter, which integrates a multispectral light source consisting of wavelengths at 850, 630, 535, and 465 nm, enabling simultaneous illumination of the entire plant (Kitić et al., 2019).

Material and Methods

The experiment represented growing arugula plants from seeds in indoor conditions in Novi Sad, Serbia (45.2396° N, 19.8227° E). At the beginning of February 2024, the arugula seeds were planted in a commercial substrate. Once the seedlings reached the height of 5 cm, they were transplanted into test pots. The seedlings were irrigated by ponding according to their specific needs until they had grown to an average height of 10 to 15 cm and had become adapted to the conditions. Following the establishment of healthy plant conditions and consistent development, solutions with varying NaCl concentrations were applied to the plants to subject them to saline stress. Treatments were applied regularly; there was an

irrigation interval of three days, while the concentrations of the saline solution were 0 (T1), 40 (T2) and 80 (T3) mM NaCl. After sixty days of exposure to salt stress, vegetation indices NDVI and EVI were evaluated using a multispectral optical device Plant-O-Meter. An infrared thermometer was used to measure the leaf's temperature simultaneously.
All statistical tests were carried out using the Statistical AnalysisSystem Statistica 6.1 version. An overall difference between sample means were tested by one-way ANOVA (for $P<0.05$). whether Tukey's HSD test was used to determine significance between the pairs of treatments.

Results and Discussion

This study investigated how different treatments affected NDVI values in arugula under early salt stress conditions. Analysis of variance revealed significant variations among treatments ($F = 18.47109$, $p < 0.05$, Table 1). Further pairwise comparisons showed that NDVI values did not significantly differ between treatments T2 and T2 ($Q = 0{,}022$, Table 2), suggesting similar responses. In contrast, significant differences were observed between T1 and T2 ($Q = 0.66$), as well as T1 and T3 ($Q=0{,}64$), indicating distinct effects of treatment T3 on vegetation indices. These results underscore NDVI's sensitivity as an indicator of salt-induced stress in arugula, providing valuable insights into treatment-specific physiological responses. Similar results were obtained in Aldakheel (2011) study, where lower NDVI values may correspond to high electrical conductivity of the soils, suggesting that soil salinity impacts vegetation health, similar to the treatment-induced variations in NDVI observed in our study. Indices based on chlorophyll absorption bands and the NIR region, such as NDVI, had a strong relation with soil salinity because these indices are related to the growth of plants, biomass, leaf area and chlorophyll content (Zhang et al., 2011).

Table 1. Summary of one-way analisys of variance performed on parameters NDVI, EVI, and leaf temperature of basil subjected to 0 (T1), 40 (T2) and 80 (T3) mM NaCl solutions ($P < 0.05$)

	One-way ANOVAtesting (df=2)	
Evaluated parameters	F- statistics	p-value
NDVI	18.471	1.85E-07
EVI	34.124	8.13E-10
Leaf temperature	3.985	0.02212

Similarly, the study explored the response of Enhanced Vegetation Index (EVI) values in arugula under various treatments subjected to early salt stress. Analysis of variance revealed significant differences among treatments ($F = 34.124$, $p < 0.05$, Table 1), highlighting diverse effects on EVI across experimental conditions. Pairwise comparisons (Table 2) revealed that treatment T1 exhibited significantly higher EVI values compared to both T2 (mean difference = 0.064, $p < 0.05$) and T3 (mean difference = 0.045, $p < 0.05$), indicating a heightened response to salt stress. Conversely, no significant difference was found between T2 and T3 (mean difference = 0.019), suggesting similar EVI responses under these treatments. These findings underscore EVI's sensitivity in detecting early physiological changes induced by salt stress in arugula, emphasizing its utility in evaluating treatment-specific plant responses in controlled agricultural environments. Lobell et al. (2010) concluded that EVI is a more reliable measure of vegetation condition and, consequently, salinity, compared to NDVI.

Table 2. Difference between means of treatments 0 (T1), 40 (T2) and 80 (T3) mM NaCl tested by multiple comparison test (Tukey HSD test, P < 0.05).

	NDVI		EVI		Leaf temperature	
Pair	Q	p-value	Q	p-value	Q	p-value
T1-T2	0,66	1.52E-07	0.064	8.13E-10	0.66	0.0451
T1-T3	0,64	0.0002126	0.045	1.45E-09	0.67	0.0400
T2-T3	0.022	0.1706	0.019	0.9666	0.01	0.9986

This study analyzed temperature variation among treatments. A total of 90 observations were distributed across three treatments. Mean temperatures were 22.1°C (T1), 21.8°C (T 2), and 21.5°C (T3), with corresponding standard deviations of 0.8909, 0.9852, and 0.7018°C, respectively. Analysis of variance revealed a sum of squares (SS) between treatments of 3.926 with 2 degrees of freedom (df), resulting in a mean square (MS) of 1.963 and an F-ratio of 2.60934 (Table 1). The associated p-value was 0.079335, indicating a trend towards significance at the $p < 0.05$ level. These results suggest potential treatment effects on temperature variation. Tian et al. (2020) concluded in their study that plants subjected to salt stress exhibited significantly higher leaf temperatures compared to the control group. These findings underscore the importance of monitoring leaf temperature as an indicator of plant responses to stressful conditions.

Conclusions

The arugula's early stress response was estimated using temperature. When a leaf is exposed to salt solution, its temperature gradually drops, which is a sign of a stress reaction. Plant-O-Meter, a portable multispectral sensor, was used to detect the spectral reflectance of plants. The two vegetational indices (VI) that were used to assess the plant response were the Enhanced Vegetation Index (EVI) and the Normalized Difference Vegetation Index (NDVI). The F-ratio values computation reveals significant differences between treatments for both derived indices. The ANOVA multiple comparison analysis highlighted significant difference in the NDVI values of T1 and T3, as well as T2 and T3. EVI values differ significantly between T1 and T2, as well as between T1 and T3 treatments. These results imply that NDVI and EVI could be used as remote sensing metrics to predict stress in arugula.

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EFFECTS OF NITROGEN FERTILIZATION ON THE YIELD OF TRADITIONAL VARIETIES AND LANDRACES OF WINTER WHEAT

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Abstract

The intensification of agriculture and modern breeding activities have reduced biological diversity and led to the nearly-complete elimination of old varieties and local landraces from farms. The aim of this study was to examine the effect of nitrogen application on the main yield components and grain yield of five old, traditional wheat landraces and varieties. The experiment was conducted as a two-factorial trial with a randomized treatment design. The following factors and treatments were examined: five winter wheat varieties - Stara Banatka, Bezostaya 1, Bankut 1205, Crnozrna, and Brkulja 4; and nitrogen fertilization with two treatments: a) control (N_0) and b) nitrogen fertilization in top-dressing based on N-min analysis (N_{109}). For all analyzed traits, the analysis of variance showed that the varieties had the largest contribution to their variability, suggesting significant genetic differences among the varieties, which can be attributed to their different origins and varietal specificity. The Crnozrna variety produced the highest grain mass per spike while the variety Bezostaya 1 had the highest 1000-grain weight. The variety Bezostaya 1 produced the highest grain yield on average. Overall, significantly higher values of yield, and yield components were achieved in the nitrogen fertilization treatment compared to the unfertilized treatment. The yield difference in favor of the fertilized treatment ranged from 2% for the variety Crnozrna to 11% for the variety Bankut 1205.

Keywords: *winter wheat, old varieties, landraces, nitrogen, yield components, yield.*

Introduction

Old, traditional wheat varieties and local landraces are considered significant sources of natural variability in important agronomic and nutritional traits, some of them with good tolerance to abiotic stress factors (Mirosavljević et al., 2021) and good ability to remobilize nutrients to the grain (Mikić et al., 2023). Their diversity can have substantial advantages in unfavorable agroecological conditions and represent an alternative to modern varieties due to better adaptability to extensive production conditions. The value of local landraces and old wheat varieties is recognized primarily from a nutritional perspective. Modern varieties, compared to old varieties and landraces, often have lower protein and mineral element content. The content of carotenoids and micronutrients (Fe and Zn) is particularly low in the grains of modern varieties. Newton et al. (2011) and Aćin et al. (2023) highlighted that local landraces and old varieties are the richest sources of micronutrients, antioxidants, carotenoids, vitamins, and other specific bioactive components, thus playing an important role in the prevention of numerous diseases. Živančev et al. (2023) noted that landraces and old varieties of bread wheat, as well as einkorn, emmer, durum, and spelt wheat, have proven to be excellent sources of tocopherols, Fe, and Zn. Local landraces, old varieties, and varieties intended for organic farming have shown relatively high protein yields under extensive

production conditions and in less favorable agroecological conditions but exhibit low performance in favorable agroecological conditions and intensive production systems (Baresel et al., 2008).
Considering that old wheat varieties and landraces used to be cultivated in farming systems with very little inputs, they could meet the present needs for ecological, sustainable, integrated, and organic production, especially on marginal lands with poor soil fertility, especially in nitrogen, in less favorable agroecological conditions and under extensive production conditions. Especially in the context of climate change and the trend toward more sustainable agricultural production systems, there is a need for the availability and use of a broader collection of genetic resources. According to Newton et al. (2011), old varieties and landraces are a very valuable but underutilized resource in modern crop production. The overall improvement of agriculture, as well as selection and breeding, has led to a constant increase in wheat yields over the years. This has been accompanied by an increased need for higher amounts of mineral nutrients in high-yielding varieties to fully express their genetic potential in intensive farming (Jaćimović et al., 2023). However, the intensification of agriculture, characterized by high chemical application, as well as modern breeding activities, has not only reduced the biological diversity of cultivated species but also led to the near-complete neglect of old, traditional varieties and local landraces. Due to the ongoing trend of reintroduction old wheat varieties and landraces, and reducing the reliance of wheat production on nitrogen fertilizers, it is necessary to re-examine their agro-morphological and productive traits, as well as their response to basic agronomic practices in trials with no and with optimal nitrogen input. Therefore, the aim of this study was to compare performances and examine the effect of nitrogen fertilization omission, as well as its application in top-dressing, on the main yield components and grain yield of five old, traditional winter wheat landraces and varieties.

Material and Methods

The research was conducted as part of the FAO project GRAINEFIT led by the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The project is focused on the conservation and sustainable use of neglected small grains genetic resources in the light of climate change. In addition, the aim of the project is to support small farms in achieving stable production and obtaining safe and high-quality grain products. The experiment was conducted during the 2022/2023 growing season, at the Agricultural school in Bačka Topola (45°47'34"N 19°35'52"E). Agro-ecological conditions are typical for the Pannonian region, with continental climate characterized by considerable temperature variations, and in recent decades, hot summers, more and more often mild winters, irregular rainfall distribution and frequent drought events. The weather data for the growing season 2022/2023 in Bačka Topola were shown on Figure 1.

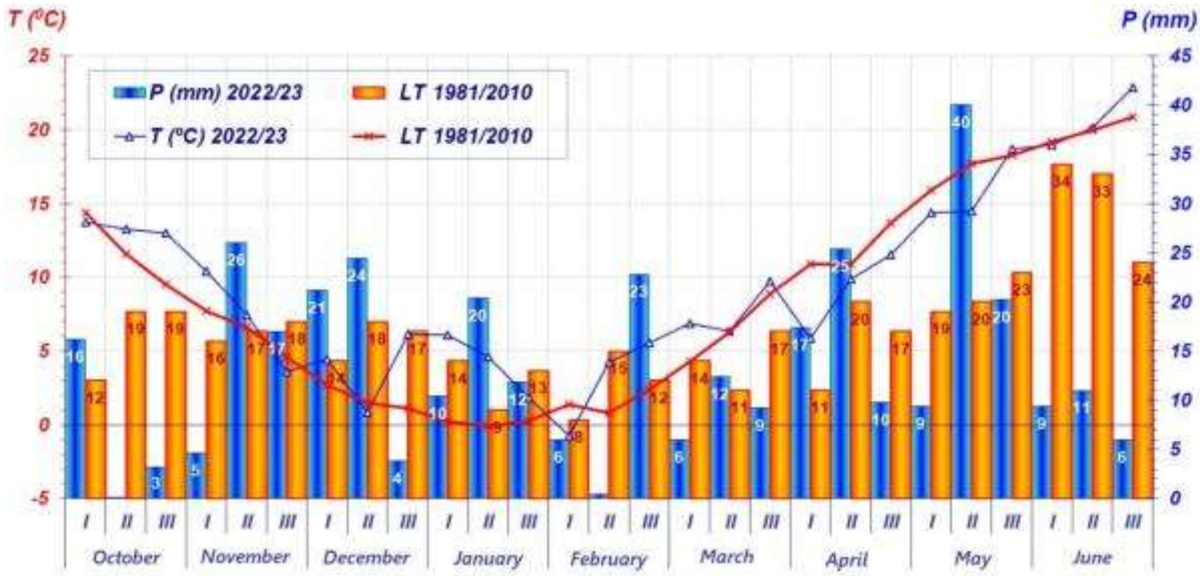

Figure 1. The average temperatures (T) and precipitation (P) compared to their long-term averages (LT) per 10-day periods from October 2022 to June 2023 in Bačka Topola

The field trial area was on meadow soil, which had been cultivated without fertilizers application for years. The soil type on the experimental plot was chernozem, formed on a loess terrace. Agrochemical analyzed determined the soil quality, as well as the mineral nitrogen content by soil profile depth, to determine the fertilization needs for the selected wheat landraces and varieties. Soil analysis for basic agrochemical properties was performed in the fall of 2022 for determination of the necessary amounts of P and K. For determination of the mineral N content in the soil according to the N-min method, soil samples were taken using a manual probe in February 2023 at layers of 0-30 cm, 30-60 cm, and 60-90 cm. Based on the soil analysis results (Table 1), the soil was slightly alkaline. According to the $CaCO_3$ content, the soil belonged to the class of highly calcareous soils, while based on humus and total N content, it was moderately humic and moderately supplied with total N. The content of available P and K was very high, so there was no need for fertilization with these elements.

Table 1. Results of basic agrochemical properties of soil testing

Agrochemical properties of soil							
Depth	**pH**		**$CaCO_3$**	**Humus**	**Total N**	**AL-P_2O_5**	**AL-K_2O**
(cm)	in KCl	in H_2O	(%)	(%)	(%)	(mg/100 g of soil)	
0-30	7.60	8.11	12.89	3.04	0.180	84.84	57.20

The results of the determination of mineral nitrogen content and distribution in the soil according to the N-min method are presented in Table 2. The total amount of mineral N in the 0-90 cm layer was 52.14 kg ha^{-1}, which served as the basis for calculating the required amount of N for top-dressing. Based on the standard calculation, it was determined that 109 kg of active N ha^{-1} was needed for top-dressing wheat on the experimental plot. It was decided to split the calculated amount into two top-dressing applications in a ratio of 70% (basic dose of N): 30% (corrective top-dressing), i.e., 76 : 33 kg of a.m. of N ha^{-1}. Given the very low mineral N content in the surface soil layer (12.90 kg ha^{-1} in the 0-30 cm layer), the top-dressing of wheat was carried out using a fast-release AN fertilizer (34% N).

Table 2. Results of mineral nitrogen (N-min) and soil moisture content analysis

Soil depth	**N-min** (kg N ha^{-1})	**Soil moisture** (%)
0-30 cm	12.90	25.6
30-60 cm	16.93	25.5
60-90 cm	22.31	23.9
Sum:	**52.14**	**75.07**

The experiment was conducted as a two-factorial, using a split-plot design with four replications and a randomized arrangement of treatments. The following factors and treatments were examined: Factor A - winter wheat varieties: S1 - Stara banatka, S2 - Bezostaya 1, S3 - Bankut 1205, S4 - Crnozrna, and S5 - Brkulja 4 (seeds obtained from the collection of the Institute of Field and Vegetable Crops); and Factor B: nitrogen fertilization in top-dressing, with two treatments: a) control treatment without N fertilization (N_0) and b) nitrogen fertilization in top-dressing based on soil N-min analysis (N_{109}).
After primary tillage (plowing to a depth of 25 cm) and pre-sowing soil preparation, sowing of the selected wheat varieties and landraces was performed on October 19, 2022. Sowing was done using a mechanical grain drill with a row spacing of 12.5 cm and a seeding rate of 500 viable seeds per m². Each variety was sown on an area of 30 m² (3 m width × 10 m length), with isolation paths of 1 m between varieties. Each main plot (i.e., variety) was divided lengthwise into two subplots: unfertilized (N_0) and fertilized with nitrogen in top-dressing based on N-min analysis (N_{109}). Each subplot was further divided into four replications, each measuring 1.5 m × 2.5 m. In the treatment planned to examine the effects of N fertilization, top-dressing of all varieties was performed twice: on February 18, 2023, at the tillering stage (base dose; 76 kg N ha^{-1}), and on March 9, 2023, before stem elongation phase (corrective top-dressing; 33 kg N ha^{-1}). The only maintenance measures applied were keeping the isolation paths and manual weed control. Harvesting of all experimental plots was carried out in late June 2023. The yields of dry grain (13% moisture) per plot were converted to t ha^{-1}. The effects of the analyzed factors and their interactions on yield components and grain yield of the examined varieties and landraces were processed using analysis of variance (ANOVA) for a two-factorial experiment conducted in a split-plot design with randomized treatment arrangements (GenStat for Windows 12th ed.). The statistical significance of treatment mean differences was determined by Fisher's Least Significant Difference (LSD) test at a significance level of 5%.

Results and Discussion

The analysis of variance for grain weight per spike, 1000-grain weight, and yield (Table 3) showed that both the analyzed varieties and nitrogen fertilization had a statistically highly significant effect on the total variability of all traits. The interaction effect of varieties and top-dressing was highly significant for grain weight per spike and grain yield, and significant for 1000-grain weight. Analysis of the contribution of individual sources of variation to the total sum of squares revealed that the varieties dominated with the largest share in the variability of all three traits, suggesting that there is a significant difference in the genetic potential for yield among the analyzed varieties, which can be attributed to their different origins and the presence of varietal specificity. The overall contribution of nitrogen fertilization and the interaction of varieties and fertilization was very low but still significant, indicating that N top-dressing also influenced the modification of the examined traits.

Table 3. Key indicators from the analysis of variance for grain weight per spike, 1000-grain weight, and grain yield in the experiment[1]

Sources of variation	d.f.	Grain weight per spike			1000-grain weight			Grain yield		
		SS (%)	F	F-pr.	SS (%)	F	F-pr.	SS (%)	F	F-pr.
Replications	3	0.3	1.04	-	0.8	1.62	-	0.5	2.12	-
Varieties (A)	4	90.1	206.24	<0.001	90.6	145.61	<0.001	94.1	279.99	<0.001
Fertilization (B)	1	3.1	28.30	<0.001	2.5	16.38	<0.001	1.2	14.81	<0.001
A × B	4	3.5	8.03	<0.001	1.9	3.12	0.031	1.9	5.65	0.002
Error	27	2.9	-	-	4.2	-	-	2.3	-	-
Total	39	100	-	-	100	-	-	100	-	-

[1] **d.f.** - degrees of freedom; **SS (%)** - sum of squares (expressed as % of the total); **F -** F-test from the analysis of variance; **F-pr.** - probability (significance) of the F-test

Statistically significant differences in grain weight per spike were recorded among all analyzed varieties (Table 4). The highest value for this trait (1.67 g) was achieved by Crnozrna, while Stara banatka produced the lowest grain weight per spike. On average across varieties, significantly higher grain weight per spike (1.34 g) was obtained with N top-dressing treatment compared to the control treatment (1.23 g). This pattern of variation between N treatments was observed in Bezostaya, Bankut, and Brkulja, while no significant differences were observed between the N_0 and N_{109} treatments in Crnozrna and Stara banatka. On average across both N treatments, the 1000-grain weight was highest in Bezostaya (37.6 g) and significantly higher compared to all other varieties. Brkulja and Crnozrna had lower but statistically similar 1000-grain weights, which were significantly higher compared to Stara banatka and Bankut. Across all analyzed varieties, significantly higher 1000-grain weight (31.1 g) was obtained with N top-dressing treatment compared to the control treatment (29.5 g). Higher 1000-grain weight with N top-dressing treatment was also observed for all individual varieties except for Crnozrna.

The highest grain yield was obtained from Bezostaya (3.66 t ha^{-1}), which was significantly higher compared to the other varieties (Table 4). Specifically, the grain yield of Bezostaya was 39% higher than Stara banatka, 32% higher than Bankut, 16% higher than Crnozrna, and 14% higher than Brkulja. On average across all five analyzed varieties, N-fertilization resulted in a significantly higher grain yield compared to the unfertilized treatment in the trial. The average yield difference in favor of N-fertilization was 0.11 t ha^{-1} (or 4%). Only Stara banatka showed a higher grain yield in the control (N_0) treatment, while for all other varieties, the yield was higher in the N_{109} treatment. The yield increased due to nitrogen application ranged from 2% for Crnozrna to 11% for Bankut.

Table 4. Grain weight per spike (g), thousand grain weight (g), and grain yield of traditional winter wheat varieties (t ha^{-1}) as influenced by analyzed varieties and nitrogen fertilization

Grain weight per spike (g)			
Varieties (A)	Nitrogen fertilization (B)		Average (A)
	N_0	N_{109}	
Stara banatka	0.93^d	0.89^d	**0.91^E**
Bezostaya 1	1.43^b	1.60^a	**1.52^B**
Bankut 1205	0.94^d	1.11^c	**1.03^D**
Crnozrna	1.68^a	1.67^a	**1.67^A**
Brkulja 4	1.17^c	1.41^b	**1.29^C**
Average (B)	**1.23^B**	**1.34^A**	**1.28**

1000-grain weight (g)			
Varieties (A)	Nitrogen fertilization (B)		Average (A)
	N_0	N_{109}	
Stara banatka	24.8^{de}	25.3^{de}	**25.0^{C}**
Bezostaya 1	36.7^{a}	38.5^{a}	**37.6^{A}**
Bankut 1205	23.8^{e}	26.0^{d}	**24.9^{C}**
Crnozrna	32.0^{c}	31.8^{c}	**31.9^{B}**
Brkulja 4	30.3^{c}	34.1^{b}	**32.2^{B}**
Average (B)	**29.5^{B}**	**31.1^{A}**	**30.3**
Wheat grain yield (t ha^{-1})			
Varieties (A)	**Nitrogen fertilization (B)**		**Average (A)**
	N_0	N_{109}	
Stara banatka	2.29^{f}	2.15^{g}	**2.22^{D}**
Bezostaya 1	3.56^{b}	3.76^{a}	**3.66^{A}**
Bankut 1205	2.35^{f}	2.61^{e}	**2.48^{C}**
Crnozrna	3.06^{d}	3.12^{cd}	**3.09^{B}**
Brkulja 4	3.03^{d}	3.23^{c}	**3.13^{B}**
Average (B)	**2.86^{B}**	**2.97^{A}**	**2.92**

The treatment means labeled with different letters in the index indicate the presence of statistically significant differences ($p \leq 0.05$; LSD test)

Numerous studies have established that wheat yield depends on several key components: plant density, number of spikes per unit area, number and weight of grains per spike, and thousand grain weight. There are complex relationships among these indicators, as increasing one parameter often leads to a decrease in another (Hristov et al., 2008; Jaćimović et al., 2012). The significance of each of these components in yield formation depends on weather conditions during critical growth stages (especially water stress), as well as on applied agronomic practices, particularly the quantities and ratios of NPK nutrients (Hristov et al., 2011; Jaćimović et al., 2020). Several studies have shown that wheat growth and yield increase with fertilizer application up to a certain level, beyond which additional fertilization generally has no further positive impact, and at very high nutrient levels, yield reduction may occur (Aćin et al., 2016).

Conclusions

Our study revealed that all examined traits of traditional winter wheat landraces and varieties, had the greatest contribution to their variability, suggesting substantial genetic differences among the varieties, attributable to their different origins and specific varietal characteristics. The highest value for grain weight per spike was achieved by Crnozrna, while the lowest was by Stara banatka. On average, the grain weight per spike was significantly higher in the N-fertilized treatment compared to the control treatment. The highest 1000-grain weight was recorded in Bezostaya 1. Overall, for all varieties, a significantly higher thousand grain weight was obtained in the fertilized treatment compared to the unfertilized treatment. The highest grain yield was obtained in Bezostaya 1, and it was significantly higher compared to other varieties. Stara banatka produced the lowest grain yield. On average, across all varieties, N-fertilization resulted in a significantly higher yield compared to the unfertilized treatment.

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RESULTS OF TESTING THE WORKING QUALITY OF DIFFERENT TECHNOLOGICAL-TECHNICAL SYSTEMS FOR PRECISION SOWING OF MAIZE

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Abstract

Precise sowing of maize can be done with different technological and technical systems. The quality of the system for precise maize sowing implies that the distance of the seeds in depth, length, and width should be adjusted to the crop being sown since the layer of soil covering the seeds should be uniform, the furrow should be properly prepared and the seeds should be well pressed and covered. Application of the system for precise maize sowing enables a uniform assembly of plants, increased yields, and reduced production costs. Considering the need to respect optimal agrotechnical deadlines, which are also very short, there is often a need to achieve higher work speeds during sowing to achieve better results. However, a large increase in movement speed opens up the possibility of seriously impairing the quality of work. The emphasis on this problem is understandable, especially considering that large farmers have high-quality tractors of higher categories. Three different systems for precise sowing of maize A, B, and C were represented in the tests. The goal of our research was to determine the quality of work of different systems for precise sowing of maize depending on the defined parameters in the production conditions of the observed area. Tests and evaluation of the quality of work of the tested systems were performed according to the DLG method of testing seeders and by the ISO 7256/1 and 7256/2 standards. Based on the obtained results, it was concluded that there is a significant influence of the defined parameters on the quality of the tested systems for precise sowing of maize, which is particularly pronounced in variants A and B, bearing in mind that the values of the QFI index at speeds greater than 10 km h^{-1} were significantly below 90%, while in variant C these values were in the range of 91 - 94% even at higher speeds.

Keywords: *Seeder, Precision sowing, Maize, Quality, Schedule indexes.*

Introduction

Maize represents one of the most important crops and in terms of representation in the structure of crop production, it occupies one of the leading places in the world (Akhiyarov et al., 2021; Banaj et al., 2023). In the Republic of Serbia, maize is grown on 1,324,368 ha with an average yield of 4.55 t ha-1 (Stat. Yearb. Serb. 2023). One of the most important technological operations in the production of maize is sowing since mistakes during sowing cannot be corrected later by other agrotechnical measures. The goal of precise and high-quality sowing is the correct and optimal number of plants per unit area (Loveland *et al.*, 2003; Findura *et al.*, 2009; Yazgi, 2016; Findura *et al.*, 2018). The correct layout and optimal number of plants per unit area are the basic prerequisite for successful sowing and maize production, and numerous field and laboratory methods have been developed to test the properties of seeders (Koller *et al.*, 2014; Narang *et al.*, 2015; Liu *et al.*, 2018). In the case of precise and high-quality maize sowing, a proper layout, sufficient vegetation space, manifestation of production potential, and high yields are achieved (Parish *et al.*, 2003;

Milenkovic *et al*., 2008). Therefore, it is understandable to strengthen the application of appropriate technological and technical systems for the precise sowing of maize. Precision sowing has been a major thrust of agricultural engineering research for many years; however, most of the research and development work has dealt with seeders for agronomic crops (Karayel *et al*., 2004). Precise sowing of maize achieves better use of available land, savings in seed material, avoidance of thinning after emergence, more uniform growth and development, and more uniform and higher yields (Fanigliulo *et al*., 2022; Turan *et al*., 2015). Improving the precision of sowing is impossible without knowing the principles of operation of all parts of the sowing mechanism and all the factors that influence possible sowing errors (Kostić *et al*., 2020). The quality of maize sowing is the most significant factor that affects a high and high-quality yield, and it is expressed as a percentage of the achieved sowing spacing according to ISO 7256/1 and 7256/2 standards (Bozdogan, 2008). The analysis of the operation of seed drills with different concepts of dosing-sowing at increased working speeds (10, 12, and 13 km h^{-1}) showed that these are working speeds that exceed the potential of the seeder (relatively low share of the sowing distance of 0.5 - 1.5 set on the level of 68.89 - 88.57 %). With increasing working speed, the share of empty spaces in the longitudinal distribution of maize seeds increases (up to 25.56%). A comparison of the same seeder at different working speeds (10, 12, and 13 km h^{-1}) showed a statistically significant difference. (Turan *et al*., 2011). The pneumatic seeder with negative pressure achieved a very good quality of maize sowing in field conditions, bearing in mind that in the group of 0.5 - 1.5 required distance, there were 94.34 - 97.64% of plants (QFI index), with values of open distances >1.5 of the theoretical (MISS index) in the range of 1.27 - 2.59 (Banaj *et al*., 2018). The seeder for precise sowing of maize achieved very good results in terms of the quality of sowing and the distribution of seeds in a row, which according to the ISO standard qualifies it to be classified in the group of very good seeders (Barać *et al*., 2023).

Material and Methods

Tests of the quality of work of various technological and technical systems for precision sowing of maize were carried out in production conditions in the vicinity of Pancevo, in 2024. The tests included the assessment of the longitudinal arrangement of maize when sowing with seed drills for precise sowing of maize depending on the defined parameters. A seeding trial was set up with local farmers, and maize was sown with three different types of drills A, B and C. Type A is the overpressure drill Panonija Becker Aeromat II, type B is the underpressure drill PSK 6, while type C is also was a seed drill with overpressure Vaderstad Tempo T6. All three drills were six-row, and the power required for traction of the tested drills was in the range of 45 - 90 kW according to the manufacturer's recommendation. Autumn plowing was carried out at 25 cm, and pre-sowing preparation with a seed drill at a depth of 15 cm for variants A and B, while for variant C plowing was carried out and then harrowing twice with a heavy harrow. Pioneer P9911 maize hybrid seeds with 95% germination were used for sowing so that they met the basic seed conditions in terms of germination and dimensions (Vitázek *et al*., 2006). The planned arrangement was 75,200 (type A), 74,000 (type B), or 73,000 plants ha^{-1} (type C), where the row spacing was 190 and 195 mm, respectively. All tested varieties worked under similar production conditions that were satisfactory, and sowing was done in April in the optimal agrotechnical period. Tests and assessments of the quality of work of the tested systems were performed according to the DLG method of testing seeders, following the ISO 7256/1 and 7256/2 standards. The quality of the work was evaluated based on qualitative indices depending on the work mode, namely: MULT index (multiple index) - represents the percentage share of achieved seed spacings in a row that are $\leq$0.5 cm from the theoretical spacing, QFI index (quality of feed index) -

represents the percentage share of realized seed spacing in a row that are >0.5<1.5 cm from the theoretical and MISS (miss index) which represents the percentage share of realized seed spacings in a row that are ≥1.5 cm larger than the theoretical spacing. Bearing in mind that locating the sown seeds is difficult, requires a lot of time, and that there is a high probability of seed spreading, the quality of sowing is evaluated and calculated only after the crop has sprouted, because the achieved assembly is the main factor of successful and profitable production (Raheman *et al*., 2003). The values of the movement, speed and other indicators were read in the tractor cabin from the display because the tractors were equipped with ISOBUS technology for advanced exploitation of agricultural machines and the autopilot T - TrimblGFX-1060. The objectives of the research was to determine the difference or similarity in the quality of the longitudinal distribution of seeds during sowing depending on the mode of operation and to achieve optimal seed distribution in the row. In the test, no emphasis was placed on the reliability and quality of production of sowing aggregates. All values were read in ten repetitions and the obtained results were processed using the Microsoft Office Excel 2007 package.

Results and Discussion

Tables 1 - 2 show the results of testing the quality of work of various technological and technical systems for precise sowing of maize. The obtained results indicate a significant influence of the defined parameters on the quality of work of the tested varieties, that is, on the longitudinal distribution of seeds in the row. The highest average values of the MULT-index of 5.47% (the share of achieved distances ≤0.5 compared to the theoretical) were recorded in variant B, at the movement speed of the sowing aggregate of 8.23 km h^{-1}, and the lowest of 1.62% in variant C, operating speed of 11.89 km h^{-1}. Analyzing the average achieved values of the MULT index by individual variants, it is noted that those of the type A seeder was in the range of 3.76 - 5.06%, of the type B seeder in the range of 3.54 - 5.47, while the values of this index in the variant C were within the limits of 1.62 - 2.93%, while the working speed regime for all tested variants was 11.89 and 8.23 km h^{-1}, respectively (table 1). Considering the values of the QFI index (range at the level of >0.5<1.5 compared to the theoretically expressed in %), it can also be noted that the defined parameters significantly impact the achieved values of this index.

Table 1. Longitudinal distribution in the rows by tested variants

Tested variant	Working speed [km h^{-1}]	Qualitative indicators of the longitudinal distribution of maize								
		MULT			QFI			MISS		
		≤0.5			>0.5<1.5			≥1.5		
		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
A	8.23	5.06	4.68	5.59	90.41	87.73	92.51	4.53	2.61	7.1
	10.16	4.64	3.98	5.11	85.91	81.34	90.13	9.45	5.79	14.22
	11.89	3.76	3.37	4.11	84.07	82.19	85.17	12.17	10.95	14.34
B	8.23	5.47	4.49	6.45	90.11	85.67	95.56	4.42	3.81	5.52
	10.16	4.35	2.88	10.16	80.89	79.17	82.44	14.76	12.31	17.95
	11.89	3.54	2.91	4.13	76.64	73.53	79.58	19.82	16.44	22.83
C	8.23	2.93	1.78	4.00	94.11	91.78	96.17	2.96	1.93	4.67
	10.16	2.21	1.69	3.15	92.73	90.77	94.14	5.16	3.82	7.76
	11.89	1.62	1.20	2.11	91.29	90.33	92.11	7.11	6.51	7.54

Thus, the lowest average values of 76.64% were achieved when sowing with a type B seeder at a working speed regime of 11.89 km h^{-1}, and the highest when sowing with a type C seeder averaged 94.11% (working speed of 8.23 km h^{-1}). Observed according to the examined varieties, the average values of QFI were in the range of 84.07 - 90.41% in the type A seeder, i.e. 76.64 - 90.11% in the type B seeder. In the case of the type C seeder, the values of this index were within the limits of 91.29 - 94.11% (table 1).

An important indicator of the quality of seed drills for precise sowing of maize is the MISS index, which represents the share of achieved row spacing at the level of $\geq$1.5 compared to the theoretical one, expressed as a percentage. The lowest values of the mentioned index were achieved with the type C seeder, which amounted to an average of 2.96%. and the highest in type B seeders were on average 19.82%. Evaluating the average values of the MISS index for all variants that were examined, it can be concluded that those of the type A seed drill varied within the limits of 4.53-12.17%, of the type B seeder within the limits of 4.42 - 19.82%, while the values of this index for the seeder type C were in the range of 2.96-7.11% (table 1).

Other authors report similar results in their research (Parish *et al*., 2003; Milenkovic *et al*., 2008; Bozdogan, 2008; Turan *et al*., 2011; Findura *et al*., 2012; Yazgi, 2016; Fanigliulo *et al*., 2022; Banaj *et al*., 2018; Barać *et al*., 2023).

Evaluating the quality of work of the examined variants, it can be stated that during the sowing of maize at increased working speeds, the quality of work is particularly impaired in the case of type A and B seeders, bearing in mind the relatively low values of the QFI coefficient (76.64 - 85.91%), with the high values of the MISS index, which points to the conclusion that operating speeds that are higher than 8 km h-1 exceed the capabilities of these two types of seed drills. For variant C, the QFI values were in the range of 91.29 - 94.11%, with low values of the MISS index, which, according to the ISO standard, qualifies this variant to be included in the group of very good seeders. Table 2 shows the average values of the achieved row spacing and statistical indicators on the influence of the defined parameters on the values of the achieved spacing. With all tested variants, it can be stated that by increasing the speed of movement, the deviations of the achieved distance in the row compared to the theoretical one also increased. Based on the test results shown in Table 2, it can be seen that the average values of the row spacing varied from 196.82 mm (seeder type C, working speed 8.23 km h^{-1}) to 212.26 mm (seeder type B, working speed 11.89 km h^{-1}).

Table 2. Achieved distance according to the investigated variant statistics indicators

Tested variant	Working speed [km h^{-1}]	Theoretical sowing distance [mm]	Achieved sowing distance [mm] Statistical indicators					Lsd	
			Mean	σ	*Cv*	*Min*	*Max*	5%	1%
A	8.23	190	197.55	14.26	4.02	180.51	222.63	4.17	5.75
	10.16	190	201.23	12.38	5.86	189.76	224.14		
	11.89	195	208.01	15.09	7.92	189.52	233.03		
B	8.23	190	198.83	6.26	3.89	191.23	208.14	4.52	6.23
	10.16	190	201.15	8.60	4.87	189.34	214.11		
	11.89	195	212.26	12.81	6.93	196.85	235.19		
C	8.23	190	196.82	5.18	2.63	191.13	204.16	2.75	3.62
	10.16	190	200.68	8.15	4.09	187.56	211.33		
	11.89	195	202.04	3.82	1.91	195.67	207.43		

The increase in the working speed resulted in an increase in the sowing distance with an increase in the standard deviation of all tested varieties, which leads to the conclusion that with an increase in the working speed of the sowing aggregates, the quality of sowing decreases. This is particularly pronounced in the case of variants A and B, bearing in mind that the variations in the average row spacing are more pronounced. In the case of variant C, the variations in the average values of the maize spacings in the row are less pronounced compared to the first two investigated variants (table 2).

The results of our research correlate with the other authors' findings (Findura *et al*., 2009; Turan *et al*., 2011; Narang *et al*., 2015; Turan *et al*., 2015).

Conclusions

Based on the obtained results, it can be concluded that there is a significant influence of the defined parameters on the quality of work of the examined types of seeders, that is, on the longitudinal distribution of maize in the row. The highest average MULT-index values of 5.47% (proportion of achieved gaps $\leq$0.5 compared to the theoretical) were recorded with variant B and the lowest of 1.62% with variant C. The lowest average values of the QFI index (gap in line at the level >0.5<1.5 concerning the theoretical expressed in %) of 76.64% were achieved when sowing maize with type B seeder, and the highest when sowing with type C seeder, on average 94.11%. The lowest values of the MISS index (percentage share of achieved row spacing at the level $\geq$1.5 compared to the theoretical) were achieved with the type C seeder and amounted to an average of 2.96%. and the highest in type B seeders were on average 19.82%. The increase in working speeds led to a deterioration in the quality of work, especially for seed drills type A and B, bearing in mind the relatively low values of the QFI coefficient (76.64 - 85.91%), with high values of the MISS index, which indicates the conclusion that working speeds that are greater than 8 km h^{-1} exceed the capabilities of these two types of seeders. With the type C planter, the QFI values were in the range of 91.29 - 94.11%, with low values of the MISS index, which qualifies this variant according to the ISO standard to be included in the group of very good planters. The average values of the row spacing varied from 196.82 mm for type C seeders to 212.26 mm for type B seeders. An increase in the working speed resulted in an increase in the sowing distance with an increase in the standard deviation.

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SERBIAN TOMATO PRODUCTION GROSS MARGIN ANALYSIS: ECONOMIC VIABILITY AND SUSTAINABILITY OF OPEN-FIELD VS. GREENHOUSE

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Abstract

The tomato production sector in Serbia is increasingly shifting towards greenhouse cultivation. This study evaluates the economic viability and sustainability of both open-field and greenhouse tomato production through a comprehensive gross margin analysis, while also considering environmental and food safety implications. Data from 211 tomato farms over four years (2020-2023) showed that greenhouse production achieved significantly higher gross margins (32,227 €/ha) compared to open-field cultivation (24,098 €/ha). In 2023, greenhouse production's gross margin was 44,749 €/ha, while open-field was 15,231 €/ha. However, the economic advantage of greenhouse production is partially offset by environmental concerns, particularly microplastic pollution from greenhouse materials, with mitigation costs ranging from 430 to 850 €/ha annually. Additionally, food safety issues arise from pest and disease management, requiring improved pest management strategies to prevent pest outbreaks and ensure compliance with maximum residue levels (MRLs) for pesticides. This research provides a holistic assessment of tomato production systems in Serbia, integrating economic, environmental, and food safety considerations. The findings suggest that while greenhouse production offers superior economic returns, its long-term sustainability hinges on addressing environmental concerns and optimizing pest management. Future research should focus on developing sustainable greenhouse materials, improving pest management strategies, and conducting long-term profitability analyses that account for fixed costs and environmental impacts. These insights are valuable for policymakers, farmers, and researchers in the agricultural sector.

Keywords: *Tomato production, Gross margin analysis, Greenhouse cultivation, Open-field farming, Microplastic pollution, Integrated pest management.*

Introduction

Tomato production is a crucial component of Serbia's agricultural sector, with both open-field and greenhouse cultivation methods being employed. Recent years have seen a shift towards greenhouse production due to higher yields and extended growing seasons. However, this transition raises questions about economic viability, environmental sustainability, and food safety.

Stanković et al. (2015) and Filipović at al. (2015, 2016, 2017) provided a comprehensive framework for analyzing gross margins in Serbian agriculture, highlighting the importance of this metric in assessing farm profitability. Building on this work, Filipovic et al. (2017) examined the economic efficiency of different vegetable production systems in Serbia, emphasizing the role of technology adoption in improving farm profitability. Krnjajić et al. (2023) and Grujić et al. (2024) further explored the environmental implications of intensive vegetable production, focusing on soil health and the potential for sustainable intensification

in Serbian agriculture. Their work provides valuable insights into the long-term sustainability of different production systems.
Our study aims to:

- Compare the gross margins of open-field and greenhouse tomato production in Serbia.
- Analyze trends in tomato farming economics over the past five years (2020-2023).
- Assess the environmental impact of greenhouse production, particularly regarding microplastic pollution and soil health.
- Evaluate food safety concerns related to tomato pests and diseases.
- Provide recommendations for the sustainable and economically viable development of the sector.

Materials and Methods

Study Design: This study employed a mixed-methods approach combining quantitative economic analysis with qualitative assessment of environmental impacts and food safety considerations. The research was conducted over a five-year period from 2019 to 2023, focusing on tomato production in Serbia's major agricultural regions.

Data Collection
Economic Data

- *Farm-level data*, data were collected from 211 tomato farms over a four-year period (2020-2023) across Serbia, especially in Belgrade, Smederevo, and Leskovac. This included information on variable costs, yields, and market prices.
- *Market data*: Daily wholesale prices of tomatoes were obtained from the three largest agricultural markets in Serbia (Belgrade, Smederevo, and Leskovac).
- *Input costs*: Prices of major inputs (fertilizers, pesticides, seeds, energy) were collected from five leading agricultural supply companies in Serbia.

Environmental Data

- *Microplastic pollution*: Soil samples were collected from 50 greenhouse farms and 50 adjacent non-farm areas.
- *Water quality*: Water samples were collected from irrigation sources and runoff points at 30 greenhouse farms bi-annually.
- *Soil health indicators*: Following the methodology outlined by Grujić et al. (2024), we assessed soil organic matter content, microbial biomass, and enzyme activities in both open-field and greenhouse production systems.

Food Safety Data

- *Pest and disease incidence*: Data were collected through farm surveys and regular monitoring (Krnjajić et al., 2023).
- *Pesticide usage and residue*: Data were obtained through farm records.

Data Analysis
Gross Margin Analysis
Following Filipović at al. (2015, 2016, 2017) approach and incorporating refinements for vegetable production, gross margins were calculated as:

Gross Margin = Total Revenue – Variable Costs

Variable costs included seeds, fertilizers, pesticides, seasonal labor, and energy costs. For greenhouse production, additional costs such as plastic replacement and heating were included.

Environmental Impact Analysis:
Microplastic quantification in soil and water samples was performed using density separation. Soil health indicators were analyzed following Grujić et al. (2024) protocols.

Statistical Analysis:
Time series analysis was used to examine trends in gross margins over the five-year period. Analysis of variance (ANOVA) was employed to compare gross margins between open-field and greenhouse production.

Results and discussion

Gross Margin Analysis

Open-field Production

- *Average yield*: 20 t/ha
- *Average selling price*: 60 RSD/kg
- *Total Revenue*: 1,200,000 RSD/ha
- *Variable Costs*: 500,000 RSD/ha
- *Average Gross Margin*: 700,000 RSD/ha

Greenhouse Production

- *Average yield*: 100 t/ha
- *Average selling price*: 70 RSD/kg
- *Total Revenue*: 7,000,000 RSD/ha
- *Variable Costs*: 3,500,000 RSD/ha
- *Average Gross Margin*: 3,500,000 RSD/ha

Trends (2019-2023)

- Greenhouse gross margins increased by 15% over the five-year period.
- Open-field gross margins decreased by 5% due to rising input costs and climate variability.
- The gap between greenhouse and open-field gross margins widened from 400% in 2019 to 500% in 2023.

Environmental Impact

- Estimated annual microplastic release from greenhouses: 25-50 kg/ha
- Microplastic mitigation costs reduced greenhouse gross margins by approximately 50,000 - 100,000 RSD/ha annually.
- Soil health indicators showed a 15% decrease in soil organic matter and a 20% reduction in microbial biomass in greenhouse soils compared to open-field soils, consistent with findings by Grujić et al. (2024).

Food Safety Concerns

- Major pests identified: *Tuta absoluta, Trialeurodes vaporariorum, Tetranychus urticae*
- Common diseases: Late blight (*Phytophthora infestans*), Early blight (*Alternaria solani*)
- Pesticide residues were generally within acceptable limits, but 5% of samples exceeded maximum residue levels (MRLs) for certain pesticides.

Economic Implications
Our results, consistent with Filipović at al. (2015, 2016, 2017) findings in other agricultural sectors, demonstrate that greenhouse tomato production in Serbia generates significantly higher gross margins compared to open-field cultivation. The 500% difference in gross

margins by 2023 explains the ongoing shift towards greenhouse production. However, it's crucial to note that gross margin analysis does not account for the higher initial investment costs associated with greenhouse construction. Filipović at al. (2015, 2016, 2017) emphasized the importance of considering these fixed costs when evaluating long-term profitability.

Environmental Considerations

The environmental impact of greenhouse production, particularly microplastic pollution, presents a growing challenge. The costs associated with mitigating microplastic pollution (50,000 - 100,000 RSD/ha annually) reduce the gross margin advantage of greenhouse production. This aligns with Filipović at al. (2015, 2016, 2017) observations on the increasing importance of environmental costs in agricultural production. The environmental impact of greenhouse production extends beyond microplastic pollution. Our findings on soil health, corroborating Grujić et al.'s (2024) research, indicate that intensive greenhouse cultivation may lead to soil degradation over time. This poses a long-term sustainability challenge that needs to be addressed through improved soil management practices.

Food Safety Implications

The management of pests and diseases in tomato production has significant implications for both gross margins and food safety. While greenhouse production offers better control over environmental conditions, the intensive use of pesticides poses risks of pesticide residue buildup, which can threaten food safety. Our findings highlight the need for improved integrated pest management (IPM) strategies in greenhouse production to mitigate these risks. Filipović at al. (2015, 2016, 2017) emphasized the importance of maintaining consumer trust through food safety compliance, which is critical for the long-term sustainability of the tomato production sector.

Conclusion

Greenhouse tomato production in Serbia offers substantial economic advantages over open-field cultivation, with higher gross margins and the potential for year-round production. However, these benefits are offset by significant environmental and food safety concerns, particularly related to microplastic pollution and pesticide residue management. Addressing these challenges is essential for the long-term sustainability of greenhouse tomato production in Serbia. Future research should focus on developing sustainable greenhouse materials, improving pest management strategies, and conducting long-term profitability analyses that account for fixed costs and environmental impacts. Policymakers should consider incentivizing environmentally sustainable practices and supporting farmers in transitioning to more sustainable production systems.

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SERBIA AGRICULTURE SECTOR TRANSFORMATION DUE TO GLOBAL, CLIMATE AND EU CHALLENGES

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Abstract

The Serbian agriculture sector is undergoing a crucial transformation due to global climate change and the demands of European Union (EU) integration. This shift requires comprehensive strategies for climate adaptation and mitigation, aligned with EU agricultural policies, to ensure sustainability, competitiveness, and economic benefits. Serbian agriculture, a significant contributor to employment and GDP, is highly vulnerable to climate variability and extreme weather events, threatening productivity and food security. To address these vulnerabilities, Serbia must adopt sustainable practices, improve regulatory frameworks, and leverage EU support. Aligning with EU standards is essential for mitigating climate risks and meeting the quality and safety standards necessary for market access. Key areas for attention include climate vulnerability, as Serbia faces increasing extreme weather events, and the need for improved agricultural practices, such as advanced technologies and sustainable farming techniques. Additionally, Serbia's regulatory frameworks require significant enhancement to meet EU standards, particularly in sustainability, food safety, and environmental protection. This paper outlines strategies for aligning with EU policies and climate adaptation, emphasizing sustainable agricultural practices like crop diversification, water management, and the use of climate-resilient crops. The benefits of this alignment include improved food security, better resource management, and increased environmental protection, contributing to the long-term sustainability and resilience of the agriculture sector. Key recommendations focus on government support, international cooperation, and sustainable development through innovation and modernization.

Key words: *Agriculture, transformation, climate change, agricultural practices, development.*

Introduction

The agricultural sector in Serbia holds a critical position within the national economy, employing approximately 690,000 individuals, which constitutes about 21% of the total labor force. Agriculture contributes around 11.9% to the GDP and occupies about 66% of Serbia's total land area, with approximately 5 million hectares dedicated to agricultural use. The rural population, which constitutes half of the country's population, depends significantly on agricultural activities. The agricultural landscape in Serbia is characterized by a large number of small farms, with around 400,000 farms, most of which are less than 5 hectares in size. Only about 100,000 of these farms operate on a commercial basis, holding 46% of the arable land. This fragmented land ownership presents challenges for large-scale agricultural productivity and modernization. It is important to emphasize Serbia's agricultural dependency and vulnerability to climate change and importance of aligning with EU regulations and standards to ensure sustainable agricultural practices and market competitiveness. The diverse agro-climatic zones across Serbia significantly influence its agricultural production. The northern region of Vojvodina, known for its fertile soils and large arable plots, primarily produces maize, cereals, sugar beet, and sunflowers, alongside substantial milk and pig farms.

Central Serbia, with its hilly terrain, supports mixed production, including high-value crops like fruits, berries, and vineyards. The southern mountainous areas are more suited for extensive and semi-extensive ruminant production.
However, the Serbian agricultural sector faces numerous challenges. The pressure on natural resources, climate change impacts, and the need for sustainable practices are critical concerns. The EU's Common Agricultural Policy (CAP) has introduced various environmental and sustainability requirements that Serbia, as an EU candidate country, must adopt. This includes the "Nitrates Directive" and "Cross-compliance" measures, which mandate farmers to adhere to environmental protection, food safety, and animal welfare standards to qualify for subsidies.
Climate change poses a significant risk to Serbian agriculture, with increasing incidents of droughts, floods, and extreme weather events causing physical damage and financial losses. Effective management of these risks requires the adoption of advanced agricultural practices, such as improved irrigation systems, sustainable crop rotations, and climate-resilient farming. Furthermore, the introduction of innovations and technologies to enhance resource efficiency and productivity is crucial. In terms of agricultural output, Serbia is a net exporter, with agricultural exports valued at €1.5 billion in 2019, contributing to 20% of the country's total exports. Key export products include fruits (plums, apples, grapes, and berries) and vegetables (such as potatoes, cabbages, melons, peppers, and tomatoes). Despite this, the sector's productivity is hampered by issues: water quality, air pollution, and low irrigation intensity.
To align with EU standards and address climate-related challenges, Serbia's agricultural policies emphasize modernization and sustainability. This includes public spending on research and development, construction of new irrigation systems, adaptation of agricultural technologies, and increased use of renewable energy sources. Additionally, Serbia's biodiversity strategy and the National Action Plan for Biodiversity and Climate Change outline measures to protect vulnerable ecosystems and enhance the resilience of agricultural production to climate shocks. The alignment with EU policies, supported by the Instrument for Pre-Accession Assistance (IPA) and IPARD program, provides financial and technical assistance to Serbian producers, processors, and local communities. These efforts are aimed at achieving sustainable agricultural growth and resilience in the face of global, climate, and EU challenges.

Materials and methods

This paper employs a mixed-methods approach, integrating quantitative and qualitative data to provide a comprehensive analysis of the Serbian agricultural sector’s transformation in response to global, climatic, and EU challenges.
Literature Review and Policy Analysis: A thorough review of relevant literature, policy documents, and strategic plans was conducted to understand the current state and challenges of Serbian agriculture. Key documents analyzed include Serbia's Nationally Determined Contribution (NDC) for 2021-2030, the Stabilization and Association Agreement with the EU, and various national agricultural and environmental policies.
Sectoral Analysis: The agricultural sector was divided into key sub-sectors, including field crops, fruits, vegetables, and livestock. For each sub-sector, production dynamics, risk factors, and climate impacts were examined. This involved analyzing data on crop yields, livestock productivity, and incidences of pests and diseases.
Risk Assessment: A risk assessment framework was developed to evaluate the vulnerability of different agricultural sub-sectors to climate-related risks. This included identifying the most significant risks such as droughts, floods, extreme temperatures, and the emergence of new pests and diseases. The impact of these risks on crop yields, livestock health, and overall agricultural productivity was quantified where possible.

Stakeholder Consultation: Input from key stakeholders, including agricultural producers, policy makers, and researchers, was gathered through interviews and consultations. This provided insights into the practical challenges faced by the agricultural sector and the feasibility of proposed adaptation and mitigation measures. The combined use of these methods provides a robust and multidimensional understanding of the transformative pressures facing Serbian agriculture and the strategies needed to mitigate risks and enhance resilience.

Results and discussion

The results of this paper provide a detailed analysis of the Serbian agricultural sector's vulnerabilities, opportunities, and the necessary measures to align with EU standards, Nationally Determined Contributions (NDCs), Sustainable Development Goals (SDGs), and the EU Green Deal. The findings are organized into three main sections: risks, possibilities, and alignment with international and EU frameworks.

Risks to the Serbian Agricultural Sector

Climate Change Vulnerability - Serbia’s agricultural sector is highly vulnerable to the adverse impacts of climate change. The analysis of historical climate data and future projections reveals several significant risks:

- **Increased Temperature**: Rising temperatures, particularly during the growing season, are expected to negatively impact crop yields. Heat stress can reduce the productivity of both crops and livestock, leading to lower yields and increased mortality rates in animals.
- **Erratic Precipitation Patterns**: Changes in precipitation patterns, including more frequent and intense droughts and floods, pose a severe threat to agricultural productivity. Droughts can lead to water scarcity, affecting both crop and livestock production, while floods can cause soil erosion and damage to infrastructure.
- **Extreme Weather Events**: The frequency and intensity of extreme weather events, such as storms and hail, are projected to increase. These events can cause significant physical damage to crops and agricultural infrastructure, leading to substantial economic losses.
- **Pests and Diseases**: Climate change is likely to alter the distribution and prevalence of agricultural pests and diseases. Warmer temperatures and increased humidity can create favorable conditions for the proliferation of pests and pathogens, further threatening crop and livestock health.

Possibilities for Adaptation and Mitigation

Adoption of Sustainable Practices

To mitigate the risks posed by climate change and align with the EU Green Deal and SDGs, Serbia must adopt sustainable agricultural practices. The following measures are critical:

- **Climate-Smart Agriculture**: Implementing climate-smart agricultural practices, such as crop diversification, agroforestry, and conservation tillage, can enhance resilience and productivity. These practices help to maintain soil health, improve water use efficiency, and reduce greenhouse gas emissions.
- **Improved Irrigation Systems**: Upgrading irrigation infrastructure to more efficient systems, such as drip or sprinkler irrigation, can help to conserve water and ensure more reliable crop production during periods of drought.
- **Soil and Water Management**: Enhancing soil and water management practices, including the use of organic fertilizers and cover crops, can improve soil fertility and structure, increase water retention, and reduce the risk of erosion.

- **Agroecological Practices**: Promoting agroecological approaches, which integrate ecological principles into agricultural systems, can help to increase biodiversity, improve ecosystem services, and enhance the resilience of farming systems to climate change.

Technological Innovations

The integration of technological innovations into agriculture offers significant opportunities for improving productivity and sustainability:

- **Precision Agriculture**: The use of precision agriculture technologies, such as GPS-guided equipment, remote sensing, and data analytics, can optimize inputs like water, fertilizers, and pesticides, leading to increased efficiency and reduced environmental impact.
- **Genetic Improvement**: The development and adoption of climate-resilient crop varieties through traditional breeding and biotechnological methods can enhance tolerance to heat, drought, and pests, ensuring more stable yields under changing climatic conditions.
- **Digital Tools**: The use of digital tools and platforms for monitoring weather patterns, soil conditions, and crop health can provide farmers with real-time information to make informed decisions, improving adaptive capacity and resilience.

Alignment with International and EU Frameworks

Nationally Determined Contributions (NDCs)

Serbia's commitment to its NDCs involves significant efforts to reduce greenhouse gas emissions from the agricultural sector and enhance its capacity to adapt to climate change:

- **Emission Reduction Targets**: Serbia has set ambitious targets to reduce its overall greenhouse gas emissions by 33.3% by 2030 compared to 1990 levels. This includes measures to reduce emissions from agricultural practices through the adoption of sustainable practices and technological innovations.
- **Adaptation Measures**: The NDC outlines various adaptation measures for the agricultural sector, including improving water management, enhancing soil conservation, and promoting the use of climate-resilient crop varieties. These measures are aimed at reducing vulnerability and increasing the resilience of agricultural systems to climate impacts.

Sustainable Development Goals (SDGs) - Alignment with the SDGs is essential for ensuring the long-term sustainability and resilience of Serbia's agricultural sector:

- **SDG 2 (Zero Hunger)**: By adopting sustainable agricultural practices and improving productivity, Serbia aims to ensure food security and promote sustainable agriculture.
- **SDG 13 (Climate Action)**: Implementing measures to mitigate and adapt to climate change aligns with the goals of SDG 13, contributing to the reduction of climate-related risks and enhancing resilience.
- **SDG 15 (Life on Land)**: Sustainable land management practices that prevent land degradation and promote biodiversity conservation are critical for achieving SDG 15.

EU Green Deal: The EU Green Deal provides a framework for Serbia to transition towards a sustainable agricultural sector:

- **Farm to Fork Strategy**: The Farm to Fork Strategy aims to create a fair, healthy, and environmentally-friendly food system. Serbia's alignment with this strategy involves adopting sustainable farming practices, reducing the use of pesticides and fertilizers, and enhancing food safety standards.
- **Biodiversity Strategy**: The EU Biodiversity Strategy aims to restore biodiversity and ensure sustainable use of natural resources. Serbia's efforts to conserve biodiversity, protect natural habitats, and promote sustainable land use practices are essential for alignment with this strategy.

- **Climate Pact and Circular Economy**: The Climate Pact and Circular Economy initiatives under the EU Green Deal emphasize reducing waste, recycling, and promoting the efficient use of resources. Serbia’s adoption of circular economy principles in agriculture can help reduce waste, enhance resource efficiency, and contribute to sustainability goals.

Key Findings

- **Climate Vulnerability**: The Serbian agricultural sector is highly vulnerable to climate change, with significant risks from increased temperatures, erratic precipitation patterns, extreme weather events, and the proliferation of pests and diseases.
- **Sustainable Practices**: Adoption of climate-smart and sustainable agricultural practices, such as improved irrigation systems, soil and water management, and agroecological approaches, is essential for mitigating climate risks and enhancing resilience.
- **Technological Innovations**: Integration of precision agriculture, genetic improvement, and digital tools can optimize resource use, improve productivity, reduce environmental impact.
- **International Alignment**: Aligning with NDCs, SDGs, and the EU Green Deal involves setting emission reduction targets, implementing adaptation measures, and adopting sustainable practices to ensure long-term sustainability and resilience of agricultural sector.

By addressing these risks and seizing opportunities for innovation and sustainable development, Serbia can enhance the resilience and productivity of its agricultural sector, ensuring alignment with international and EU frameworks and securing a sustainable future for its agricultural economy.

The discussion elaborates on the need for Serbia to enhance its legal and regulatory framework to meet EU standards. It stresses the importance of integrating climate adaptation and mitigation measures into agricultural policies. The discussion also covers the economic, social, and environmental benefits of aligning with EU policies, including better access to EU markets, improved food security, and enhanced sustainability.

Aligning Serbia's agricultural practices with EU standards is crucial for its accession process and integration into the European Union.

Conclusions

The conclusions reiterate the necessity for Serbia to adopt comprehensive strategies that align with EU policies and address climate change. It underscores the critical role of government support, financial incentives, and international cooperation in achieving these goals.

Key Points on Serbian Alignment to EU and Climate Needs

- **Legal and Regulatory Alignment**: Serbia needs to align its agricultural policies and regulations with EU standards, focusing on sustainability, food safety, and environmental protection.
- **Climate Adaptation and Mitigation**: Implementing strategies to adapt to climate change is crucial. This includes adopting sustainable agricultural practices, enhancing agrobiodiversity, and investing in climate-resilient infrastructure.
- **Economic and Social Benefits**: Aligning with EU policies will open up access to EU markets, improve food security, and provide economic benefits through subsidies and financial support.
- **Government and International Support**: Strong government policies and international cooperation are essential for successful alignment. This includes financial incentives.

Recommendations for Serbia:

1. **Promoting Climate-Resilient Agriculture:** Encourage the adoption of climate-smart agricultural practices such as agroforestry, conservation agriculture, and precision farming. These practices can enhance productivity, conserve natural resources, and mitigate climate risks.
2. **Enhancing Water and Resource Management:** Invest in irrigation infrastructure, water-saving technologies, and sustainable land management practices. Efficient water use and soil conservation are crucial for mitigating water scarcity and enhancing agricultural resilience.
3. **Supporting Smallholder Farmers:** Provide financial and technical support to smallholder farmers to enhance their adaptive capacity. Training programs on climate-smart practices, access to climate information, and market integration can empower farmers and improve their livelihoods.
4. **Strengthening Policy and Institutional Support:** Align national agricultural policies with EU requirements and climate adaptation goals. Enhance coordination among government agencies, NGOs, and international partners to implement integrated climate resilience strategies.
5. **Fostering International Cooperation:** Collaborate with EU institutions, international organizations, and neighboring countries to access funding, expertise, and knowledge-sharing opportunities. International cooperation can accelerate Serbia's progress towards sustainable agriculture and climate resilience.

Addressing climate change impacts on agriculture and rural poverty in Serbia requires holistic approaches that integrate climate resilience strategies, EU policy alignment, and support for vulnerable rural communities. By prioritizing sustainable agricultural practices, enhancing resource management, and fostering international cooperation, Serbia can mitigate the adverse effects of climate change, protect livelihoods, and achieve sustainable development goals. Continued commitment to climate action and adaptation is essential for building a resilient and prosperous future for rural communities in Serbia and ensuring food security for generations to come. In summary, Serbia's journey towards EU accession offers an opportunity to transform its agricultural sector into a resilient and sustainable engine of economic growth. By embracing EU standards, implementing innovative practices, and empowering rural communities, Serbia can navigate climate challenges and foster a prosperous agricultural sector aligned with global sustainability goals.

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BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF MAIZE INBRED LINES

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Abstract

Maize is rich in genetic and phenotypic variations, yielding a range of diverse secondary metabolites. Biochemical analysis and molecular characterization using SNP markers allowed content of secondary metabolites (carotenoids, tocopherols and phenolic acids) and genetic diversity to be evaluated in 18 maize genotypes, including nine lines from MRI gene bank and nine elite inbred lines. The contents of tocopherols, carotenoids and phenolic acids were determined by the HPLC method. Eighteen inbred lines were analyzed using 25k SNP chip (Infinium Illumina array, TraitGenetics). The elite inbred lines had greater average content of all phenolic acid, excepted gallic acid. The average contents of gamma, alfa, delta tocopherols as well as beta carotene and lutein + zeaxantin were higher in maize lines from MRI gene bank. Line 2 from MRI gene bank had high content of tocopherols and carotenoids, as well as elite inbred line 17 with the highest level of ferulic, p-coumaric, syringic, caffeic and vanillic acid. Genetic diversity parameters were calculated and the data from SNP markers were subjected to population structure analysis by STRUCTURE as well as cluster analysis in Tassel software. According to both analyses three distinct inbred groups were revealed with some discrepancies between analyses in classification of genotypes. A STRUCTURE led to a more consistent classification of genotypes based on their origins. A considerable variation of secondary metabolites observed among inbred lines could be utilized in breeding programs to increase the nutritive value of maize varieties.

Keywords: *maize, carotenoids, phenolic acids, tocopherols. genetic diversity.*

Introduction

Maize is the 3rd most important in the world production of cereal crops and is cultivated in a wide range of agroecological environments. Parallel with world's human population increasing demand for maize production as a source of food, feed, and biofuel is increasing. The main goal of maize breeding programs is to develop genotypes with high yield and little attention was paid to consumers' demands for quality (Holmes *et al*., 2019). By adopting user needs and integrating them into products, breeders will be better utilize resources, increase usage of specific maize genotypes and improve nutrition value of maize kernel (Ekpa *et al*., 2018). So, recently, many efforts have been placed in evaluating and using maize diversity for developing nutritionally-enriched high-yielding varieties.
It is well-known that maize kernels are a good source of important health-relevant phytochemicals such as carotenoids, tocopherols and phenolic acids (Ranilla, 2020). The two most abundant groups of lipid-soluble antioxidants in maize kernels are carotenoids and tochopherols and substantial variation were found among diverse maize germplasm (Chander *et al*, 2008; Drinic et al., 2019) which can be explored for breeding. The genetic variation for various carotenoids among traditional maize inbreds showed zeaxanthin and lutein were the most predominant carotenoids (Chander *et al.* 2008). On the other hand, most maize lines

naturally have a low level of α-tocopherol but are abundant in γ-tocopherol. The level of most important, α-tocopherol is about 20% of total tocopherols in maize (Rocheford *et al*, 2002), and its higher levels can be obtained through either utilization of existing natural variation (Chander *et al*. 2008) or by genetic engineering.
Other secondary metabolites found in maize kernels are the phenolic acids, which are present as soluble and insoluble bound forms. Among them, ferulic and *p*-coumaric acid can act as antioxidants in humans, ferulic acid can improve cardiovascular and kidney functions (Cicero & Gaddi, 2001) and *p*-coumaric acid minimizes the oxidation of low-density lipoprotein. Biochemical evaluation allowed genotypes to have a greater content of carotenoids, tocopherols or phenolic acid to be identified and then evaluated regarding nutritional profile.
Genetic diversity is highly desirable in maize breeding programs since it may represent an important source of genes for breeding applications and development of improved maize genotypes relevant for nutrition and health. It could be managed, maintained and explained by morphological, biochemical and molecular markers. SNP markers, a biallelic and codominant, are widely distributed and the most abundant, highly reproducible and reliable molecular markers, the most commonly used in genetic diversity assessing (Xu *et al*., 2017, Srii *et al*. 2021). Based on present knowledge and experiance, two approaches in breeding maize for increased micronutrient content in grains could be recommended: a) selection of genotypes with increased concentration of particular nutrients and boosting their content through several cycles of selection by creating synthetic populations, and b) development of hybrids from parental lines with enriched grain micronutrient content and with good combining ability (Maqbool *et al*., 2018).
In this study, content of carotenoids, tocopherols and phenolic acids as well as genetic diversity in 18 maize inbred lines were evaluated.

Material and Methods

Eighteen inbred lines, nine from Maize Research Institute Gene bank (MRIGB 1-9) and nine elite inbred lines from breeding collection (ZPL 10-18) were used in this study. The content of tocopherols, carotenoids and phenolic acids was determined by the HPLC method. Approximately 0.2 g of maize grain was used to determine tocopherols and carotenoids content, and approximately 0.5 g of maize grain for free phenolic acid. The extraction of tocopherols was achieved by adding 5 mL of 2-propanol, extraction of free phenolic acids by using 5 mL of 80% methanol, and extraction of carotenoids by adding a mixture of methanol and ethyl acetate (6:4, v/v), 2 × 5 mL. After homogenization on the digital shaker, IKA (30 min at 25 ºC) for all analyses, the extracts were centrifuged, filtered (0.45 μm nylon membrane syringe filter), and injected into the HPLC system equipped with diode array (DAD) and fluorescence detector (FLD). Carotenoid extracts before injection were evaporated to dryness under a stream of nitrogen and redissolved in the mobile phase. The same analytical column, mobile phase, and wavelengths for separation and detection of carotenoids, tocopherols, and free phenolic acids were the same as described in the study of Mesarovic *et al*., 2019. Agglomerative Hierarchical Cluster Analysis (HCA) was performed by using the PLS Toolbox software package within MATLAB (R2011a).
Using a 25k SNP chip (Infinium Illumina array, TraitGenetics), a chosen set of eighteen inbred lines from various origins was analyzed. Following the exclusion of SNPs with a missing rate and heterozygosity >10% and SNPs with minor allele frequency (MAF) <0.05 using Tassel 5 software, a total of 15289 SNPs were retained for further analysis. The same software was applied for constructing dendrogram using neighbor-joining (NJ) method. Genetic diversity parameters (minor allele frequency, observed and expected heterozigosity) were calculated using Plink 1.9 software. Utilizing the STRUCTURE v2.3.4 software

(Pritchard *et al.*, 2000), the population structure was examined. The burn-in period and MCMC length were both set at 10,000 iterations. The model was executed by changing the number of clusters (K) from 1 to 10, with 5 alterations for each K.

Results and Disscusion

Significant variation was observed in carotenoids, tocopherols and phenolic acids (Table 1). The content of Lutein+Zeaxantin varied from 22.22 to 41.57 µg/g dry weight (d.w.), with an average of 30.83 µg/g d.w. Beta-carotene ranged from 0.55 µg/g d.w. to 3.70 µg/g d.w., average 1.35 µg/g d.w. The content of β-carotene in normal yellow maize ranges from 0.3 to 4.7µg/g d.w. (Menkr *et al*. 2008). Chander *et al*., (2008) in 87 inbred lines from China revealed β-carotene content in a range of 0.016-1.726 µg/g dw,, which is lower than obtained in our study.

Table 1. Content of carotenoids and tocopherols in maize inbred lines (µg/g d.w.)

Inbred lines	Lutein+zeaxantin	β-caroten	β+γ-tocopherol	α- tocopherol	δ-tocopherol
MRIGB1	36,66	1,73	36,26	17,84	1,04
MRIGB2	41,57	3,70	65,22	14,62	3,43
MRIGB3	39,85	2,95	49,25	9,60	2,60
MRIGB4	33,92	1,23	85,92	3,17	12,22
MRIGB5	25,76	0,75	19,52	8,93	0,76
MRIGB6	27,82	1,05	30,80	7,45	2,26
MRIGB7	32,05	1,07	69,59	9,62	4,88
MRIGB8	37,13	1,93	65,87	2,89	3,64
MRIGB9	26,40	1,09	44,34	5,24	4,91
ZPL10	34,75	0,96	70,63	6,56	2,74
ZPL11	22,22	1,19	59,01	5,94	2,13
ZPL12	26,26	1,01	56,38	1,62	2,95
ZPL13	32,24	1,55	43,81	3,90	2,32
ZPL14	29,14	0,84	41,11	5,86	3,03
ZPL15	28,29	0,89	34,99	4,01	1,84
ZPL16	28,06	1,02	80,14	3,47	6,74
ZPL17	26,06	0,78	32,76	15,23	1,25
ZPL18	26,76	0,55	43,63	9,01	3,77

Among cereals, maize grain has the highest content of tocopherols and the two predominant isomers are γ-tocopherol and α-tocopherol (Rocheford *et al.,* 2002). In our study the content of α- tocopherol varied from 1.62 µg/g d.w. to 17.84 µg/g d.w., average 7.50 µg/g d.w., as well as β+γ-tocopherol from 19.52 µg/g d.w. to 85.92 µg/g d.w., average 52.09 µg/g d.w. Several studies have shown significant differences among maize inbreds for tocopherol levels. A similar range of variation inα-tocopherol content in maize kernel has been reported by Muzhingi *et al.* (2016) but Li *et al*. (2012) reported the much higher range of variation for α-tocopherol. Drinic *et al.* (2020) obtained higher average values for β + γ and α-tocopherol in study of variation of tocopherols in 120 maize inbred lines with standard (yellow and orange) kernel type as well as sweetcorn and popcorn.

The average contents of tested tocopherols as well as tested carotenoids were higher in maize lines from MRI gene bank (51.86, 8.81, 3.97, 1.72 and 33.46 µg/g d.w., respectively) than elite inbred lines (51.38, 6.18, 2.98, 0.98 and 28.20 µg/g d.w., respectively).

The content of ferulic acid varied from 0.62 µg/g d.w. to 6.91 µg/g d.w., average 2.68 µg/g d.w.and p-coumaric acid from 0.38 µg/g d.w. to 4.23 µg/g d.w., average 1.83 µg/g d.w., (Table 2). The elite inbred lines had greater average content of all phenolic acids, excepted gallic acid. Muzhingi *et al*., (2016) obtained similar results for ferulic acid distribution (between 0.23 and 6.90 µg/g d.w.) in 923 inbred lines grown in three different locations. On the other hand, higher content of *p*-coumaric acid (2.51 to 6.07 μg/g DW) was reported by Cuevas-Montilla *et al*. (2011) in nine Bolivian purple maize varieties.

Line 2 from MRI gene bank had the highest content of carotenoids and high content of tocopherols, as well as elite inbred line 17 with the highest level of ferulic, p-coumaric, syringic, caffeic and vanillic acid. Inbred lines MRIGB 4 (85.92 μg/g d.w.) and elite inbred line ZP16 (80.14 μg/g d.w.) have the highest level of β+γ-tocopherol and MRIGB 1 (17.84 μg/g d.w.) and elite inbred line ZP17 (15.23 μg/g d.w.) of α- tocopherol.

Table 2. Content of phenolic acids in maize inbred lines (μg/g d.w.)

Inbred lines	Gallic	Protocatechuic	vanillic	Caffeic	Syringic	Sinapic	P - Coumaric	Ferulic	Cinnamic
MRIGB1	12,03	2,70	2,39	1,06	1,74	0,21	1,16	1,48	6,22
MRIGB2	11,61	1,12	0,41	0,18	0,59	0,65	1,01	0,62	0,51
MRIGB3	13,82	1,98	4,15	0,75	2,11	0,18	1,97	0,85	1,78
MRIGB4	19,90	4,27	2,47	0,71	1,66	0,06	1,00	0,84	6,62
MRIGB5	14,18	2,52	1,64	0,50	0,42	0,23	0,93	2,67	14,53
MRIGB6	21,60	4,93	0,89	0,26	5,47	0,26	0,82	1,46	8,73
MRIGB7	22,88	0,73	0,72	0,38	0,52	0,40	0,38	0,65	2,52
MRIGB8	16,73	9,79	8,33	0,17	10,74	0,49	2,62	3,54	0,13
MRIGB9	13,13	6,17	4,88	1,53	6,34	0,20	2,30	3,12	10,73
ZPL10	7,07	15,84	1,50	0,71	8,11	0,11	0,87	2,54	14,80
ZPL11	18,73	19,44	3,01	0,17	10,86	0,76	2,21	4,42	7,91
ZPL12	16,28	5,12	2,84	0,27	2,60	0,19	1,89	2,46	14,44
ZPL13	14,58	7,59	4,01	1,76	24,89	0,30	3,55	6,16	9,28
ZPL14	3,19	10,33	3,95	0,93	4,28	0,12	1,01	2,86	29,05
ZPL15	14,73	12,19	5,89	0,54	2,83	0,50	2,98	2,77	21,87
ZPL16	19,61	4,93	1,95	0,22	1,39	0,26	1,85	1,68	20,17
ZPL17	19,45	8,91	15,51	2,18	29,94	0,39	4,23	6,91	15,16
ZPL18	16,36	5,63	3,90	3,25	6,47	0,11	2,11	3,26	25,98

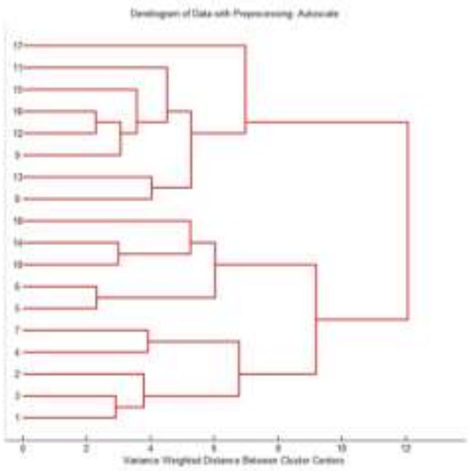

Figure 1. Dendrogram based on tested biochemical parameters in maize inbred lines

Based on the content of secondary metabolites (carotenoids, tocopherols and phenolic acids) cluster analysis was done (Figure 1). Inbred lines were grouped in two clusters at the level of similarity of approximately 10 %. Cluster I comprise two subclusters: subcluster 1. grouped five MRIGB inbred lines and subcluster 2. two MRIGB inbred lines as well three elite inbred lines grouped together. Cluster II consist of two subclasters with mixed inbred lines both from MRI gene bank and elite lines (six elite inbred lines and two inbred lines from MRIGB).

Genetic diversity parameters were calculated and the data from SNP markers were subjected to population structure analysis by STRUCTURE software as well as cluster analysis in Tassel software. Plink 1.9 software was used for calculating genetic diversity prameters. Minor allele frequency was in a range from 0.0 to 0.5 with an average of 0.29. The smallest

value for observed heterozygosity was 0 and the highest 0.06, while the average was 0.03. Expected heterozygosity was in the range from 0.11 to 0.5, with an average of 0.37. The low average value of observed heterozygosity close to zero is expected for inbred lines. Comparable, but greater average heterozygoisty (0.08) is reported in Semagn *et al*., (2012).
Cluster analysis based on SNP markers divaded inbred lines in three main groups (Figure 2). Group I consisted of five elite inbred lines and one inbred from MRIGB. Group II comprised two MRIGB inbred lines and one elite and group III formed two subgroups (A and B). Subgroup A was formed -by five inbred lines from MRIGB and subgroup B –formed by three elite inbred lines and one inbred line from MRIGB.

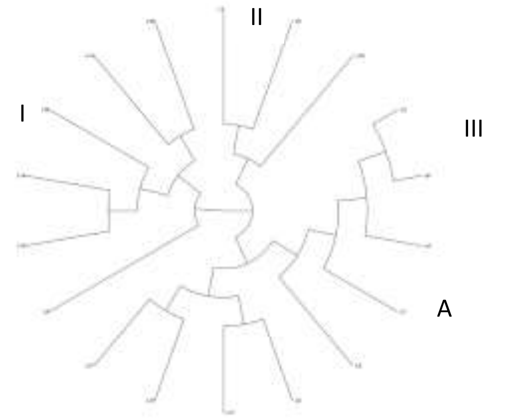

Figure 2 Cluster analysis of maize inbred lines

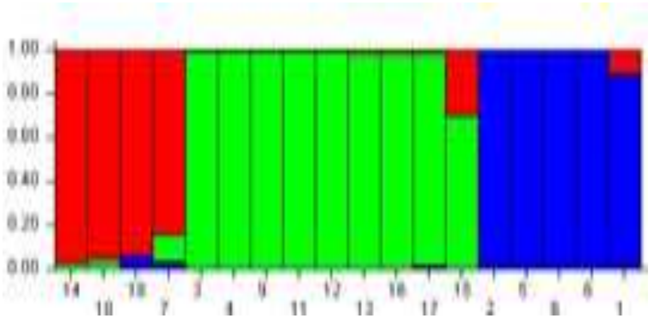

Figure 3. STRUCTURE analysis of inbred lines

Lines were subdivided into genetic clusters using a model-based approach with the package STRUCTURE (Pritchard *et al.* 2000). STRUCTURE analysis (Graf 3.), grouped inbred lines in three groups: Group 1. was formed -by three elite inbred lines and one from MRIGB, group 2. -contained nine inbred lines (3 form MRIGB and 6 elite inbred lines), and group 3. -contained five MRIGB inbred lines.
According to both analyses three distinct inbred groups were revealed with some discrepancies between analyses in classification of genotypes. A STRUCTURE led to a more consistent classification of genotypes based on their origins.

Conclusion

The presence of extensive genetic variability represents a valuable source of micronutrients, and genotypes with enhanced grain content could be used in improving of maize breeding programms. Biochemical evaluation allowed inbred lines having greater content of β carotene (MRIGB 2), L+Z (MRIGB 2 and MRIGB3), α (ZPL17) and β+γ-tocopherols (MRIGB 4 and ZPL16) to be identified and used as potential source of micronutritient for the development of nutritionally enriched maize genotypes. Significant genetical divergence among the studed~~y~~ inbred lines was determined. Inbred lines belonging to Group I after STRUCTURE analysis have the highest average level of γ-tocopherols, and inbred lines in Group III were found to be promising for β carotene, Lutein+Zeaxantin and α tocopherols, since they had high mean values for these traits. Inbred lines belonging to group II have the highest value of ferulic and p-cumarin acid. The molecular characterisation allows genetic variability to be estimated and it can be a useful tool for supporting genetic improvement when combined with biochemical traits of interest.

Acknowledgments

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ISOLATION AND CHARACTERIZATION OF PGP BACTERIAFROM *PLANTAGO LANCEOLATA* RHIZOSPHERE SOIL

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Abstract

Ribwort plantain (*Plantago lanceolata* L.) is a widespread perennial herbaceous plant species, usually found in pastures and grasslands, rarely in arable lands. It is used as a medicinal plant from ancient times because of high concentration of various secondary metabolites in leaves. But plantain also excretes many phytochemicals into its rhizosphere soil which are then used by different microbes, plant growth promoting bacteria (PGPB) among others. PGPB can enhance plant growth through different modes of action. The most important are nutrient availability improvement, control of pathogenic microorganisms, and production of plant hormones. Bacteria of genus *Pseudomonas, Bacillus, Azotobacter* and *Streptomyces* were isolated from the rhizosphere of plantain. They were fully characterized based on their morphological, physiological, biochemical and PGP traits. Out of total 12 bacterial isolates, four demonstrated production of indol acetic acid (IAA), and seven were good producer of hydrogen cyanide (HCN). Significant number of isolates showed the ability to mineralize organic phosphorus (P) compounds (six isolates) as well as to mobilize water insoluble tertiary phosphates (five isolates). *Streptomyces* isolates showed the best PGP potential, especially the isolate S4/1 with four out of five investigated PGP traits. Also, important isolates with significant PGP traits were K3/2 and K5/1 (*Pseudomonas* sp.), M3/1 (*Bacillus* sp.), and F1/2 (*Azotobacter* sp.). Further investigation of these isolates is necessary before they could be used as a potential base in creation of a bacteria based formulation(s).

Keywords: *PGP bacteria, Ribwort plantain, rhizosphere, Streptomyces.*

Introduction

Ribwort plantain (*Plantago lanceolata* L.) is a widespread perennial herbaceous plant species, usually found in pastures and grasslands, rarely in arable lands. It is used as a medicinal plant from ancient times because of high concentration of various secondary metabolites in leaves. Plantain excretes many phytochemicals into its rhizosphere soil which are then used by different microbes, including plant growth promoting bacteria (PGPB).

Rhizosphere bacteria play a principal role to plant growth and root development. These microorganisms are very diverse with numerous genus including *Azotobacter*, *Azospirillum, Rhizobium*, *Pseudomonas, Bacillus, Streptomyces, Micromonospora*, *Frankia* ect. (Bello and Utang, 2011). They growth and metabolytic activity is heavily influenced by temperature, pH, salinity, water content, as the most important abiotic factors, as well as by biotic interactions (Al-Maqtari et al., 2019).

Production of enzymes, phytohormones, vitamins, siderophores etc. by bacteria are considered as plant growth promoting (PGP) traits (Stamenov et al., 2021). Many studies proved PGP activities of bacterial isolates (Bjelić et al., 2015; Niyonzima, 2019; Krishna Samal et al., 2020; Stamenov et al., 2020). *Azotobacter* species are known as phosphate-solubilizing microorganisms because it can convert insoluble (Ca_3PO_4, $AlPO_4$, Fe_3PO_4) to available forms of phosphate (Aasfar et al., 2021). Results of Bjelić et al., (2015) showed intense indole-3-acetic acid, hydrogen cyanide and siderophores production of *Azotobacter*

sp. isolates. *Bacillus* species are known to produce protease, amylase, lipase (Al-Maqtari et al., 2019) phytase, keratinase, cellulase and pectinase (Niyonzima, 2019). The microorganisms belonging to *Pseudomonas* sp. are known to secrete cellulase and amylase (Sethi et al., 2013; Niyonzima, 2019). *Streptomyces* species produce enzyme amylase (Niyonzima, 2019), cellulase, lipase, urease, gelatinase and IAA (Stamenov et al., 2016).
Bacteria of genus *Pseudomonas, Bacillus, Azotobacter* and *Streptomyces* were isolated from the rhizosphere soil of plantain. The main aim of this research was to assess the isolates PGP traits. Prior to that, full characterization based on their morphological, physiological, and biochemical traits were performed.

Materials and methods

Bacteria were isolated from rhizospheric soil of plantain, in Trapisti, municipality Banja Luka, Bosnia and Herzegovina. Morphological identification and chemotaxonomic analyses of purified isolates were identified to genus level. Direct microscopic observation (at 1000x magnification) of the vegetative and aerial mycelium was used according to Bergey's manual of determinative bacteriology (Holt et al. 1994). Spherical thick-walled, Gram-negative cells were selected as *Azotobacter*. Rod-shaped, Gram-positive cells with endospores were chosen as *Bacillus*. Isolates which were established as gram-negative, rod-shaped cell and produced pigment fluorescein were selected as *Pseudomonas* isolates. Filamentous and Gram-positive cells were chosen as *Streptomyces* isolates.
The isolates of *Azotobacter*, *Bacillus*, *Pseudomonas* and *Streptomyces* were inoculated on F-agar, tripton soya agar (TSA), King's B and synthetic medium, respectively (Stamenov et al., 2021). Growth conditions were tested at different temperatures (5, 28 and 45°C), salt concentrations (3, 5 and 7%) and pH levels (5, 7 and 9) for 2-5 days. The width of the colony was measured and compared to the control.
Utilization of carbon sources was determined by using Hugh-Leifson medium (peptone 2 g/L, K_2HPO_4 0.3 g/L, NaCl 5 g/L, 10 g/L of carbon sources (glucose, galactose, xylose, saccharose, lactose or fructose) bromtimol blue 0.03 g/L and agar 3 g/L) (Stamenov i sar., 2020). Change of colony colour from greenish to yellow was proof of a positive reaction (Hugh and Leifson, 1953).
According to Frey-Klett et al. (2005), bacterial cultures were streaked on tryptic soy agar with glycine. To test hydrogen cyanide (HCN) production, a sterile filter paper soaked in 2% Na_2CO_3 and 0.5% picric acid (2,4,6-trinitrophenol) solution was placed in each Petri dish. Dishes were sealed with parafilm and incubated at 28°C. Development of color from yellow to dark brown indicated HCN production. Production of indol-3-acetic acid (IAA) by bacterial isolates was examined according to the Etesami et al. (2015). Development of pink color in tubes after addition of Kovac´s reagent proved the presence of indole in the medium and capacity of bacterial isolates to produce the enzyme tryptophanase. The ability of bacteria to produce siderophores was tested according to Schwyn and Neilands (1987), on chrome-azurol S (CAS) medium. The change of colour from blue to orange indicated ability of bacteria to produce siderophores. The ability to mineralize organic phosphorus compounds was tested on a modified Menkina substrate (Liu et al., 2011). Pikovskaya (1948) medium was used to examine the ability of bacteria to solubilize insoluble $Ca_3(PO_4)_2$. Phosphate mineralization and solubilization was verified by the appearance of a transparent zone around bacterial colony, respectively. Effectiveness of phosphate solubilization and mineralization of phosphorus organic compounds was evaluated according to zone diameter.
Ability to produce cellulase was tested on CMC agar (carboxymethyl cellulose agar). Solution of Congo-red (1 mg/cm^3 H_2O) was poured over bacterial colonies after incubation period. The

solution was decanted after 15 min and the colonies were overflowed with 1M NaCl. A transparent zone around the colonies was the proof of the cellulase activity.
Lipase activity was determined by growing the isolates on the nutrient medium (peptone 10 g/L, NaCl 5 g/L, $CaCl_2xH_2O$ 0.1 g/L and agar 15 g/L) with Tween 80. Discoloured zone around the isolates colony was the proof of lipolytic activity. Christensen’s urea agar (urea 20 g/L, NaCl 5 g/L, glucose 1 g/L, peptone 1 g/L, KH_2PO_4 2 g/L, phenol red indicator 0.012 g/L and agar 15 g/L) was used to examine the urease activity. Since urea is unstable, it was filtered through syringe filter (0.2 μm) and added separately, after autoclaving. The appearance of the red color was proof of the urea decomposition. Amylase activity was examined by flooding iodine on colonies grown on starch agar (starch 10 g/L, KH_2PO_4 0.5 g/L, K_2HPO_4 0.5 g/L, $MgSO_4 \times 7\ H_2O$ 0.2 g/L, agar 15 g/L). Absence of a halo zone around the colony confirmed hydrolysis of starch. Nutrient medium with pectin was used to test pectinase production by bacteria (Soriano et al., 2000). After 24h, colonies were overflowed with lugol. A discolored zone around the colony was proof of the pectinase activity.

Results and Discussion

From the rhizosphere of ribwort plantain, different bacteria were isolated. Out of 50 isolates, 12 were chosen for detailed characterization. The bacteria isolates were denoted as follows: genus *Streptomyces* (S4/1 MPA, S4, S5), *Pseudomonas* (K1/1, K1/2, K1/3), *Bacillus* (MPA 1/1, MPA(UB)1/1, MPA(UB)1/2, MPA(UB)1/3) and *Azotobacter* (F1/1, F1/2). These strains were further studied for different biochemical, physiological and PGP properties.
Influence of temperature, salt and pH levels on bacterial colony growth are presented in Table 1.

Table 1. Effect of different temperature, salinity and pH levels on growth

Isolates	Temperature (°C)			Salt (%)			pH value		
	5	28	45	3	5	7	5	7	9
S4/1 MPA	-	+++	-	+++	++	+	+++	+++	++
S4	-	-	++	+++	-	-	+++	-	++
S5	-	-	+	+	-	-	++	-	-
K1/1	-	+	-	-	+	-	++	-	++
K1/2	+	++	+++	++	+	-	+++	++	+++
K1/3	-	++	-	-	-	-	+	+++	+
MPA 1/1	-	+	+	++	++	++	++	++	++
MPA(UB)1/1	-	+	-	++	-	-	+++	++	+++
MPA(UB)1/2	-	+	++	++	-	-	++	++	++
MPA(UB)1/3	-	+	+	+	+	-	++	++	++
F1/1	+	++	+	++	++	+	+++	++	+++
F1/2	+	++	+	++	++	+	+++	++	+++

Legend: intense growth (+++), optimal growth (++), minimal growth (+), absence of growth (-);

For the most isolates, optimum temperature for the growth was at 28°C. Similarly, bacteria isolates showed good growth at 45°C, except S4/1 MPA, MPA(UB)1/1 and bacteria of the genus *Pseudomonas* (K1/1 and K1/3). The results showed good tolerance of *Azotobacter* isolates to all salt concentrations. Isolates S4/1 MPA (*Streptomyces sp.*) and MPA 1/1 (*Bacillus sp.*) showed optimal colony growth, too. The majority of bacteria isolates grew better and formed larger colonies at lower salt concentration contrary to the highest NaCl concentration where only four isolates grew. All isolates had intense or optimal growth at pH 5. Similar results were observed at pH 7 (except for S4, S5 and K1/1) and pH 9 (except for S5). Strain S5, belonging to the genus *Streptomyces*, showed higher sensitivity compared to the other isolates (Table 1). The ability of bacteria to grow in extreme conditions such as low or high pH, salinity or temperature allows them to survive and to adapt to diverse surroundings (Stamenov et al., 2016). Based on this, strain K1/2, MPA 1/1, F1/1 and F1/2 showed good resilience to adverse environments. Results of Krishna Samal et al. (2020) indicated similar tolerance properties of *Azotobacter* isolates to different salts (2, 5 and 7%) and pH levels (6, 7, 8 and 9). According to Stamenov et al. (2021), *Bacillus* isolate B85 formed colonies at, both 10°C and 45°C as well as at salt levels ranging from 5% and 7%. The ability of *Bacillus* and *Pseudomonas* isolates to grow at extreme temperatures and at high concentrations of NaCl is reported by Stamenov et al. (2020).

Utilization of various carbon sources by rhizospheric bacteria are presented in Table 2. None of 12 isolates utilize all six examinated carbon sources. However, three out of 12 bacteria isolates (S4/1 MPA, F1/1, F1/2) utilize five carbon sources. According to the Parveen et al. (2018) bacteria isolate WRPA26 showed best potential of various carbon sources utilization. This strain successfully utilized glucose, citrate, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose. On the other hand, study of Krishna Samal et al. (2020) showed good utilization of glucose, maltose, inositol, mannose and rhamnose by *Azotobacter* isolates (A2 and A3). Results of this research indicate that *Pseudomonas* isolate (K1/3) poorly utilized carbohydrates. On the contrary, Stamenov et al. (2020) observed good utilization of different carbon sources (glucose, galactose, fructose, saccharose and xylose) by *Pseudomonas* isolates.

Table 2. Utilization of different carbon source

Isolates	Glucose	Galactose	Xylose	Sacharose	Lactose	Fructose
S4/1 MPA	+	+	+	+	-	+
S4	-	+	+	+	-	+
S5	-	-	-	-	-	-
K1/1	+	+	+	-	-	+
K1/2	+	-	-	-	-	+
K1/3	-	-	-	-	-	+
MPA 1/1	+	-	-	+	-	+
MPA(UB)1/1	+	-	-	+	-	+
MPA(UB)1/2	+	-	+	+	-	-
MPA(UB)1/3	+	-	+	+	+	-
F1/1	-	+	+	+	+	+
F1/2	-	+	+	+	+	+

Legend: colony growth (+), absence of growth (-)

Both *Azotobacter* isolates (F1/1, F1/2), *Bacillus* (MPA(UB)1/1, MPA(UB)1/2, MPA(UB)1/3), *Streptomyces* S4/1 MPA and *Pseudomonas* K1/1 produced HCN (Table 3). According to Stamenov et al. (2021), 74.2% of total bacteria isolated from common thyme rhizosphere were able to produce HCN. On the contrary, in the study of Parveen et al. (2018), all examined bacterial isolates from wheat rhizosphere were found negative for HCN production. Hydrogen cyanide production by rhizospheric bacteria could induce suppression of many pathogenic bacteria and fungi.

Indole-3-acetic acid (IAA) is the main auxin phytohormone. Auxins participate in the regulation of basic plant physiological processes such as cell division, tissue differentiation and response to stress conditions (Parmar et al., 2023). All isolates of the genus *Streptomyces* (S4/1 MPA, S4 and S5) showed intense production of IAA contrasted to one isolate of genus *Azotobacter* (F1/2) which showed minimal production. Interestingly, pseudomonads and bacilli did not produce IAA. Many results indicated IAA production by more than 54%

(Stamenov et al., 2021) and 70% (Parveen et al., 2018) of the total number of isolated rhizobacteria.
All isolates were screened for production of siderophores but none were found (Table 3). In the study of Stamenov et al. (2016), only one isolate of the *Azotobacter* sp. was found to be positive for siderophores production. On the contrary, results of Parveen et al. (2018), showed potential of all investigated bacterial isolates for siderophore production where solubilization efficiency varied from 72.7% to 250%.
Phosphorus is one of the most important macronutrient for plant growth and development. All isolates of the genus *Azotobacter* (F1/1, F1/2) showed ability to mineralize phosphorus organic compounds (Table 3). Also, two isolates *Streptomyces* (S4/1 MPA, S4) and two isolates of *Bacillus* (MPA(UB)1/2, MPA(UB)1/3) showed similar activity. This is in accordance with the results of Stamenov et al. (2016). The authors showed that two *Azotobacter* isolates (A1 and A2) utilized organic phosphorous.

Table 3. PGP traits and enzyme production ability

Isolates	PGP traits*					Enzyme production**				
	HCN	IAA	S	P_{min}	P_{mob}	C	L	U	A	P
S4/1 MPA	+	+++	-	+++	+++	-	+	-	-	-
S4	-	+++	-	+++	+++	-	+	-	-	-
S5	-	+++	-	-	++	-	-	-	-	-
K1/1	+++	-	-	-	-	-	-	-	-	-
K1/2	-	-	-	-	+	+	-	-	-	+
K1/3	-	-	-	-	-	+	-	-	-	-
MPA 1/1	-	-	-	-	-	+	-	-	-	-
MPA(UB)1/1	++	-	-	-	-	+	-	-	-	-
MPA(UB)1/2	++	-	-	+	-	+	-	-	-	+
MPA(UB)1/3	++	-	-	+	-	+	-	-	-	+
F1/1	++	-	-	++	-	+	-	+	-	+
F1/2	+++	+	-	++	+	-	-	+	-	+

*Legend for HCN, IAA and S: (+++) intense production, (++) optimal production, (+) minimal production, (-) not detected; legend for Pmin and Pmob: (+) 4 mm day^{-1}, (++) 4-5 mm day^{-1}, (+++) >5 mm day^{-1}, (-) not detected; hydrogen cyanide production (HCN), siderophore production (S), mineralization of phosphorus organic compounds (Pmin.), phosphate solubilization (Pmob.), **cellulases (C), lipases (L), ureases (U), amylases (A), pectinases (P)

The results of our study showed that only 5 out of 12 isolates could solubilise inorganic phosphates. These were isolates of *Streptomyces* (S4/1 MPA, S4 and S5), one isolate of *Pseudomonas* (K1/2) and one isolate of *Azotobacter* (F1/2). Microbial solubilization in soil is alternative, eco-friendly process for enhancing bioavailability of plant nutrients. According to Parveen et al. (2018), more than 50% of investigated bacterial isolates were able to solubilize phosphates. The PGP features could affect plant growth indirectly by increasing soil fertility via solubilization of inorganic phosphorus, or directly by secretion of HCN, IAA etc.
The isolate F1/1 had the ability to produce three (cellulases, ureases and pectinases) out of five examined enzymes (Table 3). Some *Pseudomonas* and *Bacillus* isolates denoted as K1/2, MPA(UB)1/2 and MPA(UB)1/3 showed cellulases and pectinases production capacity. Similar result was found by Niyonzima (2019). That study indicated the ability of *Bacillus* sp. MSL2, *Bacillus megaterium* BMS4 and *Pseudomonas fluorescens* to produce cellulose degrading enzymes while *Bacillus subtilis* proved as an excellent producer of pectinases. All isolates were screened for amylases production but it was found that none were able to produce this enzyme (Table 3). Meanwhile, according to the Al-Maqtari et al. (2019), most of amylases are of microbial origin (*Bacillus subtilis*).

Conclusion

Plantain rhizosphere soil harbors many, potentially beneficial bacteria genus/species that can survive in conditions of elevated salt concentration. They also showed excellent ability to grow in wide range of pH level (varying from acidic to alkaline condition). Out of total 12 fully characterized bacterial isolates, four demonstrated production of indol acetic acid (IAA), and seven were good producer of hydrogen cyanide (HCN). Six isolates showed the ability to mineralize organic phosphorus (P) compounds as well as to mobilize water insoluble tertiary phosphates (five isolates). *Streptomyces* sp. isolates showed the best PGP potential, especially the isolate S4/1 with four out of five investigated PGP traits. Also, important isolates with significant PGP traits were *Pseudomonas* sp. K3/2 and K5/1, *Bacillus* sp. M3/1 and *Azotobacter* sp. F1/2. Further investigation of these isolates is necessary before they could be used as a potential base in creation of a bacteria based formulation(s).

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THE INFLUENCE OF AUXIN OF BACTERIAL ORIGIN ON THE SEED QUALITY PARAMETERS OF SOME FIELD CROPS

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Abstract

The effect of four concentrations of auxin of bacterial origin (0 - control without auxin, 0.1, 5, and 50 μg ml^{-1}) on germination and early seedling growth in five field crops was investigated. The genetic materials for this study included the maize hybrid 'ZP 684', the wheat variety ‘Evropa 90’, the soybean variety 'Galina', the field pea variety 'Kosmaj', and the sunflower hybrid 'NS Oskar'. Energy germination and germination were recorded according to ISTA rules. After 10 days, root length, shoot length, and seed vigor were determined. Energy germination did not depend on auxin, but germination did, except for maize. Wheat, soybean, and field pea had significantly higher germination at 5 and 50 μg ml^{-1} auxin and sunflower at 50 μg ml^{-1} auxin compared to the control. The results showed that the auxin of bacterial origin promoted early seedling growth in all crops compared to the control. Compared to the control, 0.1 and 5 μg ml^{-1} auxin increased root length in maize, 0.1, 5, and 50 μg ml^{-1} in wheat and sunflower, 5 and 50 μg ml^{-1} in soybean, and 50 μg ml^{-1} in field pea. Compared to the control, the auxin treatments increased shoot length in the crops studied, except for field pea, where the 50 μg ml^{-1} auxin did not contribute to the increase in this trait. Compared to the control, all treatments with auxin increased seed vigor in maize, soybean, field pea and sunflower, while this was observed in wheat when treated with 5 μg ml^{-1} auxin. Auxin-treated seeds are therefore expected to have the advantage of surviving under unfavorable environmental conditions caused by abiotic and biotic stress. Auxin-treated seedlings were more vigorous and better developed.

Keywords: *Early seedling growth, Germination, Auxin of bacterial origin.*

Introduction

Microbiological natural biostimulants have great potential for plant production worldwide. Numerous countries such as the members of the EU, the USA, China, India, and Japan are trying to increase the use of microbiological inoculants and thus biostimulants through legal regulations and incentives in agriculture. The development of ecological awareness and the good results of the application of PGPBs in agriculture led to their use in other areas of human activities, i.e. forestry, horticulture, bioremediation, and recultivation. The use of PCR techniques enables the detection and control of species and strains of PGPR bacteria involved in the synthesis of phytohormones.

High-quality seed processing is crucial for profitable crop production. The number of plants per unit area under natural field conditions and thus the crop yield per unit area in agriculture depends on how successful and high quality the seed will develop. According to Bajguz and Piotrowska-Niczyporuk (2023), 12 groups of plant hormones (phytohormones, plant growth regulators) have been identified in plant species: auxins, gibberellins, cytokinins, ethylene, abscisic acid, salicylic acid, brassinosteroids, jasmonates, polyamines, melatonin, peptide, and terpenoid lactones hormones. These hormones are necessary for the growth and development of plants. Numerous chemical and bacterial biostimulants containing hormones are available on the world market under various trade names for the preparation of seed and planting material. Chemical biostimulants contain synthetic auxins and can be harmful to the plant if used inadequately. Compared to natural auxins, they are degraded slowly in excessive amounts, remain longer in the plants, and are often lethal (Pazmiño *et al*., 2012). Their higher concentration leads to deformation and twisting of the leaf tissue due to uncontrolled and distorted cell division and collapse of the tissue structure, which eventually leads to the death of the plant (Grossmann, 2010). Unfortunately, the overuse of synthetic auxins during the flowering stage of crops, especially herbicides containing synthetic auxins, has reduced pollinator visitation and caused pollinator mortality (Bohnenblust *et al*., 2016). It is precisely for these reasons that research into the use of natural microbiological biostimulants for pre-sowing seed treatment has begun worldwide. The research to invent new bacterial biostimulants is based on testing different species and strains of bacteria and their combinations to find the most effective preparation to improve the quality of seed germination. Unlike chemical biostimulants, natural biostimulants contain microorganisms that produce phytohormones, and their quantity is balanced so that there is no fear of undesirable side effects (Saharan and Nehra, 2011). The basis of natural biostimulants is primarily plant growth-promoting bacteria (PGPB), which synthesise hormones that have a positive effect on seeds, seedlings and plants (Backer *et al*., 2018). According to Singh and Purohit (2008) PGPB hormones are natural analogues of plant hormones. About 80% of pathogenic and beneficial soil bacteria produce auxin and belong to different genera such as *Pseudomonas*, *Bradyrhizobium*, *Rhizobium*, *Mesorhizobium*, *Azospirillum*, *Serratia*, *Bacillus* and *Azotobacter* (Ahemad and Kibret, 2014; Gupta *et al*., 2015). Indole-3-acetic acid (IAA) is the most studied auxin produced by PGPB (Woodward and Bartel, 2005). Auxins produced by PGPR have a positive effect on seed germination (Miransari and Smith, 2014), root growth by improving water and nutrient availability (Arora *et al*., 2013), vegetative organ growth, photosynthesis, pigment formation, metabolite biosynthesis, biomass production and yield (Spaepen and Vanderleyden, 2011) and increases resistance to stress conditions (Gamalero and Glick, 2011).

The main objective of this paper is to study the effects of four concentrations of auxin of bacterial origin (0 - control without auxin, 0.1, 5, and 50 $\mu g\ ml^{-1}$) on seed germination and seedling growth of five crops (maize, wheat, soybean, field pea, and sunflower).

Materials and methods

Bacillus spp. were isolated from the rhizosphere of maize using the classical microbiological method. Subsequently, species identification was performed by cytological-morphological and biochemical analyses based on Bergey's Manual of Systematic Bacteriology, i.e. cell morphology, analysis of fermentation products, enzymatic activity, cell wall structure, and other phenotypic tests as a starting point for the preliminary classification of the genus *Bacillus*. Final genetic identification was performed by DNA amplification using the polymerase chain reaction (PCR) method by amplification of the 16S rRNA gene. Prior to DNA extraction, each isolate was subcultured in LB broth for 24 hours at room temperature. DNA extraction was performed using the Quick-DNA™ Fungal/Bacterial kits (Zymo Research, Irvine, USA). For PCR analysis, universal primers for 16s, 27F (5'- AGA GTT TGA TCM TGG CTC AG-3'), and 1492R (5'- TAC GGY TAC CTT GTT ACG ACT T-3') were used to determine the identity of 2 isolates. Reactions were performed in a final volume of 30 μL and contained the EconoTak® Plus 2 × Master Mix, 0.5 mM of each primer and 10 ng of genomic DNA. The following PCR amplification conditions were set in a thermal cycler: 5 minutes at 94°C, then 35 cycles at 94°C/30 seconds, 30 seconds at 55°C and 60 seconds at 72°C with a final extension of 7 minutes at 72°C. PCR products were mixed with Safe-Orange G dye and run on a 1.5% agarose gel in 1 × Tris-boric acid-EDTA (TBE) buffer at 80 V/1.2h. The gels were visualized with a UV transilluminator (UV-26, MRC LAB). Amplicons were purified using the PCR product purification kit QIAquick PCR Kit/250 (Qiagen GmbH, Hilden, Germany) and then sent to MACROGEN for sequencing. The resulting sequences were screened for homology with previously sequenced genes in the GenBank database using the National Center for Biotechnology Information Blast Nucleotide Search Program (BLASTN). A 100% sequence match was found for *Bacillus subtilis* and *Bacillus megaterium*.

Colorimetric determination of auxin concentration was performed according to the Salkowski method (De-Bashan *et al*., 2008). The Avena test was used to confirm the biological activity of the isolated auxins (Nitsch and Nitsch, 1956; Sirois, 1966). The analysis of auxin (indole-3-acetic acid) was confirmed and quantified by tandem mass spectrometry - MRM experiment (Đorđević *et al*., 2017).

The four concentrations of auxin of bacterial origin (0, 0.1, 5, and 50 μg ml^{-1}) were analyzed. Certified seeds of five crops (maize hybrid 'ZP 684', the wheat variety 'Evropa 90', the soybean variety 'Galina', the field pea variety 'Kosmaj' and the sunflower hybrid 'NS Oskar') were used.

The seeds were disinfected with 2% NaClO and washed 3 times in sterile distilled water. Germination tests were performed by placing 100 seeds in sterilized Petri dishes in a phytotron at a temperature of 20°C on filter paper soaked with 10 ml of a suitable medium in four replicates. According to ISTA (2024), the seed is germinated when the root length is 2 mm. Stem and root length were determined after 10 days on 20 randomly selected seedlings in each treatment and replication. Seed vigor was calculated using the following formula:

Seed vigor = (shoot + root length) × germination.

Statistica version 13 (StatSoft, Tulsa, Oklahoma, USA) was used to analyze the data. The mean values were compared with Tukey's test at a 5% level.

Results and discussion

It is generally known that the *Bacillus* genus produces indole-3-acetic acid, siderophores, and hydrogen cyanide. Different isolates produced different amounts of auxin (indole-3-acetic acid). The colorimetric and Avena tests showed that *B. subtilis* had a higher auxin concentration than *B. megaterium* (Table 1).

Table 1. The concentration of auxin produced (colorimetric test and Avena test).

Species of Bacteria	Auksin, μg ml^{-1}	
	Colorimetric test	Avena test
B. subtilis	14.50	14. 36
B. megaterium	12.78	12.21

During germination, the seed goes through a phase of imbibition (the seed absorbs water and swells, enzymes are activated and the seed begins to respire) and seedling emergence (begins with the emergence of the primary root). During germination of maize, wheat and field pea, the epicotyl elongates and the cotyledon(s) remain in the soil (hypogeal germination), while in soybean and sunflower, the hypocotyl grows above the soil (epigeal germination).

Our results showed that auxin had no significant effect on the germination energy of the tested crops compared to the control treatment (Table 2). In general, germination energy values were high in wheat (ranging from 90.2 at 0 μg ml^{-1} to 92.0 at 50 μg ml^{-1}), soybean (ranging from

88.0 at 0 μg ml^{-1} to 93.5 at 50 μg ml^{-1}), field pea (ranging from 90.0 at 5 μg ml^{-1} to 91.5 at 0.1 μg ml^{-1}) and sunflower (ranging from 88.8 at 0.1 μg ml^{-1} to 92.2 at 50 μg ml^{-1}).

Table 2. Effect of auxin of bacterial origin on seed germination and seedling growth of maize, wheat, soybean, field pea, and sunflower.

Parameters	Auxin concentration effects (A)	Crop				
		Maize hybrid 'ZP684'	Wheat cultivar 'Evropa 90'	Soybean cultivar 'Galina'	Field pea cultivar 'Kosmaj'	Sunflower hybrid 'NS Oskar'
Germination energy, %	0 μg ml^{-1}	37.8	90.2	88.0	90.8	89.3
	0.1 μg ml^{-1}	42.0	91.5	90.8	91.5	88.8
	5 μg ml^{-1}	40.0	91.8	89.2	90.0	91.8
	50 μg ml^{-1}	43.0	92.0	93.5	91.0	92.2
Germination, %	0 μg ml^{-1}	96.8	92.9^{b}	91.8^{c}	96.8^{b}	91.5^{b}
	0.1 μg ml^{-1}	98.2	93.8^{ab}	94.0^{bc}	98.5^{ab}	92.8^{ab}
	5 μg ml^{-1}	98.8	95.5^{a}	94.8^{ab}	100.0^{a}	92.8^{ab}
	50 μg ml^{-1}	98.5	95.8^{a}	97.0^{a}	99.8^{a}	94.7^{a}
Root length, cm	0 μg ml^{-1}	18.29^{c}	11.65^{d}	18.94^{b}	19.57^{b}	14.80^{b}
	0.1 μg ml^{-1}	22.79^{a}	18.49^{b}	18.70^{c}	22.54^{b}	19.50^{a}
	5 μg ml^{-1}	18.97^{b}	20.54^{a}	20.46^{a}	21.49^{b}	19.80^{a}
	50 μg ml^{-1}	18.28^{c}	16.2^{c}	20.47^{a}	24.71^{a}	20.88^{a}
Shoot length, cm	0 μg ml^{-1}	6.80^{d}	15.94^{d}	7.57^{d}	7.06^{b}	8.03^{d}
	0.1 μg ml^{-1}	9.03^{b}	17.28^{c}	9.33^{c}	8.42^{a}	9.93^{b}
	5 μg ml^{-1}	8.96^{c}	17.84^{b}	9.49^{b}	8.38^{a}	8.43^{c}
	50 μg ml^{-1}	10.16^{a}	18.36^{a}	10.61^{a}	6.34^{c}	10.17^{a}
Seed vigor	0 μg ml^{-1}	2427.5^{c}	2561.7^{b}	2432.3^{d}	2576.4^{b}	2158.4^{c}
	0.1 μg ml^{-1}	3126.3^{a}	3356.2^{ab}	2634.9^{c}	2852.6^{a}	2729.5^{b}
	5 μg ml^{-1}	2758.1^{b}	3663.4^{a}	2837.7^{b}	2987.0^{a}	2618.5^{b}
	50 μg ml^{-1}	2801.3^{b}	3309.2^{ab}	3014.8^{a}	2897.2^{a}	2939.0^{a}

Crop	Germination energy	Germination	Root length	Shoot length	Seed vigor
Maize	0.286^{ns}	0.596^{ns}	0.000**	0.000**	0.000**
Wheat	0.127^{ns}	0.007**	0.000**	0.000**	0.000**
Soybean	0.283^{ns}	0.001**	0.000**	0.000**	0.000**
Field pea	0.870^{ns}	0.005**	0.000**	0.000**	0.002**
Sunflower	0.128^{ns}	0.051*	0.000**	0.000**	0.000**

ns - not significant, * $p \leq 0.05$, ** $p \leq 0.01$.

Auxin of bacterial origin significantly increased germination, except in maize. The lowest germination was observed in the control treatment without auxin (wheat 92.9%, soybean 91.8%, field pea 96.8%, and sunflower 91.5%). In terms of germination, the crop seed meets the requirements established by the Rulebook on the quality of seeds of agricultural plants (Official Gazette of SFRY 47/1987) according to which the minimum germination for maize is 85%, wheat 82%, soybean 70%, field pea 75% and sunflower 80%. The hormone auxin regulates seed dormancy and germination (Li *et al*., 2016) primarily by mediating the ABA signaling pathway (Ye *et al*., 2016). Also, auxin had a significant effect on the root length of the tested crops compared to the control treatment, except for soybeans. Maize, wheat, field pea, and sunflower had the least root length in the control (18.29 mm, 11.65 mm, 19.57 mm, and 14.80 mm, respectively). However, soybean had the lowest root length (18.94 mm) in the 0.1 μg ml^{-1} treatment with auxin. The highest root length was obtained in maize (22.79 mm) in the treatment with 0.1 μg ml^{-1} auxin, in wheat (20.54 mm) in the treatment with 5 μg ml^{-1} auxin, in soybean in the treatment with 5 (20.46 mm) and 50 μg ml^{-1} auxin (20.47 mm), in field pea (24.71 mm) and sunflower (20.88 mm) in the treatment with 50 μg ml^{-1} auxin. Auxin increased shoot length in the crops, except for field pea, where treatment with 50 μg ml^{-1} did not increase the trait. In contrast, maximum shoot length was achieved in maize (10.16 mm), wheat (18.36 mm), soybean (10.61 mm), and sunflower (10.17 mm) when treated with 50 μg ml^{-1} auxin. Field pea had significantly higher shoot lengths in the treatments with 0.1 (8.42 mm) and 5 μg ml^{-1} (8.38 mm) auxin than in other treatments. Auxin influenced seed vigor. The highest seed vigor was obtained in maize (3126.3) in the treatment with 0.1 μg ml^{-1} auxin, in wheat and field pea in the treatment with 5 μg ml^{-1} auxin (3663.4 and 2987.0, respectively), and in soybean and sunflower in the treatment with 50 μg ml^{-1} auxin (3014.8 and 2939.0, respectively). Similar to our research, auxin of bacterial origin increases germination and the length of roots and shoots of maize seedlings (Lwin *et al*., 2012; Noumavo *et al*., 2013), wheat seedlings (Akbari *et al*., 2007; Fatima *et al*., 2009), soybean seedlings (Cvijanović *et al*., 2020) and sunflower seedlings (Miladinov *et al*., 2015; Miklič *et al*., 2016). The hormone auxin promotes root growth and thus tolerance to drought stress by improving nutrient uptake (Kurepa and Smalle, 2022). When plant seeds are inoculated with auxin (IAA-synthesising PGPB), they have a more developed root system and a greater height of the aerial part, a larger leaf area, and biomass, and ultimately a higher yield due to the elongation of primary roots and the formation of lateral and adventitious roots (Malhotra and Srivastava, 2009; Puente *et al*, 2010; Glick, 2012). Accordingly, young plants with a better developed root system are more stable because they are more connected to the substrate and their ability to absorb water and nutrients from the substrate increases. Auxin stimulates plant cell division, plant tissue expansion and differentiation, thereby stimulating seed germination and seedling rooting, the rate of xylem and root system development, and the control of vegetative growth processes (Hussain, 2010; Spaepen and Vanderleyden, 2011). Stanojević *et al*. (2014) and Đorđević *et al*. (2017) also indicated that bacterial auxin has a stimulatory effect on wheat and maize seedlings as it affects the elongation of roots and stems. In addition, inoculation of maize seeds with PGPB bacteria in the field increased the abundance, activity, and biodiversity of soil microorganisms, the resistance of stems to lodging and grain yield, and reduced the number of infertile plants (Mandic *et al*., 2016; Mandić *et al*., 2018).

Conclusion

Auxin of bacterial origin showed an effect on seed germination (except for maize) and seedling growth. Compared to the control, the significantly higher germination of wheat, soybean, and field pea was observed at 5 and 50 μg ml^{-1} auxin, and sunflower at 50 μg ml^{-1} auxin. Compared to the control, the significantly higher root length of maize was observed at 0.1 and 5 μg ml^{-1} auxin, wheat and sunflower at 0.1, 5, and 50 μg ml^{-1} auxin, soybean at 5 and 50 μg ml^{-1} auxin, and field pea at 50 μg ml^{-1} auxin. Auxin treatments significantly increased shoot length in the crops compared to the control, with the exception of 50 μg ml^{-1} auxin in field pea. In addition, auxin treatment significantly increased seed vigor in maize, soybean, field pea, and sunflower, and 5 μg ml^{-1} auxin in wheat. Accordingly, auxin of bacterial origin can be used to improve germination and seedling growth and can be a cost-effective commercial agricultural input.

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THE INFLUENCE OF HEAVY METALS ON THE GROWTH OF THE WHEAT STEM

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Abstract

Heavy metals are significant environmental pollutants because they represent potential metabolic inhibitors. The aim of the research was to determine the content and influence of heavy metals on tree development in the varieties *Pobeda* and *Ljiljana*. The tolerance of certain wheat genotypes to high concentrations of heavy metals was determined through comparative tests in vessels. The experiment was set up in vessels where two different concentrations of heavy metal mixture, 50 ppm and 250 ppm, were added under controlled conditions. Higher concentrations of heavy metalmixture had a negative effect on wheat stem height. Higher concentrations of heavy metals significantly reduced plant growth in both cultivars.

Key words: *Atomic absorption Spectrophotometry, Heavy metals, Wheat steam, Triticum sp.*

Introduction

Current agricultural practices involve widespread use of chemical pesticides and mineral fertilizers known for their ability to cause negative impacts on human health and degrade the natural environment (Rastija, 2022). Contamination alongside roads was evident through significant negative correlations between metal concentrations in soil and plants samples and distance from the road edge. Only cadmium concentrations in soil and plant samples were not associated with roadside pollution. Pollution of plants and soil alongside roads did not extend muchbeyond 20 meters from the road (Kanmani and Gandhimathi, 2013).

Differences in stress-induced effects of elevated concentrations of toxic metals indicate differenttolerance of plants to metals. Based on the results of depth variation of heavy metal concentration,it is concluded that heavy metal concentrations present in solid waste samples depend on the site conditions, as well as on the conditions prevailing at the sampling sites. Based on the average concentration, the concentration of heavy metals in the collected solid waste sample was determined in the following order: Mn > Cu > Pb > Cd (Zhao et al., 2017).

Research initiated in the 1960s has shown that soils in urban and industrial areas contain extremelyhigh concentrations of heavy metals. Plants are most sensitive to industrial pollution, which is whythey are successfully used as indicators to assess environmental pollution and establish adequate biomonitoring. The aim of this study is to determine the content of heavy metals in the leaves of Plantain (Plantago major L.) in the Tuzla area, as well as to assess the influence of thermal power
plants and industrial facilities on surrounding areas. It was found that the content of heavy metals in Plantain leaves often did not decrease with increasing distance from the dominant anthropogenicsources of heavy metal emissions (Bektić et al., 2020).

At the cellular level, the consequences of prolonged exposure to high metal concentrations can include membrane disintegration, ion loss, lipid peroxidation, DNA/RNA degradation, and ultimately cell death. For plants to develop and grow normally, they must maintain

concentrationsof essential elements within optimal values - a state of homeostasis (Stojanović, 2017).
Studies have shown that it is not the total concentration but the reactive fraction of heavy metals in the soil that dictates their toxicity to plants, microbes, and human beings. Exchangeable forms of heavy metals that are soluble in water are much more reactive and biologically available than precipitated species (Kim et al., 2015). However, the distribution of heavy metal species is influenced by numerous environmental factors, especially pH, redox potential, clay content, Fe/Mnoxide content, organic matter content, and the presence of other cations and anions in the solid phase (Pietrzykowski et al., 2014). To accurately assess the bioavailability of heavy metals in the soil, factors of influence must be taken into account.
In soil and sediment, heavy metals exist in various forms, such as: dissolved ions (e.g., $Cu^{2+,}$ Cd^{2+}, $CrO4^{2-}$, $Cr_2O_7^{2-}$ and MnO_4^{2-}), organic complexes (e.g., Cu^{2+}, Pb^{2+}, and Hg^{2+} bindingto dissolved organic matter), exchangeable ions (e.g., Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, and Pb^{2+}) adsorbed onto soil solid particles, and those (co-) precipitated as part of soil particles (e.g., $Cd_3(PO_4)^2$, ZnS, $PbCO_3$, and $HgSO_4$). These three forms maintain thermodynamic equilibrium in activities and mutual concentration with insoluble precipitate as the dominant species (Liu et al.,2019).

Materials and Methods

The study on the influence of heavy metal mixture concentration on the dynamics of heavy metal accumulation in wheat plants and plant growth was conducted through experiments in pots in the greenhouse of the Faculty of Agriculture in Zemun, where controlled conditions of temperature and humidity were maintained. The vegetative trial was arranged in three repetitions, with a total of 36 pots in which two varieties of wheat, *Pobeda* and *Ljiljana*, were sown. Before sowing, the pots were filled with 2 kg of dry extract of Novobalt, which was subsequently contaminated with a mixture of heavy metal chemical compounds in the form of solutions of the following compounds: zinc in the form of zinc acetate-$Zn(CH_3COO)_2$ x $2H_2O$, lead in the form of lead acetate $C_4H_6O_4Pb$ x $3H_2O$, chromium in the form of chromium trioxide-CrO_3, copper in the form of copper sulfate-$CuSO_4$, and cadmium in the form of cadmium nitrate-$Cd(NO_3)_2$ x $4H_2O$.
The following concentrations of solutions were applied: 0 ppm (control), 50 ppm, 250 ppm
In addition to the heavy metal content in wheat plants, the height of the plants from the soil level was first measured. Afterward, the heavy metal content in wheat plants was determined. The *Pobeda* and *Ljiljana* wheat varieties were chosen because they are predominantly grown in the southern Banat region. Twelve seeds were sown in each pot at a depth of 5 cm. Samples from the trial in pots were analyzed during the tillering and heading phases, where four plants were taken from each pot. After sampling, the roots were manually separated from the wheat stem. Subsequently, the plant mass-stems were washed with distilled water and kept for several hours in 0.1 M HCl to remove soil and mineral oxides from the surface. Then, the plant mass was ground and dried in a dryer at 80˚C. A 1 g sample was taken and poured with 20 ml of 60% HNO_3. Mild boiling was performed for 2 hours. After cooling, 3 ml of H_2O_2 was added, followed by boiling for 15 minutes. The peroxide procedure was repeated. After cooling, 2 ml of $HClO_4$ was added, and mild evaporation was performed until dense white fumes of perchloric acid appeared (Jones and Case, 1990). After cooling, 5 ml of 5M HCl was added, and then the samples were quantitatively transferred into normal 50 ml vessels. The vessels were filled up to the final volume with distilled water. The solution was filtered through quantitative filter paper. Readings were performed using atomic absorption spectrophotometry (Varian Spectr AA 220FS instrument) in acetylene/air flame. The analysis of the obtained data was conducted using the statistical softwarepackages STATISTICA 8 for Windows and SPSS Statistics 17.0.

Results and Discussion

Plant height

The variety *Pobeda* had a slightly lower average height (39.40 cm), while the variety *Ljiljana* had a higher average height (44.50 cm). The average height of the tree per sample for the variety *Pobeda* varied from 34.00 to 42.60, and for the variety *Ljiljana* from 35.50 to 50.00 (tables 1 and 2).

Table 1. Plant Height, *Pobeda* Variety, cm

Treatment	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	42.60	7.9889	14.6647
50 ppm	41.60	8.1768	15.0097
250 ppm	34.00	1.8371	3.3723
Mean	39.40		

Table 2. Plant Height, *Ljiljana* Variety, cm

Treatment	Zn, Pb, Cr, Cu, Cd	LSD 5%	LSD 1%
Control	50.00	1.8371	3.3723
50 ppm	46.00	14.5818	26.7668
250 ppm	35.50	5.1143	9.3881
Mean	44.50		

During metal mixture contamination at a concentration of 50 ppm, the *Ljiljana* variety had a plant height of 46.00cm, while the *Pobeda* variety had a slightly lower height of 41.60 cm. At aconcentration of 250 ppm, the plant height for the *Ljiljana* variety was 35,50 cm, while for the *Pobeda* variety, it was lower at 34.00 cm.

The process of photosynthesis is most sensitive to the toxic effect of lead. Long-term exposure causes inhibition of chlorophyll biosynthesis (Ernst et al., 2000; He et al. 2014). Studies have shown that lead negatively affects photosynthesis, transpiration, and stomatal conductivity when its concentration in the soil exceeds 300 ppm (Fu and Wang, 2015). Differences in the effects caused by stress due to elevated concentrations of toxic metals indicate varying plant tolerance tometals (Zhao et al., 2017).

Under the influence of higher concentrations of lead in plants are main processes inhibited, suchas germination, growth and development, photosynthesis process, water intake, mineral diet andenzymatic activity (Agami and Mohamed, 2013; He at al., 2016; Sharma and Dubey, 2005).

The content of heavy metals in the stem of wheat

The content of heavy metals in the *Pobeda* and *Ljiljana* varieties varies significantly (Tables 3 and 4). According to their average content in wheat plants, the heavy metals investigated in thePobeda variety can be ranked in the following order:

zinc > cadmium > copper > lead > chromium

The lead content of the *Pobeda* variety in the wheat stem is on average 3.77 mg kg $^{-1}$ and varies from 2.46 mg kg $^{-1}$ (control) to 5.45 mg kg $^{-1}$ (variant with 250 ppm). The cadmium content of the *Pobeda* variety in the wheat stem is on average 4.99 mg kg $^{-1}$ and varies from 0.65 mg kg $^{-1}$ (control) to 9.12 mg kg $^{-1}$ (variant with 250 ppm).

According to their average content in the wheat stem of the *Ljiljana* variety, the investigated heavymetals can be arranged in the following order:

zinc > cadmium > copper > lead > chromium

The lead content of the *Ljiljana* variety in the wheat stalk is on average 3.08 mg kg $^{-1}$ and varies from 2.14 mg kg $^{-1}$ (control) to 4.33 mg kg $^{-1}$ (variant with 250 ppm).

The cadmium content of the *Ljiljana* variety in the wheat stem is on average 9.05 mg kg $^{-1}$ and varies from 0.39 mg kg $^{-1}$ (control) to 17.31 mg kg $^{-1}$ (variant with 250 ppm).

Table 3. Content of Heavy Metals in Wheat Plants, *Pobeda* Variety mg kg^{-1}

Treatment	Zn	Pb	Cr	Cu	Cd	Ftest	LSD5%	LSD1%
Control	19.39	2.46	1.23	4.49	0.65	38832.27**	0.1197	0.1655
50 ppm	22.80	2.78	1.63	4.13	5.20	14839.17**	0.2168	0.2998
250 ppm	49.10	5.45	1.75	5.54	9.12	49105.52**	0.2678	0.3703
Mean	30.43	3.77	1.54	4.72	4.99	-	-	-

Table 4. Content of Heavy Metals in Wheat Plants, *Ljiljana* Variety mg kg^{-1}

Treatment	Zn	Pb	Cr	Cu	Cd	F test	LSD5%	LSD1%
Control	29.99	2.14	0.58	4.65	0.39	38832.27**	0.1197	0.1655
50 ppm	69.93	3.13	0.64	5.05	9.45	37006.43**	0.4607	0.6372
250 ppm	79.13	4.33	1.23	5.41	17.31	58501.43**	0.4087	0.5652
Mean	59.68	3.2	0.82	5.04	9.05	-	-	-

Conclusion

The vegetative trial in pots was conducted with different concentrations of heavy metal mixtures. Increasing the concentration of metal mixtures had a negative impact on plant height. Higher concentrations of heavy metals significantly reduced plant growth in both varieties. The results of the statistical analysis (analysis of variance) show that the experimental factor - the concentration of heavy metal mixtures for soil contamination - had a very significant impact on the content of heavy metals in wheat plant parts, as evidenced by the calculated F-values.

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Zhao S., Fan Z., Sun L., Zhou T., Xing Y., Liu L. (2017*)*. Interaction effects on uptake and toxicity of perfluoro-alkyl substances and cadmium in wheat (*Triticum aestivum L.*) and rapeseed (*Brassica campestris L.*) from co-contaminated soil. Liaoning: Ecotoxicology andEnvironmental Safety. 137:194-201.

FORAGE YIELD AND WEEDNESS OF THE INTERCROPPED SPRING VETCH AND OATS

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Abstract

Intercropping of annual legumes and cereals reduces the weediness of the plot and increases the forage yield. Legumes have a beneficial effect on the soil, leaving respectable amounts of nitrogen and maintaining soil fertility. On the other hand, cereals reduce the laying of legumes, representing their support. The aim of the research is to determine the influence of combined sowing of common vetch and oats in different ratios on forage yield and weediness. The experiment was set up in 2022, in the village Radoinja, municipality of Nova Varoš. The trial variants were spring vetch in pure sowing, oats in pure sowing, vetch 75% : oats 25% and vetch 50% : oats 50%. Average forage and hay yields of pure crops and mixtures did not differ significantly. The share of weeds in the pure vetch crop was high, on average 80%. In the mixtures of vetch and oats, the share of weeds was significantly reduced compared to the pure vetch crop. The lowest number of weed plants was in the pure oat crop and in the mixture with 50% vetch and 50% oats.

Keywords: *vetch, weeds, oats, yield, intercropping*.

Introduction

Annual legumes are one of the most important plant groups in conventional agricultural production systems and are an indispensable component of sustainable agricultural systems, i.e. organic production. A greater proportion of annual legumes in forage production systems can be ensured by growing them in a mixture with small grains. As a result of the complementarity between the components in the use of agroecological resources, the advantages of such mixtures are mainly reflected in higher forage yields compared to the yields of pure crops of the components (Bedoussac and Justes, 2010; Chapagain and Riseman, 2014). Better control of weed flora as well as biotic and abiotic stress are further potential advantages of such mixtures compared to pure plant components (Anil *et al.*, 1998; Semahegn, 2022). In forage production systems, mixtures of annual legumes and cereals have a number of advantages over their pure counterparts. One of the most important is the supply of nitrogen to the soil, which comes from nitrogen fixation, and thus the preservation of its chemical properties. Annual legumes generally have a relatively low forage yield potential, especially in regions with low rainfall. They are also difficult to harvest as they tend to lie down. The complementarity of legumes and cereals in terms of utilization of agroecosystem resources (nutrients, light, water) is often manifested in higher yields and better forage quality when grown in intercropped compared to pure legume and cereal crops (Balabanli *et al.*, 2010).

In all production systems, annual legumes as components of intercrops provide a far more sustainable source of nitrogen through symbiotic nitrogen fixation (Crews and Peoples, 2004). Cultivation of legumes and cereals as intercrops can also minimize nitrogen losses, which are more pronounced with pure legumes, mainly through the uptake of inorganic nitrogen by the cereals and the reduced rate of mineralization during the decomposition of organic matter, due

to the higher C/N ratio in cereals (Hauggaard-Nielsen *et al.*, 2003). The intercropping of legumes and cereals can provide numerous advantages, such as: increased biomass yield and utilization of soil resources, increased yield stability, improved utilization of nutrients, water and light, as well as control of weeds, diseases and pests (Ton, 2021; Semahegn, 2022).
The aim of the work was to determine the degree of forage yield and weediness of a intercropped of spring vetch and oats at different combinations.

Materials and methods

The experiment was carried out in 2022 in the village Radoinja, near Nova Varoš (43^0 30'41" N, 19^0 42'59" E, 790 m above sea level) in Serbia. The soil on which the experiment was carried out is mountain black soil on limestone (calcomelanosol), up to 70 cm deep, the arable layer is up to 30 cm. The parent substrate is limestone, loose and present in the subsoil horizon. The soil has a neutral reaction (pH KCl = 6.90), rich in humus (5.15%), very carbonated (18.25%), rich in nitrogen (0.25%), well supplied with potassium (30.90 mg/100 g) and very poor in phosphorus (5.00 mg/100 g).
The climate in the area of the municipality of Nova Varoš is determined by the altitude and relief. The lowest temperatures are in January, and the hottest months are July and August. The daily air temperatures during July and August are very high, but the nights are cool, so the average values are not high (16.6 and 18.2 ℃). The average air temperature in 2022 was 8.8 ℃. The sum of precipitation during 2022 was 724.9 mm. The most precipitation in 2022 was during May and September. The least amount of precipitation was in October, March and July.
The basic tillage of the soil was done in autumn by plowing to a depth of 25 cm. With the basic treatment, 300 kg ha^{-1} of NPK fertilizer formulation 16:16:16 was introduced. The experiment was set up according to a completely randomized block system in 3 repetitions, with the size of an elementary plot of 5 m^2 (5 x 1 m). The trial variants were: vetch - pure crop, 120 kg ha^{-1} (A1); oats - pure crop, 120 kg ha^{-1} (A2); vetch + oats - 75%+25%, 90 kg + 30 kg ha^{-1} (A3) and vetch + oats - 50%+50%, 60 kg + 60 kg ha^{-1} (A4).
Sowing was done on March 27, 2022, with an inter-row distance of 20 cm, and the row distance was 4 cm, at a depth of 4-6 cm. The spring vetch cultivar Ovčepoljska and the oat cultivar Lovćen were used for testing.
The weed flora and the number of weeds were determined twice. The first weed count per 1 m^2 was done when the vetch was 20 cm high, and the second before mowing the crop. Mowing of the green mass of the crop was carried out in the phase of full flowering of vetch on July 8, 2022.
After mowing, the forage yield was measured. The forage yield was recalculated based on the mass of fresh forage from the elementary plot and expressed in t ha^{-1}. Then, a sample of 1 kg of green mass was taken using the method of random selection, and the weight share of vetch, oats and weeds was determined from it. Then the sample was left to dry naturally at room temperature. After drying, the share of vetch, oats and weeds in dry matter was calculated. The dry matter content was determined as the proportion of the dry sample in relation to the fresh green sample. Hay yield (t ha^{-1}) was determined by multiplying the total amount of green biomass with the content of dry matter.
The results were processed by the one-factor analysis method (ANOVA), using the SPSS 4.5 program. The significance of the differences in treatment means was tested by the LSD test.

Results and discussion

Based on the results of the first weed count, 11 weed species were recorded (Table 1). Redroot pigweed (*Amaranthus retroflexus* L.) was the most abundant in the crops, followed by white goosefoot (*Chenopodium album* L.), potato weed (*Galinsoga parviflora* L.), Persian speedwell (*Veronica persica* L.) and field sowthistle (*Sonchus arvensis* L.).
The higest number of weed plants was detected in the pure vetch crop (173 plants m^{-2}), while in the pure oat crop there were 129 plants per 1 m^2. The number of weed plants in the mixtures decreased compared to the pure vetch crop (144 and 110 plants per m^2). The fewest weeds were in the mixture with 50%+50% of vetch and oats. With the increase in the share of oats in sowing, the number of weed plants also decreased.

Table 1. Floristic composition of weeds and number of plants per m^2

Weed name	First count				Second count			
	A1	A2	A3	A4	A1	A2	A3	A4
Redroot pigweed (*Amaranthus retroflexus* L.)	127	60	79	64	77	33	44	29
White goosefoot (*Chenopodium album* L.)	10	39	16	12	8	11	7	8
Potato weed (*Galinsoga parviflora* L.)	17	6	11	9	0	0	0	7
Persian speedwell (*Veronica persica* L.)	12	3	12	11	0	0	0	0
Field bindweed (*Convolvulus arvensis* L.)	4	3	1	4	0	11	0	0
Field buttercup (*Ranunculus arvensis* L.)	1	9	0	4	0	4	0	0
Field sowthistle (*Sonchus arvensis* L.)	1	0	17	0	13	4	16	4
Chickweed (*Stellaria media* L.)	1	1	7	3	4	0	0	0
Charlock mustard (*Sinapis arvensis* L.)	0	3	0	0	0	0	0	0
Birthwort (*Aristolochia clematitis* L.)	0	1	1	3	0	0	4	4
Pale smartweed (*Polygonum lapatifolium* L.)	0	4	0	0	0	4	0	0
In total	173	129	144	110	105	67	71	52

A1-vetch, A2-oats, A3- vetch 75% + oats 25%, A4- vetch 50% + oats 50%

Based on the results of the second count, the highest number of weed plants was also counted in the pure vetch crop (105 plants) (Table 1). There were 67 plants in the pure oat crop, and 71 and 52 plants in the mixtures. The least amount of weed plants was in the 50% vetch + 50% oat treatment, due to strong oat competition.
During the second count, 9 weed species were identified. Among them, the most numerous were redroot pigweed (*Amaranthus retroflexus* L), white goosefoot (*Chenopodium album* L.), field bindweed (*Convolvulus arvensis* L.) and field sowthistle (*Sonchus arvensis* L.).
Based on the results of the first and second weed counts, the highest weediness was in the pure vetch crop. In the mixture with 75% vetch and 25% oats, there were fewer weeds than in the vetch pure crop, more compared to the pure oats crop. The mixture with 50% vetch and 50% oats had the fewest weeds.

Average forage and hay yield of pure crops of vetch and oats, as well as their mixtures, did not differ significantly. The lowest forage and hay yield was recorded with pure vetch (23.38 t ha^{-1} and 7.40 t ha^{-1}), and the highest with a pure oat crop (30.61 t ha^{-1} and 9.69 t ha^{-1}) (Table 3). The results are in agreement with the results of other authors (Lithourgidis *et al.*, 2006; Tuna *et al.*, 2007; Ansar *et al.*, 2013). On the basis of previous research, related to the methods of combining intercropping of cereals and grain legumes and the impact on yield and yield stability, it was determined that cultivation in a mixture is a significantly more reliable method of production than individual cultivation of this forage crops (Raseduzzaman and Jensen, 2017).

Table 2. The forage yield, hay yield and the share of dry matter in the total yield of spring vetch and oat crops

	Forage yield (t ha^{-1})	Hay yield (t ha^{-1})
A1- vetch	23.38	7.40
A2- oats	30.61	9.69
A3- vetch + oats 75%:25%	27.33	8.65
A4- vetch + oats 50%:50%	30.15	9.54

Based on the obtained results, the share of vetch in the pure crop and mixtures did not differ significantly. The share of vetch had values from 8.75 to 20.61%. The proportion of oats in the A3 treatment was significantly lower compared to the pure oat crop (A2) and to the A4 treatment (Table 3).

Table 3. Share of vetch, oats and weeds in hay

	Vetch	Oats	Weeds
A1- vetch	20.61a	-	79.39a
A2- oats	-	95.44a	4.56c
A3- vetch + oats 75%:25%	22.44a	57.29b	20.27b
A4- vetch + oats 50%:50%	8.75a	83.73a	7.57bc

Values marked with different lowercase letters per column are significantly different at the P≤0.05 level according to the LSD-test

The share of weeds was the highest in the pure vetch crop (79.39%). A large share of weeds in a pure crop of vetch is a consequence of later sprouting and dead vetch stem in 2022. Weeds have taken over the surface of the soil and overgrown the vetch. The lowest share of weeds was recorded on the variant with oats in pure sowing. On the variants with combined sowing of vetch and oats, a significantly lower share of weeds was recorded compared to the pure vetch stand. In treatments A3 and A4, the share of weeds was significantly lower compared to the pure vetch crop. Treatment A4 had 7.52% weeds and treatment A3 20.27% weeds, and they did not differ significantly for this trait. Simić *et al.* (2018) also came to similar research. Intercropping, i.e. growing crops in a mixture, is a good measure to reduce weediness (Dolijanović and Simić, 2015).

Oats had favorable conditions for growth and development in 2022 and sprouted before the weeds and suppressed the weeds with their further intensive growth. For this reason, there are the fewest weeds in the pure oat crop. As the share of oats in the mixture increased, the share of weeds decreased.

Conclusion

Based on one year results (2022), forage and hay yield on varieties with pure crops and mixtures did not differ significantly. The share of weeds in hay was the highest in the pure crop of vetch, the lowest in the pure crop of oats, and in the mixtures it was significantly lower compared to the pure crop of vetch. The number of weed plants was the highest in the pure crop of vetch, and lowest in the pure crop of oats and in varieties with 50%+50 vetch and oats in both counts. The lowest share of weeds was recorded on the treatment with 50% of vetch and oats. Cultivation of intercropping leguminous and grain crops can provide numerous advantages, such as: increased biomass yield and adequate utilization of land resources, increased yield stability as well as more effective control of crop weediness.
Because, both crop production and weed infestation depend in a high level of the meteorological conditions of the year, the investigations will continue in next years.

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EFFECT OF DIFFERENT MICROBIAL FERTILIZERS ON THE MORPHOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF MAIZE

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Abstract

The maize cultivation trial was conducted at the Research and Experimental Field "Radmilovac" of the Faculty of Agriculture in Belgrade in 2022 on the soil type luvic chernozem in completely randomized blocks. The cropping system included tillage with a disk harrow at 25-30 cm with complete incorporation of winter wheat crop residues and tillage with a harrow before sowing. Basic fertilizer was applied in the fall at 500 kg ha^{-1} NPK (15:15:15). The following microbiological fertilizers were used for top dressing in spring: Biofertilizer ("Slavol", manufacturer "Agrounik" Serbia) with 5 l ha^{-1} in two treatments and Eko lame with 10 l ha^{-1} in 3 treatments. The top dressing in the control variant was done with nitrogen fertilizer AN at the rate of 60 kg ha^{-1} N. Maize varieties (ZPSC 666) were used. The maize was grown in a six crop rotation. Statistical analysis confirmed that top dressing had a greater effect on morphological, productive characteristics and grain yield of maize. The highest grain yield was achieved in the variant with the “Eko Lame” fertilizer (5900 kg ha^{-1}) and the lowest in the control treatment (3500 kg ha^{-1}). The differences achieved were statistically very significant. In addition to the analysis of the grain yields obtained, a similar trend was found by comparing the morphological and productive characteristics of the maize plants obtained. The correlation-regression analysis of the obtained data showed a statistically positive dependence of the grain yield on the height of the plant, the length and width of the maize cob, and on the mass of grains per plant and the kernel weight.

Keywords: fertilizer, yield, maize, top dressing.

Introduction

Maize (*Zea mays* L.) is the most important cereal in Serbia in terms of agriculture. In Serbia, maize was cultivated on an area of 923 thousand hectares with a production of 6.6 million tons and an average productivity of 7200 kg ha^{-1} in recent years. Compared to the ten-year average (2013-2022), maize production is 5.0% higher. In Serbia, maize productivity is low compared to global productivity, which is due to several limiting factors. Among other things, weed infestation and poor weed control methods lead to yield losses. In addition, the timing of sowing and the selection of maize hybrids should be better coordinated, especially taking into account climatic changes. Adhering to the principles of crop rotation and increasing the frequency of cultivation have an invaluable positive impact (Dolijanović et al., 2017). The reduction in soybean acreage and the lack of another, alternative crop for the simplest three-field rotation has an impact on the decline in yields of maize and also winter wheat.

One of the most important issues in modern maize cultivation is the question of fertilizer selection and application. This agrotechnical measure must first and foremost be adapted to the type and quality of the soil, the selected hybrid and the intensity of production. The role of

mineral fertilizers and manure is invaluable. As there is less and less liquid manure in recent times, the use of microbiological fertilizers is a rational solution. They play a key role in the mineralization of organic compounds, the mobilization of poorly soluble inorganic compounds as well as in the supply of nutrients to plants and in yield formation. To feed the growing population, the increase in grain production depends critically on the supply of chemical fertilizers that can compensate for nutrient deficiencies (Zhang et al., 2019). However, the excessive use of chemical fertilizers can reduce nutrient use efficiency and potentially lead to soil acidification (Guo et al., 2010), eutrophication (Zhang et al., 2011), greenhouse gas emissions and N leaching (Li et al., 2016). Previous research has shown that the use of microorganisms in crop production increases the accessibility and efficiency of nutrients, which can reduce the need for mineral fertilizers by 50% without reducing crop yield (da Costa et al., 2013). Microbial fertilizers help plants to grow healthy and stay strong as they contain fertilizers and beneficial microorganisms. Microbial fertilizers (MF), also known as organic fertilizers, are environmentally friendly as they are mainly composed of food and agricultural wastes (animal manure, straw, etc.) and beneficial microorganisms (Sahin and Yilmaz, 2023).
The aim of this work is to investigate the effect of microbial fertilizers (by seed and foliar treatments) on grain yield and yield components of maize, in addition to the basic fertilizer applied in autumn.

Material and methods

The trial on maize cultivation with low-input technology was conducted in 2022 at the research and experimental field "Radmilovac" of the Faculty of Agriculture of the College of Belgrade (Serbia) on the soil type luvic chernozem in completely randomized blocks. The cropping system included tillage with a disk harrow at 25-30 cm with complete incorporation of the previous crop residues and tillage with a harrow before sowing. The basic fertilization was carried out in autumn with 500 kg ha^{-1} NPK (15:15:15). The following microbial fertilizers were used for top dressing in spring: Biofertilizer ("Slavol", manufacturer "Agrounik" Serbia) with 5.0 l ha^{-1} in two treatments and Eko lame 10 l ha^{-1} in three treatments. Top dressing in the control variant was carried out with the nitrogen fertilizer AN at a rate of 60 kg ha^{-1} N. The maize varieties (ZPSC666) were used. Cultivation was carried out in a six-crop rotation (winter wheat-maize-summer barley+red clover-red clover-soybean-sunflower). Maize was sown on April, 15. The size of a one-year crop rotation field (field) was about 10 ares.
The application of microbial fertilizers and herbicides in maize cultivation was carried out according to the schedule shown in Table 1. The seeds were treated 24 hours before sowing and the other treatments were applied over the leaves using a hand sprayer designed for this type of experiment.

Table 1. Schedule of application of microbiological preparations and application of herbicides

Preparation/ Date	Seed treatment	First treatment-foliar	Weediness evaulation	Herbicide	Second treatment -foliar	Third treatment-foliar
Eko lame	14.04.	17.05.	30.05.	31.05.	03.06	17.06
Slavol	14.04.	17.05.	30.05.	31.05.	03.06	-

For study of the morphological and productive characteristics of maize, on each treatment the samples of 10 maize plants were taken on September 13. These plants were used for

analysis the stalk height, cob diameter, number of rows of grains in the cob, number of grains, cob length, weight of 1000 seeds, grain yield per plant and per unit area (per ha).
The data obtained were statistically evaluated using analysis of variance and correlation analysis with the Pearson coefficient, taking into account the microbial fertilizers as factors, while the LSD test was used for the individual comparisons.
The meteorological conditions during the growing season of maize, especially the optimum average air temperatures, had a significant effect on the formation of high yields. The data in Table 2 show that low rainfall in May and June had an impact on yield reduction this year. The higher rainfall in September had an effect on later ripening and higher moisture content of maize grains in varieties without microbiological fertilization.

Table 2. Average air temperatures and amount of precipitation from April to September at Radmilovac

Months	Temperature (°C)	Precipitation (mm)
April	12.3	80.1
May	20.3	32.2
June	24.8	43.3
July	25.8	63.9
August	25.0	89.7
September	18.0	98
Average/Sum	21.03	407.2

Results and discussion

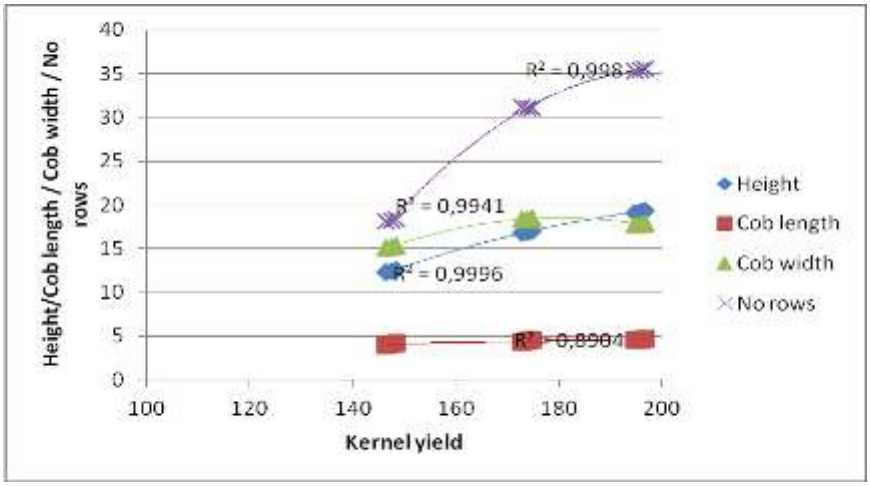
Based on the data on the influence of the studied preparations on the morphological and productive characteristics of maize plants , we can see that the application of the preparations influences the increase in the value of all the studied characteristics (Table 3). It can be seen that all investigated traits were statistically significantly or very significantly different in the fertilizer application variants compared to the control variant. The growth and development of maize plants is partly determined by the activity and abundance of microorganisms introduced into the rhizosphere by fertilizers (Wang et al., 2024), as well as by the application of microbiological foliar fertilizers. The results showed the lower moisture content of the grain when using the Eko-Lame preparation, what is important advantage for easier storage, a lower risk of storages diseases and pests, as well as for reduction in the cost of drying the grain.

Table 3. The influence of investigated microbiological fertilizers on the morphological and productive properties of maize

Parameters/Treatments	Control	"Eko Lame"	Slavol	Average
Plant height (cm)	147.4[a]	195.7[c]	173.8[b]	172.3
Cob length (cm)	12.4[a]	19.2[c]	16.9[b]	16.2
Cob width (cm)	4.1[a]	4.6[c]	4.4[b]	4,4
No of rows of grain in the cob	15.2[a]	17.8[b]	18.4[c]	17.1
No of grains in the row	18.2[a]	35.4[c]	31.1[b]	28.2
Grain weight per plant (g)	53.6[a]	151.7[c]	121.3[b]	108.9
Mass applause (g)	11.5[a]	29.3[c]	24.8[b]	21.9
Weight 1000 seeds (g)	227.1[a]	254.8[b]	250.7[b]	244.2
Seed moisture (%)	16.9[a]	16.3[a]	18.5[b]	17.2
Grain yield (kg/ha)	3500[a]	5900[c]	4900[b]	4767

Values of means followed by the same letter are not significant.

All morphological and productive characteristics of the maize plants showed higher values when using the Eko Lame preparation compared to Slavol, although the number of treatments, which is higher with the first preparation, must also be taken into account. Grain yield, the most important parameter in this study, was statistically significantly increased by the application of microbiological fertilizers (Table 3). In a study on the inoculation of maize with nine Azospirillum strains, a yield increase of 24–30 % was observed (Hungria et al., 2010), with an increase in maize grain yield of 17 (Cavallet et al., 2000) and 20-21 % (Zambrano Gavilanes et al., 2023). In the research Pacheco et al. (2021), was found that microbial inoculants under field conditions, clearly showed that microbial inoculants increased maize productivity while improving P (Phosphorus) use efficiency and reducing P concentration in the grain. Furthermore, the use of these microbial inoculants proved to be compatible with conventional agricultural management practices. This is very important as rock phosphate (a high quality P source used in conventional agriculture) is a non-renewable, finite resource whose reserves are rapidly being depleted, so prices are expected to increase dramatically in the near future (Dias et al., 2015).
The correlation analysis revealed a statistically very high dependence of maize grain yield on the height of the maize plants and the length and width of the maize cob (Figure 1). Only developed maize plants with a growth habitus that can carry a large ear or even two ears can guarantee grain yields in the field that come close to the genetic fertility potential of these hybrids. In the experiments, Srećkov et al. (2023) found the strongest interdependence between grain yield and the number of grains in a row and, for the other traits studied, between ear length and the number of grains in a row.



Graph 1. Correlation-regression analysis of certain characteristics of maize

Conclusion

The results of this work show that microbial fertilizers have a positive effect on the tested characteristics of maize in a dry climate without irrigation. Microbial fertilizers which were used contain microbes that promote plant growth at the beginning of the growing season and increase grain yield. The applied microbiological fertilizers in in one-year research showed a positive effect on increasing grain yield on treatment with Eco-lime (5.900 kg ha^{-1}) on treatment with Slavol (4.900 kg ha^{-1}) in comparison to control (3.900 kg ha^{-1}), In this study established that grain yield of maize the mostly varied in dependance to the height of the maize plant and the length and width of the cob.

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EFFECT OF AMMONIUM THIOSULFATE NITROGEN FERTILIZER ON QUALITATIVE PARAMETERS OF AROMATIC PLANTS

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Abstract

The yield of medicinal plants and the quality of their active compounds are influenced by various factors, including soil composition and nutrient content. The extent of yield increase depends on soil fertility, which can be enhanced with fertilizers. The aim of this study was to evaluate the effect of applying a nitrogen fertilizer based on ammonium thiosulfate on qualitative properties of selected plants. The content of chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b*, and essential oils was investigated in selected spice and aromatic plants, including *Mentha* × *piperita* 'Danica', *Ocimum basilicum* 'Dark Green', and *Melissa officinalis*. All selected species were grown in two variants: the Control variant was grown without the use of fertilizer, and the ATS variant, where a nitrogen fertilizer based on ammonium thiosulfate was applied. The fertilizer was applied to the soil in two repetitions at a dose of 2x 6 ml/m². Two harvests were conducted, and the content of chlorophyll *a* and *b* in the fresh biomass was determined spectrophotometrically, while the content of essential oils in the dry biomass was determined by steam distillation. Results suggest that the application of nitrogen fertilizer did not significantly affect the levels of chlorophyll *a*, chlorophyll *b*, or essential oils. The experiment was a part of a larger research project that primarily examined indicators of crop yields when nitrogen fertilizer was applied. The findings serve as the foundation for prospective future scientific research, from the perspective of quality parameters.

Keywords: *mint, basil, lemon balm, ammonium thiosulfate, chlorophyll a, chlorophyll b, essential oil.*

Introduction

Aromatic plants and their bioactive compounds have been utilized for thousands of years in traditional medicine, and contemporary efforts persist to study and apply them in pharmacology (Rasool et al., 2020). For centuries, humans have depended on aromatic plants for sustenance, flavouring, and healing. Various parts of these plants—seeds, leaves, flowers, fruits, stems, and roots - are rich in bioactive compounds, offering significant therapeutic properties (Sun and Shahrajabian, 2023). Aromatic plants not only enhance the flavour and aroma of food and aid in preservation but also possess medicinal properties (Gonçalves et al., 2020). They contain essential oils, phenolic compounds, fibre, vitamins, and minerals (Fierascu et al., 2021). The family *Lamiaceae* comprises more than 7,000 plant species. Many of these plants contain valuable essential oils and include medicinal, aromatic, honey-producing, and ornamental species (Khoury et al., 2016). *Mentha* × *piperita* 'Danica', *Ocimum basilicum* 'Dark Green', and *Melissa officinalis* all belong to this plant family. There are many factors which can influence the yield of herbs and a quality of content substances, such as soil structure and a content of nutrients. The extent of yield increase depends on soil fertility which can be increased by the usage of fertilizers (Kováčik, 2013). Fertilization and nutrition of spice, aromatic, and medicinal plants require a specific approach due to differences in cultivation methods, duration, and nutrient requirements throughout their growth period. Important principles include considering agroecological conditions and individual plant nutrient needs, calculating fertilizer doses based on soil chemical analysis, and protecting soil quality and the environment. Factors such as soil type, rainfall, and soil pH must also be considered. Fertilizers should be applied no later than six weeks before harvest for medicinal plants (Habán et al., 2013). Industrial fertilizers, made chemically or physically from mineral raw materials, provide essential nutrients that improve soil fertility, yield, and product quality. These nutrients are present in various inorganic or organic compounds in different forms, concentrations, and ratios, contributing to better quality, longer shelf life, disease resistance, and resilience to adverse weather conditions (Varga and Ducsay, 2015). Fertilizing medicinal herbs with nitrogen (N) and sulphur (S) is essential for their optimal growth and development. Nitrogen is crucial for the formation of leaves and biomass, as it is a key component of chlorophyll (Habán et al., 2013). Symptoms of nitrogen deficiency include stunted growth, thin stems, yellowing leaves, and reduced chlorophyll and protein synthesis (Kalina, 2005; Varga and Ducsay, 2015). Sulphur is essential for amino acids and protein synthesis (Kováčik, 2013). Fertilizers combining both nutrients are often used to meet the plants' simultaneous needs for nitrogen and sulphur, ensuring better flavour, health value, and overall quality of the herbs (Kalina, 2005). This study aims to determine the effect of ammonium thiosulfate-based nitrogen fertilizer application on the content of chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b*, and essential oils in selected spice and aromatic plants, including *Mentha* × *piperita* 'Danica', *Ocimum basilicum* 'Dark Green', and *Melissa officinalis*.

Material and Methods

Plant material

In this study, three species of aromatic plants were cultivated: *Ocimum basilicum* 'Dark Green', *Mentha* × *piperita* 'Danica' and *Melissa officinalis*. The seeds were purchased from the company Semo, the mint seedlings were taken from cultivar collection of Botanical Garden, SUA in Nitra.

Fertilizer characterization

The ammonium thiosulfate-based nitrogen fertilizer used in this study is a liquid containing sulphur, characterized by a clear to yellow-brown colour. According to Stercorat company, this fertilizer comprises of ammonium thiosulfate, bisulphite, sulphate, and water, with at least 11% of total nitrogen content, 11% of ammoniacal nitrogen, and 25% sulphur by weight. With a pH of 6.5-7.8, it provides rapidly absorbable ammoniacal nitrogen and dual oxidation state sulphur, ensuring optimal plant availability. The fertilizer decomposes to provide both immediate and sustained sulphur supply. Recommended application rates are 15-230 litres per hectare annually, and it can be applied via foliar spray, band placement, pre-plant soil preparation, furrow irrigation, drip irrigation, or hydroponics. For medicinal, aromatic, and ornamental plants, the suggested dosage is 30-60 litres per hectare. The goal is to enhance yield, durability, and plant vitality (Stercorat, 2016).

Characterization of an experimental site soil and climatic conditions

The Nitra region is located in the southwestern part of Slovakia in the Danubian Lowland. It is known for its high-quality soil types and subtypes, including brown soils, chernozems, black soils, and fluvisoils, making it one of the most productive agricultural areas in Slovakia. It has a warm climate with long, hot, dry summers and short, mild, dry winters, making it one of the warmest and driest areas in Slovakia. Average annual precipitation ranges from 500-600 mm, and the average annual temperature is around 9-10°C (Hreško, 2006). Soil samples were taken in spring 2019, showing in Table 1 a medium nitrogen content, and given the nitrogen fertilizer testing, no additional fertilization was applied.

Table 1: Soil analysis of the experimental area

	pH	Nan	Nutrient content ($mg.kg^{-1}$) (Mehl.III)					Cox (hummus)
		$mg.kg^{-1}$	P	K	S	Ca	Mg	%
2019	6.89	13.0	77.5	495.0	5.0	5200	964.0	3.85

The data of climatic conditions (Table 2) monitored at the experimental site for 2019 were provided by the Institute of Landscape Engineering of Slovak University of Agriculture in Nitra using meteorological station.

Table 2: Evaluation of temperature and precipitation during experiment according to air temperature and climatic normal 1961 - 1990

Month	Temperature 1961 - 1990 [ºC]	t [ºC] 2019	Characteristic – 2019	Precipitation 1961 – 1990 [mm]	Precipitation 2019 [mm]	Characteristic - 2019
III.	4.8	8.1	very hot	31.6	15.6	very dry
IV.	10.4	9.4	normal	41.6	21.4	dry
V.	15.2	9.3	extremely cold	56.0	134.8	extremely wet
VI.	18.3	18.7	normal	66.2	29.0	very dry
VII.	20.0	21.9	warm	59.3	21.0	very dry
VIII.	19.7	22.3	very warm	54.2	83.7	very wet

Plant cultivation and fertilization

Sowing and cultivation of seedlings took place in the greenhouses of University Botanical Garden. The cultivation was carried out on small experimental plot located in demonstration garden of Institute of Horticulture, Slovak University of Agriculture in Nitra in 2019. Basil was sown on March 21st. Seedlings were transplanted into pots on April 3rd, and later planted outdoors on May 27th. Plants were spaced 35 x 40 cm apart. Mint was planted on May 27th, from separated cuttings rooted plant spaced 30 x 30 cm apart. The plot with lemon balm plants spaced 30 x 30 cm apart was fixed up on April 16th. Dried parts of the plants were removed, and the soil was loosened to remove weeds. During the growing season, the plot was managed as needed to remove weeds and soil crust. The crop was irrigated as necessary in the early morning hours. The first application of nitrogen fertilizer to the selected plants was carried out on June 3rd, at a dose of 6 ml/m². After the first harvest, the second application of nitrogen fertilizer was conducted on July 4th, at a dose of 6 ml/m².

Plant harvest and postharvest processing

The two-phase harvest of aboveground parts was carried out manually. The first harvest was conducted on July 3rd at the beginning of flowering, with plants being trimmed approximately 10 cm above the soil surface. The second harvest was performed on August 12th. After harvesting, individual plant species were weighed, and the chlorophyll content was determined from average samples. The remaining samples were then spread out on screens and air dried at room temperature. Well-dried foliage was used for essential oil content analysis.

Determination of chlorophyl *a* and *b* content

These analyses took place in the premises of the Institute of Horticulture and at the AgroBioTech Research Centre in Nitra. An average 1 g sample of fresh plant material was prepared and homogenized in small amount of acetone (80 %) (Heidolph Silent Crusher M, Heidolph Instruments, Schwabach, Germany). After homogenization, the acetone extract was carefully filtered using the fritted glass filters S4. The prepared extract was quantitatively transferred into the volumetric flask (50 mL) and filled with acetone to the final volume. The intensity of extract colour was measured at 649 nm for chlorophyll *a* and at 665 nm for chlorophyll *b* (spectrophotometer, PHARO 200, Spectoquant, Darmstadt, Germany). The zero position was controlled by pure acetone at 750 nm wavelength. The possible dispersion value was deducted from individual absorbance values. The calculation of chlorophyll content was performed according to Hegedűsová et al. (2018).

Determination of essential oils content

This analysis was carried out by distillation with water vapour. Before the distillation, the dry drug of the plants is grinded into smaller fractions and 20 g sample is weighed and then transferred into distillation flask with distilled water and brought up to a boil when the distillation begun. 10 minutes after the volume of distillate was consistent the heating was turned off and the essential oils content in splitting funnel is counted. The essential oil content is calculated according to Hegedűsová et al. (2018).

Statistical analysis

A statistical analysis was performed by using of the Statgraphics Centurion XVII (StatPoint Inc., The Plains, VA, USA). Obtained results were evaluated by analysis of variance (ANOVA), and average values were tested by the least significant difference (LSD) test performed at the significance level of 95 % (at $p < 0.05$).

Results and Discussion

Chlorophyll *a* and *b* content

The average values of chlorophyll *a* and *b* content in tested plant species, are shown in Table 3. The highest chlorophyll a content (102.13 mg/100g) was found in *Mentha* × *piperita* 'Danica' in the first harvest with ATS fertilizer, while the lowest (34.60 mg/100g) was in *Ocimum basilicum* 'Dark Green' with ATS in the first harvest. The highest chlorophyll *b* content (65.32 mg/100g) was measured in *Mentha* × *piperita* 'Danica' in the second harvest with ATS, while the lowest (14.79 mg/100g) was in *Ocimum basilicum* 'Dark Green' in the first harvest with ATS. The only significant difference in chlorophyll *b* content was measured in *Mentha* × *piperita* 'Danica' in second harvest with ATS treatment. Gažíová (2019) found no significant effect of ATS on leaf chlorophyll *a* or *b* content, a finding confirmed by our results for soil-applied nitrogen fertilizer. Polyticka and Goldz (2002) reported nitrogen fertilizers affecting chlorophyll content, but our data did not support these findings. Csámpai (2018) noted higher chlorophyll *a* in the first harvest of *Ocimum basilicum* 'Dark Green', with our results showing slightly higher levels in the second harvest.

Table 3. Chlorophyll *a* and *b* content [mg/100g fresh weight]

Species	Chlorophyll *a*				Chlorophyll *b*			
	1st harvest		2nd harvest		1st harvest		2nd harvest	
	Control	ATS	Control	ATS	Control	ATS	Control	ATS
Ocimum basilicum	39.63 ± 1.83a	34.60 ± 3.17a	40.63 ± 0.08a	44.39 ± 1.11a	17.15 ± 0.56b	14.79 ± 1.30b	18.54 ± 0.97b	20.17 ± 1.02b
Mentha × *piperita*	97.98 ± 16.98a	102.13 ± 9.24a	73.16 ± 3.20a	76.52 ± 0.07a	55.09 ± 9.85c	57.27 ± 6.10c	51.87 ± 6.68c	65.32 ± 2.52**b**
Melissa officinalis	59.58 ± 1.31a	59.84 ± 11.34a	60.77 ± 0.99a	59.71 ± 3.46a	30.21 ± 0.57b	34.32 ± 6.00b	31.40 ± 0.07b	31.28 ± 2.23b

*a, b - Different letters in a **row** indicate a statistically significant difference*

The chlorophyll *a/b* ratio is a critical indicator of plant stress and damage, with a lower chlorophyll *a* content suggesting greater plant damage (Paučová, 2019). Our measurements (Table 4) showed the highest chlorophyll *a/b* ratio in the Control variant of *Ocimum basilicum* 'Dark Green' during the first harvest (2.31 mg/100g). The lowest ratio was in the ATS variant of *Mentha* × *piperita* 'Danica' during the second harvest. Polyticka and Goldz (2002) found that chlorophyll *b* content in *Ocimum* species is half that of chlorophyll *a*, which was confirmed by our data. The ATS variant's chlorophyll *a/b* ratio was lower or equal to the Control. Statistical analysis showed these differences were not significant, suggesting minimal plant damage from nitrogen fertilizer. The only significant difference, confirmed statistically, was in *Ocimum basilicum* 'Dark Green,' with a 16% decrease in the ATS variant.

Table 4. Ratio of chlorophyll *a* and *b* content in fresh weight

Species	**1st harvest**		**2nd harvest**	
	Control	**ATS**	**Control**	**ATS**
Ocimum basilicum	2.31**a**	1.94b	2.19a	2.2a
Mentha × piperita	1.78a	1.78a	1.41a	1.17a
Melissa officinalis	1.97a	1.74a	1.94a	1.91a

a, b - Different letters in a ***row*** *indicate a statistically significant difference*

Content of essential oils

The average essential oil content for both variants in both harvests is shown in Table 5. *Mentha* × *piperita* 'Danica' had the highest essential oil content at 2.85% in the first harvest with nitrogen fertilizer, while the lowest content was 0.18% in *Melissa officinalis* in the first harvest with ATS application. The only statistically significant difference (0.91%) was in *Mentha* × *piperita* 'Danica,' with the Control variant containing 1.94% and ATS 2.85% essential oils in the first harvest. Other species showed minimal differences.

Table 5. Content of essential oils in dry weight [%]

Species	**1st harvest**		**2nd harvest**	
	Control	**ATS**	**Control**	**ATS**
Ocimum basilicum	0.54 ± 0.05a	0.68 ± 0.18a	0.59 ± 0.06a	0.54 ± 0.00a
Mentha × piperita	1.94 ± 0.09**b**	2.85 ± 0.02a	2.72 ± 0.03a	2.70 ± 0.00a
Melissa officinalis	0.20 ± 0.02a	0.18 ± 0.00a	0.29 ± 0.10a	0.29 0.03a

a, b - Different letters in a ***row*** *indicate a statistically significant difference*

Gažíová (2019) found no significant changes in essential oil content with ATS, confirmed by our soil application results. Hussain et al. (2008) noted that harvest timing influenced essential oil content in mint, with higher content in the first harvest (May, 23°C) compared to the second harvest (November, 18.5°C), decreasing from 12.2 g/kg to 10.5 g/kg in *Mentha* × *piperita*. In a study by Ostadi et al., it was found that the highest essential oil content (2.7 %) was present in the first harvest of plants treated by 50% chemical fertilizer in combination with nano chelated fertilizer. The lowest essential oil content (1.4 %) was determined in plants with no fertilizer treatment in the second harvest (Ostadi et al., 2020).

Conclusions

This study was a part of an extensive body of work that was mainly concerned with qualitative factors and found that, in the case of the studied species, nitrogen fertilizer was beneficial. Producers of fertilizer were also curious about any impact it might have on qualitative criteria that are economically significant. The study in two harvests show that the effect is variable and does not reach statistical significance.

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EFFECTIVENESS OF BIOSTIMULANTS ON THE QUANTITATIVE AND QUALITATIVE PARAMETERS OF BLUEBERRY (*VACCINIUM CORYMBOSUM*)

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Abstract

The research aim was the evaluation of biostimulants (Agriful, Tecamin Max and Tecamin Brix) effect on selected qualitative and quantitative properties of six varieties of highbush blueberry (Duke, Huron, Draper, Bluecrop, Calypso a Liberty). The study was carried out during the growing season of 2023 in the planting of the Botanical Garden in SAU Nitra, Slovakia. It can be concluded that the application of biostimulants caused a statistically evident increase in total yields on average by 87.2%. The increase occurred due to the increase in the number of fruits, while the length and weight of the fruits did not change. Furthermore, we can state that the application of biostimulants under given conditions did not have a demonstrable effect on the change of most of the measured quality indicators, except the content of malic acid, the content of total acids and the pH value. These three indicators are significantly correlated with each other. Since malic acid makes up approximately 90% of the content of total acids, we can conclude that the decrease in its content, as well as the decrease in the content of total acids, was caused precisely by the application of biostimulants, which resulted in a lower production of secondary metabolites (in this case malic acid), which are formed during cellular stress. Biostimulants created a situation in the plant where the level of stress hormones was apparently lower and did not force the cells to defend themselves by producing secondary metabolites and therefore also malic acid.

Key words: *blueberry, biostimulant, total yield, malic acid.*

Introduction

Blueberries are deciduous shrubs of the genus *Vaccinium* (family Ericaceae; subfamily Vacciniaceae). The genus *Vaccinium* comprises about 450 species, including cranberry (*V. macrocarpon* Ait.), bilberry (*V. myrtillus* L.), lingonberry (*V. vitis-idaea* L.), and huckleberry (*V. parvifoium*), which are valued for their sweet flavoured and nutrient-rich fruits (Song 2012). Berries with the highest nutritional density is the blueberry, which is rich in antioxidants, fiber, and vitamins. Flavonoids and phenolic acids are an extensive group of compounds with functional effects that decrease the risk of heart disease, diabetes, and cognitive impairment. (Silva 2020, Tobar-Bolanos 2021, Kalt 2020).
It is commonly acknowledged that blueberry production and sales will continue to rise gradually for the foreseeable future due to the health advantages of blueberries and their increased demand worldwide. (Yue 2017, Gallardo 2018).
Enhancing the nutritional quality of fruit by adding more bioactive chemicals is another major goal (Mezzetti 2016). Environmental elements like light, temperature, and water availability have a big impact on this (Xu 2019, Pinto-Morales 2022). Knowledge of the environmental factors and growing conditions that positively influence bioactive accumulation will provide an opportunity to develop agronomic practices that can enhance the nutritional quality of blueberry fruits (Krishna 2023). Prior research has demonstrated that growing blueberries

without fertilizer results in essentially nonexistent yields; as such, fertilization is required to guarantee sufficient output. (Ciucu Paraschiv 2023).
Substances or microorganisms that support and defend the physiological functions of plants are known as biostimulants (du Jardin 2015); while elicitors induce physiological defence responses in plants (Ali 2021). Upon detecting the signals, plants initiate cellular pathways to expedite the synthesis of chemicals and enzymes associated with antioxidant capability, consequently elevating the concentration of health-promoting bioactive substances (Mannino et al. 2021; Teklić et al. 2021).
The positive impact of organic fertilizers on the physical, chemical, and biological characteristics of soil and crops has been demonstrated by several studies (Manea et al., 2019, Khan et al., 2020, Wajid et al., 2020). In addition to enhancing soil quality, biostimulating substances have an indirect impact on plant physiology and metabolism. Seaweed extract-treated plants have stronger growth and a better-developed root system that allows for more widespread nutrient uptake. They also absorb and assimilate nutrients more quickly than untreated plants. (Lenart 2023). According to Van Oosten et al. (2017) biostimulants aid in the proper utilization of water and minerals by plants, promote their growth, and mitigate the effects of abiotic stressors.
The specialty crop business is very interested in using biostimulants such as natural seaweed extracts and humic compounds (fulvic and humic acids) to increase plant development and yield. This is especially true in the cultivation of blueberries (*Vaccinium* sp.) (Vargas, 2015).
Due to their role as signaling mediators, elicitors and/or biostimulants can produce stimuli that increase the concentration of bioactive substances without compromising quality and production. (du Jardin 2015; Ahmad et al. 2019; Ali 2021).

The aim of the study was to evaluate the effectiveness of the selected biostimulants (Agriful, Tecamin Max and Tecamin Brix) on the selected qualitative and quantitative properties of blueberry (*Vaccinium cormybosum*) in growing season 2023.

Material and Methods

The implementation of the study was carried out during the growing season of 2023 in the semi-productive blueberry-planting of the Institute of Horticulture, FHLE SAU in Nitra, Slovakia. The planting was carried out in the spring of 2022. The plants are planted in containers with a diameter of 0.5 m and embedded in the ground in their entire volume. The spacing is 4.0x1.0m. The space in the row is mulched with chips from conifers, any weeds were removed mechanically. The interrows are sown with a special mixture of grasses designed for intensive fruit orchards and, as needed, were mowed several times per vegetation after reaching a height of 0.2-0.3m. The plants were irrigated by drip irrigation from its own well. Irrigation hoses are placed on the surface of the soil and are doubled. The intensity of irrigation is 2.3 l/hour, the spacing of the drippers is 0.5 m. Irrigation was started automatically (Irritrol Total Controll-R TC15EX-R, Riverside, California, United States) according to a predetermined schedule.
There are a total of 6 varieties in planting, Duke, Huron, Draper, Bluecrop, Calypso and Liberty. The number of plants in a row from one variety is 20, except for the Calypso variety, which has 10. The application of biostimulants was on 10 plants and the remaining 10 plants served as a control. Varieties are planted and arranged in the planting according to the date of ripening, from the beginning of July (Duke) to the beginning of September (Liberty). Within the objectives, the following parameters was measured after harvest: fruit size, fruit weight, total sugars, total acids, total soluble solids, glucose, fructose, malic acid, pH level.

Three commercial biostimulants were used. Agriful (Agri Tecno Brand, Spain) - containing 25% fulvoacids, 45% other organic substances, 4.5% nitrogen, 1% phosphorus and 1% pottasium. Tecamin Max (Agri Tecno Brand, Spain) - 14.4% aminoacids, 12% free L-aminoacids, 7% nitrogen, 60% organic compounds. Tecamin Brix (Agri Tecno Brand, Spain) – 10% sea algae extract, 18% potassium, 0.2 boron, aminoacids.
According to the plan, Agriful was applied by watering in the number of 6 times and a dose of 50ml/10l of water, Tecamin Max was applied by spraying in the number of 3x in a dose of 30ml/10l of water and Tecamin Brix was applied also by spraying in the number of 3x in a dose of 30ml per 10l water. The spray was applied using a Stihl SR 200 back-mounted motorized sprayer. The application date was determined according to the weather from the end of April to the beginning of July. The dosage was determined based on an agreement with the sponsor of the study. Harvesting of the fruits was done gradually, according to the ripening of the fruits. We picked all the fruits from each bush, the harvest was done once.

Results and Discussion

All applications were applied according to the plan, there was no major disruption to application plans. All applications were implemented from 26.4.2023 to 6.7. 2023. The sprays were applied when there was no wind, or with only a very light wind. The temperature during application was from 11 to 18°C. The time of application was in the morning, the weather was clear to partly clear.
The harvest dates were as follows: Duke, 10.7., Huron 17.7., Draper 1.8., Bluecrop 21.8., Calypso 28.8. and Liberty 4.9.2023.
Table 1 shows the average harvests from 10 bushes . We would like to remind, that this is a planting in the first year after planting, so, of course, the yields are lower than with in full-bearing period. In addition, no fertilizers were applied, either by watering, spraying, or fertigation, in order to eliminate as many influences as possible outside of biostimulants. We can state that the yield increase depending on the varieties was from 31.8 to 178.5%, on average 87.2%. The yield increase was statistically significant for all varieties ($p < 0.05$).
In agriculture, these biostimulants are widely used as soil supplements, and they usually work best when combined with fertilizers. Unfortunately, the majority of research on biostimulants has been conducted on annual crops, which has left a gap in our knowledge of their full potential when it comes to perennial crops like blueberries. Previous research found that applying humic materials to blueberries increased plant growth in the first two years after planting (Vargas, 2015).

Table1. Results of quantitative indicators after the application of biostimulants.

Variety	**Total yields**			**1 berry diameter**		**1 beery weight**	
	control	**biostimulants**	**increase**	**control**	**biostimulants**	**control**	**biostimulants**
	(g)	***(g)***	***(%)***	***(mm)***	***(mm)***	***(g)***	***(g)***
Duke	1184	2338	97.5	13.5	12.9	1.32	1.13
Huron	480	1337	178.5	12.6	14.2	1.06	1.54
Draper	936	2015	115.3	14.5	14.2	1.63	1.52
Bluecrop	1197	1940	62.1	13.5	11.2	1.33	1.2
Calypso*	546	753	37.9	12.5	14.6	1.54	1.44
Liberty	1861	2453	31.8	13	11.8	1.23	1.11
priemerné zvýšenie			**87.2**	**Ø 13.3**	**Ø 13.1**	**Ø 1.35**	**Ø 1.32**

By variety Calypso the data were from 5 bushes.

When evaluating the average diameter and weight of the berries, we can state that the application of biostimulants did not result in a statistically significant increase in the length and weight of the berries. The reported differences are not statistically significant ($p < 0.05$).
Humic acids did not improve blueberry root growth, fruit yield, or fruit quality (Nunez 2023). Growers are increasingly interested in using humic acid (HA) for blueberry production. However, previous research describing blueberry responses to HA applications is scarce. In 'Legacy' northern highbush blueberry (NHB, Vaccinium corymbosum), HA application did not affect root growth, shoot growth, fruit yield, or fruit quality (Shoebitz et al., 2016; Shoebitz et al. 2019). In contrast, HA application promoted root growth in 'Draper' NHB (Bryla and Strik, 2015). Prior research has demonstrated that growing blueberries without fertilizer results in essentially nonexistent yields; as such, fertilization is required to guarantee sufficient output. (Ciucu Paraschiv 2023).

We present the measured analytical parameters in table 2. Overall, it can be concluded that the application of biostimulants in the given characteristics did not had a demonstrable effect on the change of most of the measured indicators, except for the content of malic acid, the content of total acids and the pH value, when there was a demonstrable decrease in these indicators.

Table 2. Results of qualitative indicators after the application of biostimulants

variety/variant		**fructose**	**glucose**	**TSS**	**malic acid**	**pH**	**total acids**	**total sugars**
		(g/l)	***(g/l)***	***(°Brix)***	***(g/l)***		***(g/l)***	***(g/l)***
Duke	control	65.1	56.82	12.98	8.20	3.12	9.26	128.83
	biostimulants	65.54	56.08	12.96	6.79	3.21	7.53	129.25
Huron	control	55.91	47.53	10.82	9.45	2.99	10.89	111.08
	biostimulants	63.01	55.57	12.30	7.13	3.12	8.06	126.34
Draper	control	69.94	63.49	13.84	8.42	3.05	9.84	138.52
	biostimulants	64.22	54.17	12.25	8.46	3.07	9.55	123.62
Bluecrop	control	64.19	51.59	12.06	7.64	3.14	8.35	121.31
	biostimulants	66.74	53.21	12.53	7.01	3.22	7.43	125.54
Calypso	control	59.82	52.14	11.76	10.20	2.93	12.02	118.04
	biostimulants	64.24	55.32	12.46	7.11	3.14	7.98	126.76

The fructose content in the control variant was from 55.91g/l to 69.94g/l, while in the treated variant it was from 63.01g/l to 66.74g/l. The average fructose content in the control variant was 62.99g/l, while it was 64.75g/l in the treated variant. The measured differences within the evaluation at the species (not variety) level are not statistically significant ($p < 0.05$), therefore we can conclude that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties did not cause a demonstrable increase in fructose in blueberry fruits.
The results observed in the study by the author Vrhovsek et al. (2012) show that the variety Draper achieved the most significant concentrations of fructose, among other varieties, reaching a value of 82.8 g/l. This finding agrees with our own measurements
The glucose content in the control variant was from 47.53g/l to 63.49g/l, while in the treated variant it was from 53.21g/l to 56.08g/l. The average glucose content in the control variant was 54.32g/l, while it was 54.87g/l in the treated variant. The measured differences within the evaluation at the level of the species (not the variety) are not statistically significant (p <

0.05), we can therefore conclude that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties did not cause a demonstrable increase in glucose in the blueberry fruits.

From the studies of the authors Vrhovsek et al. (2012), the range of glucose in fruits ranges from 22.4 g/l to 48 g/l, but our research shows different values, from 47.53 g/l to 63.49 g/l. In their research, Madrera et al. (2021) identified and reported glucose content values for the variety Duke. Specifically, their study showed that the glucose content in the cranberry fruits of this variety reached a value of 35.51 g/l. These low values reported by both authors, compared to our measured higher values, we attribute to the fact that the fruits we studied were collected at a later stage of maturity. At this advanced stage of ripening, more fructose and glucose accumulate in the fruit, which also leads to a higher content of total sugars in the cranberry fruit. Zhang et al. (2020) identified in their study a range of glucose content in cranberry fruits that ranged from 33.8 g/l to 61.3 g/l. At the same time, it is important to note that these results are consistent with our own measured values.

The content of TSS in the control variant was from 10.82°Brix to 13.84°Brix, while in the treated variant it was from 12.25°Brixl to 12.96°Brix. The average content was 12.29°Brix for the control variant, while it was 12.50°Brix for the treated variant. The measured differences within the evaluation at the level of the species (not the variety) are not statistically significant ($p < 0.05$), therefore we can conclude that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties did not result in a demonstrable increase in the content of refractometric dry matter in blueberry fruits.

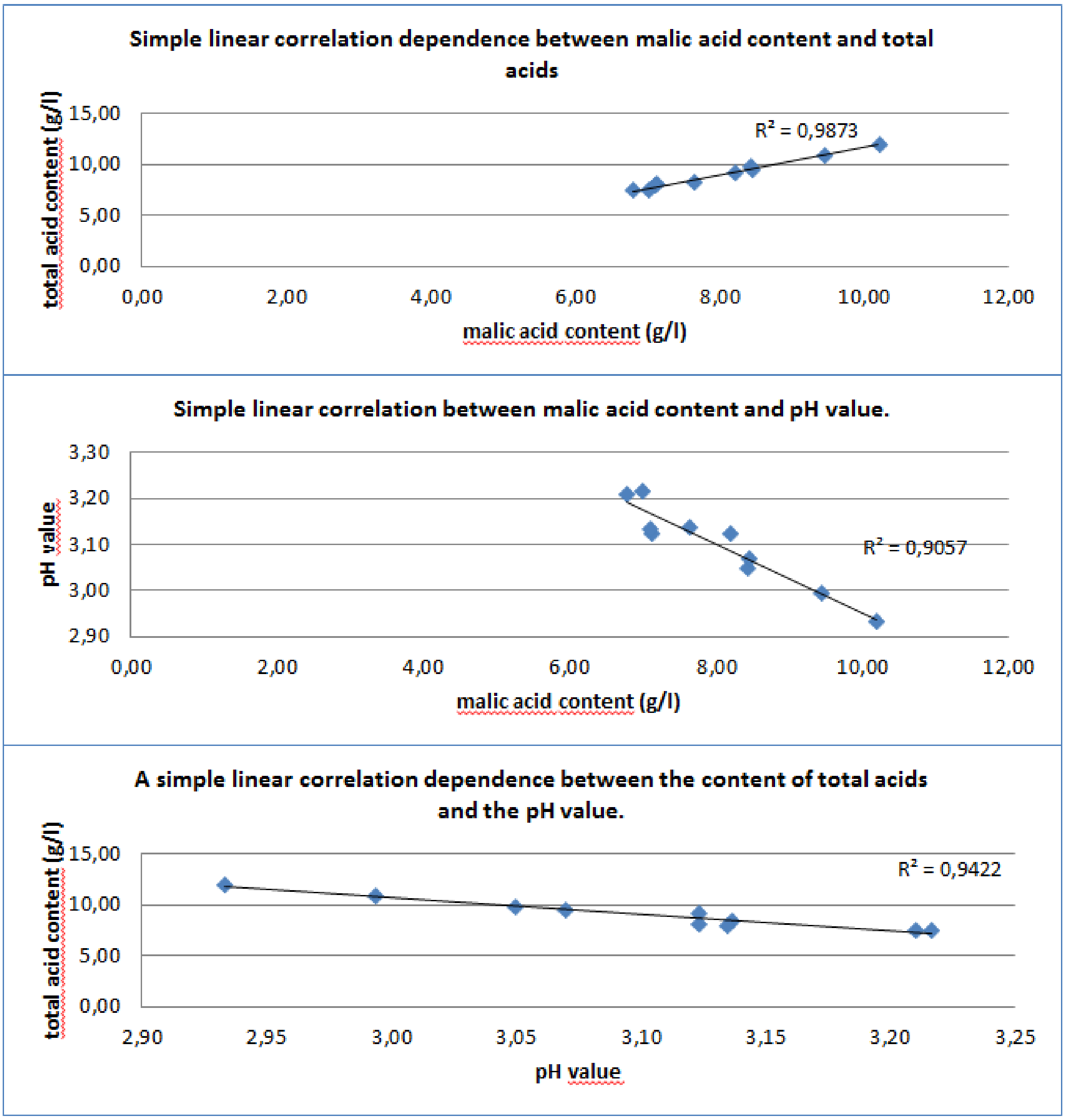
Based on our findings, it can be observed that the refractometric dry matter values obtained by us differ slightly from those reported by Zdunić et al. (2016) in their work. In this study, the range of refractometric index of dry matter of cranberries is presented in the range from 9.7 to 11.6 °BRIX. Our own measurements report data that show little deviation compared to these benchmarks. This variability can result from a variety of factors, including the genetic characteristics of the variety, environmental conditions, and measurement methods. Our study showed values that are somewhat different compared to the values of Sinelli et al. (2008), who reports an average content in cranberry fruits of 8.42 °Brix. He noted that the content of refractonic dry matter in cranberries can be higher and depends on the stage of maturity and varietal characteristics of the fruit.

The content of malic acid in the control variant was from 7.64g/l to 10.2g/l, while in the treated variant it was from 6.79g/l to 8.46g/l. The average content was 8.78g/l in the control variant, while it was 7.3g/l in the treated variant. The measured differences in the assessment at the species level are statistically significant ($p < 0.05$), we can therefore state that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties resulted in a demonstrable decrease in the content of malic acid in the blueberry fruits.

The decrease in malic acid content in cranberry fruits may be the result of a complex set of factors that include the genetic characteristics of a specific variety, the variability of environmental conditions and the methods used in the measurement (Vrhovsek et al., 2012). Michalska (2015) states in her study that during the ripening process of cranberries, their acidity gradually decreases, which reduces the titratable acidity. Further in his study, he claims that the dominant organic acid responsible for the acidity of cranberry fruits is malic acid.

Regarding the pH value, we can state that it was from 2.93 to 3.14 in the control variant, while it was from 3.07 to 3.22 in the treated variant. The average value was 3.05 for the control variant, while it was 3.15 for the treated variant. The measured differences in the assessment at the level of species (not varieties) are statistically significant ($p < 0.05$), therefore we can conclude that the application of the mentioned biostimulants in the

mentioned concentrations and the mentioned varieties resulted in a demonstrable change in the pH value of the blueberry fruits.
We found the highest pH in the treated variant of the Bluecrop variety with a value of 3.22 and the Duke variety with a value of 3.21. The lowest measured pH values for the control variant of the Calypso variety were 2.93. According to research by Madrera et al. (2021) found the Duke variety to have a pH value of 2.72 pH, a difference of 0.21 from our own findings.
The content of total acids in the control variant was from 8.35g/l to 12.02g/l, while in the treated variant it was from 7.43g/l to 9.55g/l. The average content was 10.07g/l in the control variant, while it was 8.11g/l in the treated variant. The measured differences in the assessment at the species level are statistically significant ($p < 0.05$), we can therefore state that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties resulted in a demonstrable reduction in the content of total acids in the blueberry fruits.
According to research by Nookaraju (2010) and Li et al. (2019) it is clear that the balance between sweetness and acidity plays a fundamental role in the process of determining the taste and quality of fruits. This fact emphasizes the importance of acidity as an important factor influencing the sensory properties of fruits, together with their sweetness. Zhang et al. (2020) state in their study that the range of total acid content ranges from 2.5 g/l to 14.2 g/l with an average occurrence at the level of 8.63 g/l. These values are consistent with our own observations, which showed a range from 7.43 g/L to 12.02 g/L.
The content of total sugars in the control variant was from 111.08g/l to 138.52g/l, while in the treated variant it was from 123.62g/l to 129.25g/l. The average content of total sugars in the control variant was 123.56g/l, while it was 126.30g/l in the treated variant. The measured differences within the evaluation at the species (not variety) level are not statistically significant ($p < 0.05$), therefore we can conclude that the application of the mentioned biostimulants in the mentioned concentrations and the mentioned varieties did not result in a demonstrable increase in total sugars in the blueberry fruits.
Authors Crespo et al. (2010) state in their study that the most dominant sugars in cranberry are glucose and fructose, while their accumulation in berries is significantly influenced by genetic factors and variability of climatic conditions, especially light intensity. According to Zdunić et al. (2016), the observed range of total sugar content in cranberries ranges from 68 g/l to 78 g/l. These data are inconsistent with our own findings in the control variant. A scientific study published by Leeuwen et al. (2012), states that the average sugar content in cranberries usually ranges from 80 g/l to 100 g/l. We explain this difference by the fact that the fruits that were subjected to our study were collected at an advanced stage of maturity. At this stage of ripening, higher amounts of fructose and glucose accumulate in the fruit, which can lead to an increase in the total sugar content of the cranberry fruit.
The synergistic effect of technology with biostimulation, based on seaweed extracts, on the percentage of large fruit and on qualitative yield was shown by Kaplan et al (2013). An experiment conducted by Lenart et al (2022) showed that algal fertilizers increase the antioxidant activity of highbush blueberry fruit, the content of anthocyanins and total polyphenols, which is related not only into better fruit health promoting properties but also into better storage. Graph 1 demonstrates a simple linear correlation between the content of malic acid, total acids and pH value. This knowledge is important from the point of view of the provenance of the measured values, or their reduction after the application of biostimulants. In this case, malic acid appeared as a secondary metabolite formed at a certain level of stress. Since we observe a decrease in the content of malic acid, total acids and pH after the application of biostimulants, we conclude that the biostimulants reduced the production of stress hormones, which we consider a positive reaction.



Graph 1. Linear correlation between selected analyzed parameters.

Conclusion

Overall, it can be concluded that the application of biostimulants in the given characteristics caused an increase in total yields, depending on the varieties, from 31.8 to 178.5%, on average by 87.2%. The yield increase was statistically evident for all varieties. When evaluating the average length and weight of the berries, we can state that the application of biostimulants did not result in a statistically significant increase in the length and weight of the berries. The stated differences are statistically unprovable. The increase in yields after the application of biostimulants occurred due to an increase in the number of fruits, while the length and weight of the fruits did not change.

Furthermore, we can state that the application of biostimulants did not have a demonstrable effect on the change of most of the measured quality indicators, except for the content of malic acid, the content of total acids and the pH value, when there was a demonstrable decrease in these indicators, respectively at the pH value, its increase. These three indicators in this study are significantly correlated with each other (graph 1). Since malic acid makes up approximately 90% of the content of total acids, we can conclude that the decrease in its content, as well as the decrease in the content of total acids, was caused precisely by the application of biostimulants, which resulted in a lower production of secondary metabolites (in this case, malic acid), which arise during cellular stress. Biostimulants created a situation in the plant where the level of stress hormones is apparently lower and does not force the cells to defend themselves by producing secondary metabolites and therefore also malic acid.

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EFFECT OF CULTIVAR AND PROCESSING METHOD ON QUALITY OF CHIPS MADE FROM TURNIP, RUTABAGA AND RADISH

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Abstract

Turnip, rutabaga and radish are less-known, or less-grown, vegetable species in Slovak Republic. All these species belong to Brassicaceae family which is known by important health-promoting properties. However, all root vegetables have not a long-term storage ability, mainly turnip or radish. Plant material for analyses came from field experiment realized at SUA in Nitra in 2022. Within study, following cultivars were used: turnip - Amelie F1, Oasis F1, Purple Top White Globe, Tonda a Coletto Viola; radish - black skin - Kulatá Černá, Nero Tondo d'Iverno, white skin/purple pulp - Red Meat; rutabaga - Dalibor, Magres. Lyophilization (freeze drying at -58°C; 72 hours) and conventional drying (60°C; 10 hours) of roots were tested in connection to antioxidant activity (DPPH; FRAP; ABTS) and polyphenol content of processed chips. Statistical evaluation of gained results revealed significant differences among cultivars and processing method in AOA and TPC of processed chips. The highest TPC was confirmed in radish cultivar 'Red Meat' (lyophilization 10312.40 mg GAE.kg^{-1} d. w.; drying 9044.02 mg GAE.kg^{-1}) with purple pulp of roots. The highest AOA, because of polyphenol abundance, were confirmed by all three methods in mentioned radish cultivar 'Red Meat'. TPC and AOA in lyophilized samples were significantly higher than in conventionally dried samples of tested crops. On the other hand, lyophilization is method more demanding to energy costs for processing, as well as costs for lyophilizer buying compared to common drying. If qualitative increase of chips is evaluated in connection to mentioned costs, conventional drying can be marked as suitable method for root processing.

Keywords: *Root vegetables, Cultivar, Processing, Antioxidant activity, Polyphenols.*

Introduction

Turnip, rutabaga and radish belongs to less known, or less-grown, root vegetables in Slovak Republic. These species are just grown in local/home gardens (Meravá, 2021). According to Grubben (2004), turnip (*Brassica rapa* ssp. *rapa*) is reportedly the oldest vegetable species from *Brasssicaceae* family. Turnip was described in 356-323 years BC in area of the Near East and Persia. Sun (2015) describes Europe, Near East and Middle Asia as probable origin area. Nowadays, it is currently introduced world-widely. Rutabaga (*Brassica napus* var. *napobrasssica*) comes probably from Scandinavia, Czech Repulic or Russia. Rutabaga was originally grown as feeding crop for animals and it was consumed by people just in famine period (Morgan, 2012). Welbaum (2015) indicates that first reference about its domestication is approximately from the end of 16th and beginning of 17th century from England. In recent period, it is more intensively grown northern European countries especially (Pokluda et al., 2022). First reference about radish (*Raphanus sativus* var. *longipinnatus*), mainly black radish, comes from Egypt approximately 2780 years BC when it was offered to workers on pyramides. Lately, it was spread to the world (Singh *et al.*, 2017).

Turnip, rutabaga and radish belong to the *Brassicaceae* family. All *Brassica* species are known for their health-promoting properties due to the abundance of glucosinolates and polyphenols (Swastika *et al.*, 2018; Sanlier and Guler Saban, 2018; Kapusta-Duch *et al.*, 2012). These metabolites are responsible for providing a unic and distinctive taste (Wieczorek *et al.*, 2019). Moreover, consumption of *Brassica* vegetables rich on glucosinolates and polyphenols are known by interesting potential for prevention and treatment of various, chronic diseases, e.g. obesity, type 2 diabetes, cardiovascular diseases (including hypertension or stroke), cancer or osteoporosis (Chen *et al.*, 2018; López-Chillón *et al.*, 2019; Raiola *et al.*, 2018; Silva Dias, 2019; Favela-González *et al.*, 2020).
Quality of raw or processed vegetables is key factor from aspect of final consumers (Rahman *et al.*, 2021). According to Pokluda *et al.* (2022), turnip, rutabaga and radish can be stored 2-5 months in cooling room with low temperature (0-1 °C) and high air humidity (90-95%). On the other hand, mentioned storage conditions is not able to apply for local grower due to absence of cooling room with exact atmosphere. Thus, production of chips (or other products) is possible solution for processing of higher yield which is not able to sell directly. Methods for chips processing are various in dependent to quality keeping or energy costs, e.g. heat (conventional) drying, infrared radiation drying, freeze-drying (lyophilisation) or deep frying (Frîncu *et al.*, 2022). Conventional drying and lyophilisation are probably two most-used methods for production of vegetable chips.
The aim of this study was to compare the effect of mentioned drying methods to quality of chips (antioxidant activity, polyphenols) made from turnip, rutabaga and radish.

Material and Methods

Plant material for analyses came from field experiment realized at Slovak University of Agriculture in Nitra (Slovakia) in 2022. The climate of experimental area is characterized by warm and dry summer and slightly warm, dry or very dry winter.
In realized study, tested cultivars were following: turnip - Amelie F1, Oasis F1, Purple Top White Globe, Tonda a Coletto Viola; radish - black skin - Kulatá Černá, Nero Tondo d'Iverno, white skin/purple pulp - Red Meat; rutabaga - Dalibor, Magres.
Within sample preparation, ten roots of each tested cultivar were taken for chips processing. Roots were precisely washed and cleaned from all impurities. Sequentially, roots were quartered and opposite parts of root were taken for chips processing and its analyses. Root samples were cut to the slices with diameter of 1 mm. Two treatments of chips processing were tested. In conventional (heat) drying method, slices were dried at 60^{0} C during 10 hours. In lyophilization, slices were dried at -58^{0} C during 72 hours. The antioxidant activity of produced chips was measured spectrophotometrically by using of DPPH (Brand-Williams *et al.*, 1995), FRAP and ABTS (both Paulová *et al.*, 2004) methods. Its values were expressed in ųmol TE per gram (TE - Trollox equivalent). The polyphenol content was determined by spectrophotometric method of Lachman *et al.* (2003) and expressed in mg GAE per kilogram (GAE - gallic acid equivalent).
The statistical analysis of obtained results was performed by using of the Statgraphic Centurion XVII (StatPoint, USA). Results were evaluated by analysis of variance (ANOVA) and average values were tested by LSD test performed at the significance level of 95% ($p <0.05$).

Results and Discussion

Phenolic compounds represent a large group of phytochemicals wide-spread in the plant kingdom. Depending on their structure, they can be classified in various groups. The most

wide-spread polyphenol group in turnip, rutabaga and radish, as well as in other Brassicaceae species, are flavonoids and derivates of hydroxycinnamic acid (Cartea *et al.*, 2011).
Statistical analysis of obtained results showed significant differences of total polyphenol content (TPC) among cultivars of tested crops and methods of chips processing. In case of conventional drying, TPC values were ranged from 2267.78 (rutabaga 'Magres') to 9044.02 mg GAE.kg^{-1} (radish 'Red Meat'). In lyophilization treatment, TPC values were ranged from 2650.45 (rutabaga 'Magres') to 10312.40 mg GAE.kg^{-1} (radish 'Red Meat').

Table 1 Polyphenol content in turnip, rutabaga and radish chips (mg GAE.kg^{-1} ±SD)

Species	Cultivar	Conventional drying	Lyophilisation
Turnip	Amelie F1	3384.72 ±33.75^{j}	4154.83 ±25.76^{m}
	Oasis F1	2748.63 ±27.61^{e}	3336.07 ±28.09^{i}
	Purple Top White Globe	2732.54 ±9.01^{e}	3388.50 ±36.51^{j}
	Tonda a Coletto Viola	3231.74 ±13.32^{h}	4024.06 ±33.16^{l}
Rutabaga	Dalibor	3529.40 ±26.85^{k}	4349.27 ±44.04^{n}
	Magres	2267.78 ±8.98^{a}	2650.45 ±22.51^{d}
Radish	Kulatá černá	2532.33 ±8.94^{c}	3044.33 ±9.52^{g}
	Nero Tondo d´Inverno	2435.92 ±13.15^{b}	2895.40 ±36.52^{f}
	Red Meat	9044.02 ±22.74^{o}	10312.40 ±41.93^{p}

Note: SD - standard deviation; Values with different letters among rows and columns are significantly different at p <0.05 by LSD in ANOVA.

Table 2. Antioxidant activity of turnip, rutabaga and radish chips measured by DPPH method (µmolTE.g^{-1} ±SD)

Species	Cultivar	Conventional drying	Lyophilisation
Turnip	Amelie F1	24.69 ±0.32^{c}	28.56 ±0.13^{f}
	Oasis F1	28.16 ±0.36^{f}	31.27 ±0.27^{i}
	Purple Top White Globe	30.09 ±0.33^{gh}	33.36 ±0.35^{j}
	Tonda a Coletto Viola	28.39 ±0.26^{f}	31.67 ±0.09^{i}
Rutabaga	Dalibor	27.16 ±0.18^{e}	30.20 ±0.55^{h}
	Magres	26.41 ±0.09^{d}	29.73 ±0.28^{g}
Radish	Kulatá černá	19.06 ±0.09^{a}	21.67 ±0.25^{b}
	Nero Tondo d´Inverno	21.69 ±0.06^{b}	24.95 ±0.07^{c}
	Red Meat	51.67 ±0.12^{k}	54.41 ±0.21^{l}

Note: SD - standard deviation; Note: Values with different letters among rows and columns are significantly different at p <0.05 by LSD in ANOVA.

The antioxidant activity (AOA) is often used for expression of nutritional benefit of individual vegetables, fruits and other plant products (Prenzler *et al.*, 2022). This important parameter of product quality can be determined by various methods based on different principle (Shahidi and Zhong, 2015). Statistical analysis of obtained results showed significant differences of AOA among cultivars of tested crops and chips processing in all used AOA determination methods (table 2-4).
DPPH radical scavenging assay is probably the most frequently used methods of AOA determination and it offers the first approach for evaluating of AOA. It is a method based on single electron transfer with mechanism of hydrogen atom transfer being only a marginal

reaction pathway in the assay (Prior et al., 2005). DPPH method is based on electron donation of antioxidants to neutralize DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical (Hegedűsová *et al.*, 2018). In conventional drying (table 2), AOA was varied from 19.06 (radish 'Kulatá černá') to 51.67 ųmolTE.g^{-1} (radish 'Red Meat'). In lyophilization treatment, AOA were ranged from 21.67 (radish 'Kulatá černá') to 54.41 ųmolTE.g^{-1} (radish 'Red Meat').

Table 3. Antioxidant activity of turnip, rutabaga and radish chips measured by ABTS method (ųmolTE.g^{-1} ±SD)

Species	Cultivar	Conventional drying	Lyophilisation
Turnip	Amelie F1	12.30 ±0.14^{ij}	14.54 ±0.20^{i}
	Oasis F1	9.61 ±0.16^{d}	12.45 ±0.12^{j}
	Purple Top White Globe	10.88 ±0.16^{g}	12.91 ±0.07^{k}
	Tonda a Coletto Viola	9.37 ±0.14^{c}	11.59 ±0.16^{h}
Rutabaga	Dalibor	10.51 ±0.20^{f}	12.20 ±0.17^{i}
	Magres	9.76 ±0.10^{d}	11.75 ±0.05^{h}
Radish	Kulatá černá	7.42 ±0.07^{a}	8.85 ±0.08^{b}
	Nero Tondo d´Inverno	10.23 ±0.09^{e}	11.54 ±0.11^{h}
	Red Meat	58.74 ±0.08^{m}	61.06 ±0.08^{n}

Note: SD - standard deviation; Note: Values with different letters among rows and columns are significantly different at p <0.05 by LSD in ANOVA.

Table 4. Antioxidant activity of turnip, rutabaga and radish chips measured by FRAP method (ųmolTE.g^{-1} ±SD)

Species	Cultivar	Conventional drying	Lyophilisation
Turnip	Amelie F1	14.53 ±0.04^{j}	17.43 ±0.39^{l}
	Oasis F1	11.32 ±0.06^{e}	13.10 ±0.73^{gh}
	Purple Top White Globe	12.66 ±0.27^{fg}	15.79 ±0.24^{k}
	Tonda a Coletto Viola	14.00 ±0.16^{i}	15.53 ±0.10^{k}
Rutabaga	Dalibor	12.57 ±0.19^{f}	14.28 ±0.33^{ij}
	Magres	9.64 ±0.10^{d}	13.25 ±0.29^{h}
Radish	Kulatá černá	6.70 ±0.19^{a}	7.46 ±0.27^{b}
	Nero Tondo d´Inverno	6.39 ±0.15^{a}	8.52 ±0.17^{c}
	Red Meat	34.87 ±0.19^{m}	38.42 ±0.24^{n}

Note: SD - standard deviation; Note: Values with different letters among rows and columns are significantly different at p <0.05 by LSD in ANOVA.

According to Prior *et al.* (2005), ABTS method is based on measurement of antioxidant ability to scavenge the stable radical cation ABTS^{+} (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)). In this method (table 3), AOA values were ranged from 7.42 (radish 'Kulatá černá') to 58.74 ųmolTE.g^{-1} (radish 'Red Meat') in conventional drying treatment, respectively from 8.85 (radish 'Kulatá černá') to 61.06 ųmolTE.g^{-1} (radish 'Red Meat')
Prior *et al.* (2005) indicate that FRAP method is based on measurement of ferric ion (Fe_3^{+}) to ferrous ion (Fe_2^{+}). In conventional drying (table 4), AOA was ranged from 6.39 (radish 'Nero Tondo d´Inverno') to 34.87 ųmolTE.g^{-1} (radish 'Red Meat'). In lyophilization treatment, AOA were ranged from 7.46 (radish 'Kulatá černá') to 38.42 ųmolTE.g^{-1} (radish 'Red Meat').

TPC values were varied among tested cultivars and crops; thus, it is not possible to evaluate the richest source of polyphenols from tested crops. Radish cultivar 'Red Meat' was significantly different from other tested cultivars. In this cultivar, the expressively higher TPC was determined. It should be caused by purple color of root pulp because of abundance of anthocyans as it was described by Bozdogan and Yasar (2011). Our results confirmed fact that cultivar belongs to the most important factors influencing on the quality of root vegetables (Önder *et al.*, 2020; Bystrická *et al.*, 2015; Sengul *et al.*, 2011), or horticultural products generally. According to obtained results, AOA has connection to TPC in roots of tested crops. The highest AOA value was found by all three analysis methods in radish cultivar 'Red Meat' with purple pulp color. A similar finding about AOA and TPC correlation was also shown in the experiments with other crops, e. g. red beet (Šlosár *et al.*, 2020), potato (Gupta *et al.*, 2015), sweet potato (Ji *et al.*, 2015) or carrot (Scarano *et al.*, 2018).

Our results indicated that processing method of vegetables or fruits has a significant impact on final product properties. Using of lyophilization resulted in significantly higher TPC compared to the conventional drying in all tested cultivars. Alean *et al.* (2016) describe that polyphenol degradation during crop processing in very depending on temperature and drying period. These factors effect to the irreversible oxidation processes of polyphenols and they can be also affected by cell destruction. Higher TPC in lyophilised chips compared to heat-drying method was presented also in the study of Jia *et al.* (2019) with Asian persimmon. On the contrary, authors found approximately the same results of chips sensorial evaluation in both tested drying methods.

According to obtained results and discussion with other studies, lyophilization can be marked as more preserve method for keeping of bioactive substances in product processing. On the other hand, important factor is also energy demanding of used processing method; definitely, financial costs have impact to price of marketed products which belongs to key factors of final consumer. It is known that process of lyophilization is significantly longer that coventional heat drying. Because of not so significant differences between tested methods, heat drying appeared as more suitable for smaller grower which would like to process excessive (non-longer storable) yield of root crops.

Selection of cultivars used for chips production is depending on various factors. Mostly, it is taste, favour, structure and nutritional value of final product. Nowadays, interest in vegetable chips, as healthier alternative to potato chips, is increasing. They are suitable for direct consumption, as well as additive for food decoration with aim to increase of nutritional value in food cooking. From rutabaga and turnip tested cultivars, it can be recommended to use 'Dalibor' and 'Amelie F1'. From radish tested assortment, cultivar 'Red Meat' can be definitely recommended because its nutritional value. Additive advantage of this cultivar is purple color of pulp, visually interesting for using in modern, creative gastronomy.

Conclusions

Turnip, rutabaga and radish belongs to less known, or less-grown, root vegetables in Slovak Republic. All these species be to the *Brassicaceae* family. Crops from this family are known for their health-promoting properties due to high antioxidant activity and abundance of polyphenols or glucosinolates. Quality of processed vegetables is key factor from aspect of final consumers. Methods for chips processing are various in dependent to quality keeping or energy costs. Conventional drying and lyophilisation are probably two most-used methods for production of vegetable chips. Production of chips (or other products) is possible solution for processing of higher yield which is not able to sell directly. Obtained results confirmed significant impact of cultivar and processing method to quality of prepared chips. From all tested cultivars, radish cultivar 'Red Meat' can be recommended for chips processing because of its nutritional value and unique, visually interesting pulp color. It is known that process of lyophilization is significantly longer and more financially demanding that conventional heat drying. Differences among tested processing methods were not so significant; thus, heat drying appeared as more suitable for smaller grower which would like to process excessive (non-longer storable) yield of root crops.

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CORRELATION ANALYSIS OF CORM DENSITY AND FERTILIZATION TREATMENTS ON SAFFRON GROWTH AND YIELD IN SEMI-ARID CLIMATE

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Abstract

Saffron (*Crocus sativus* L.), an autumn-blooming plant that produces a highly valuable spice, is primarily cultivated in Mediterranean climates. In Lebanon, it is mainly grown in the North Bekaa Valley. Recently, it has been introduced to the central part of the Bekaa Valley. To evaluate the response of saffron to organic matter and potassium-nitrogen chemical fertilizer in the semi-arid climate of Lebanon, an experiment was conducted at a private farm in Deir el Ghazel. The study, carried out from 2019 to 2022, utilized a split-plot design based on randomized complete blocks with three replications over three consecutive crop growth seasons. The correlation coefficient analysis revealed a strong and significant relationship between initial corm density and various treatments (R=0.975). These two factors significantly influence second-year parameters such as flower number (R_{treat}=0.928; R_d=0.922) and saffron weight (R_{treat}=0.914; R_d=0.916). They also moderately affect factor of flower number (R_{treat}=0.631; R_d=0.6). Their influence slightly weakens in the third year, with flower number showing a moderate significant correlation (R_{treat}=0.613; R_d=0.582), saffron weight showing a weak but significant correlation (R_{treat}=0.377; R_d=0.346), and factor of flower number maintaining a moderate significant correlation (R_{treat}=0.576; R_d=0.553). Notably, the individual corm weight at the end of the experiment was moderately but negatively impacted by both factors (R_{treat}=-0.514; R_d=-0.469), while the final corm number was strongly positively affected by both factors (R_{treat}=0.757; R_d=0.715).

Keywords: *Crocus sativus, Lebanon, organic matter, potassium-nitrogen fertilization.*

Introduction

Saffron (*Crocus sativus* L., Iridaceae) is widely recognized as one of the most valuable and expensive medicinal plants globally (Nazarian *et al*., 2024). Initially confined to specific geographic areas, its significance has led to its cultivation worldwide (Mathew, 1999). Typically grown in arid and semi-arid regions, saffron is planted in early fall and completes its growth cycle by mid-spring (Pirasteh-Anosheh and Kamgar-Haghighi, 2019). Successful production depends on key soil characteristics, such as good nutrient content, suitable bulk density, a well-developed flexible structure, proper drainage, and adequate water-holding capacity (Shahandeh, 2020).
The economic yield of saffron varies significantly across different regions, with no clear pattern or explanation for these variations (Pirasteh-Anosheh *et al*., 2023). This variation is hypothetically attributed to soil properties, which significantly influence saffron flower yield and highlight the importance of soil conservation for high-quality production (Aghhavani Shajari *et al*., 2018; Cardone *et al*., 2020).

In areas where saffron is cultivated, the absence of specific fertilizer recommendations is notable due to variations in soil organic matter, climate, and agricultural practices (WBSE, 2006). Some researchers argue that fertilization might not be crucial in fertile soils, as saffron corms can store ample nutrients to support plant growth. This perspective is reinforced by the relatively low nutrient absorption observed in saffron (Shahandeh, 2020). Thus, the economic yield, particularly the stigmas, relies more on corm nutrient reserves than direct soil conditions. When evaluating saffron's nutrient requirements, it is essential to consider both stigma and corm yield (Douglas *et al*., 2014).
Optimizing saffron growth and yield requires emphasizing fertilization and proper nutrition to balance vegetative and reproductive growth (Koocheki, 2004). Studies highlight that soil physicochemical and biological properties are critical indicators for designing crop yield plans and guiding the selection of manure to improve soil properties and crop yield (Ma *et al*., 2021).
Various studies have investigated the effects of different nitrogen fertilizers on saffron growth and production. Kirmani *et al*. (2014) reported a substantial increase in saffron yield and corm output with the application of nitrogen at 90 $kg.ha^{-1}$. Similarly, Omidi *et al*. (2009) demonstrated the positive effects of nitrogen chemical and bio-fertilizers on saffron yield and quality components. Potassium, the second most vital nutritional element for spice crops, enhances nitrogen utilization, protein production, size and weight, oil content, and coloration (Fatematuzzohora and Karim, 2020). In saffron, potassium acts as a flowering stimulator and plays a crucial role in providing nutritional support during the reproductive growth stage (Jabbari *et al*., 2017; Khayyat *et al*., 2018).
The objective of this paper is to provide an overview of how various fertilization regimens and saffron planting densities correlate with saffron growth and yield characteristics.

Material and Methods

Experimental site:
The experiment took place over three years in Deir el Ghazel, Lebanon, using 6000 healthy saffron bulbs (2.8 cm diameter) selected from 9000 bulbs at Qaa Farm in northern Lebanon.
The pedo-climatic conditions of the experimental site:
According to ICARDA, Deir el Ghazel has a hot, dry season from May to September and very cold weather for the rest of the year. Average rainfall is 450 mm, with 80% occurring between November and March, and no rain from June to September.
Experimental set up:
The experiment assessed the effects of "Bulb Density," "Vegetal Organic Matter" (VOM), and "Potassium Nitrogen Fertilization" (KNO_3) on saffron. At two density levels, 30 and 56 bulbs per unit area, KNO_3 was applied at 0, 5, 10, and 15 units, with VOM included at 1% in selected treatments. Using a Randomized Complete Block Design (RCBD), the study included 16 treatments across two bulb density categories with 3 replicates each. Controls had no KNO_3 and no VOM. Fertilizers were manually applied in February 2020, January 2021, and February 2022.
Statistical analysis:
IBM SPSS Statistics 20 software was used for Pearson's correlation analysis to examine how the fertilization regime and saffron density affect the growth and yield indicators.

Results and Discussion

Table 1 shows a strong positive correlation (R = 0.975) between fertilization regimes and initial corm density which imply their mutual codependence in potential yield improvement.

However, their influence was altered notably over two years. In the second year, both fertilization regimes and corm density were highly positively correlated with flower number (R_{treat} = 0.928; R_d = 0.922) and saffron weight (R_{treat} = 0.914; R_d = 0.916), and moderately correlated with flower number (R_{treat} = 0.631; R_d = 0.6) and leaf length (R_{treat} = 0.554; R_d = 0.633). In the third year, the correlations decreased, showing moderate positive correlations with flower number (R_{treat} = 0.613; R_d = 0.582) and weaker correlations with saffron weight (R_{treat} = 0.377; R_d = 0.346) and flower number (R_{treat} = 0.576; R_d = 0.553).
According to the literature, saffron production can be influenced by different factors, including planting method, irrigation method and applying fertilizers (Fallahi *et al*., 2021), climatic factors (like air temperature and precipitation), and cultivation site conditions (Cardone *et al*., 2019). Focusing specifically on the impact of fertilization—both chemical and organic—on saffron growth and yield, our results demonstrate that fertilization type and dosage significantly affect saffron growth and yield, varying by year. This finding contrasts with Pirasteh-Anosheh *et al*. (2023), who found no significant correlation between chemical fertilizer amounts and saffron yield, though they noted that higher bulk density was linked to higher yields. In contrast, Maktabdaran *et al*. (2018) identified a significant linear relationship between nitrogen percentage and yield.
Planting density generally boosts yield, with more corms per area leading to more flowers and stigmas, as seen in the second year. This effect, however, diminishes by the third year. Douglas *et al*. (2014) also noted that planting density can influence crop productivity and yield components, particularly the number of flowers per square meter. When evaluating factors that impact saffron growth and yield, it is crucial to consider the strong correlation between saffron’s behavior and climatic factors, such as temperature, precipitation and extreme weather events (Kouzegaran *et al*., 2020). This correlation is evident in the results of both years. The temperature remained relatively consistent between the two years; however, in the third year, additional rain just before and during the production season restrained growth compared to the second year, when there was no rain just before and during the production period.

Table1. Pearson correlation analysis of growth and yield indicators affected by fertilization and saffron density

<table>
<tr><th></th><th>Treat/d</th><th>F nb2</th><th>SW2</th><th>F nbF2</th><th>L L2</th><th>F nb3</th><th>SW3</th><th>F nbF3</th><th>L L3</th></tr>
<tr><td rowspan="2">Treat</td><td rowspan="4">.975**
<.001</td><td>.928**</td><td>.914**</td><td>.631**</td><td>.554**</td><td>.613**</td><td>.377**</td><td>.576**</td><td>0.086</td></tr>
<tr><td><.001</td><td><.001</td><td><.001</td><td><.001</td><td><.001</td><td>0.008</td><td><.001</td><td>0.562</td></tr>
<tr><td rowspan="2">d</td><td>.922**</td><td>.916**</td><td>.600**</td><td>.633**</td><td>.582**</td><td>.346*</td><td>.553**</td><td>0.112</td></tr>
<tr><td><.001</td><td><.001</td><td><.001</td><td><.001</td><td><.001</td><td>0.016</td><td><.001</td><td>0.449</td></tr>
</table>

F nb: Flower number; SW: Saffron weight; F nbF: Factor of flower number; L L: Leaf length; 2: second year; 3: third year. ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Table 2 shows that in the final third year, corm parameters were variably affected by fertilization regimes and densities. Total corm weight had a weak but highly significant positive correlation with both factors (R_{treat} = 0.398; R_d = 0.386). In contrast, the final number of corms was strongly positively correlated with high significance (R_{treat} = 0.757; R_d = 0.715) with the factors. Notably, individual corm weight was moderately negatively correlated with both fertilization and density factors, with high significance (R_{treat} = -0.514; R_d = -0.469), suggesting that different fertilization types, dosages, and densities lead to variations in individual corm weight. Additionally, the corm multiplication factor was weakly negatively correlated with fertilization (R_{treat} = -0.354) and more significantly negatively correlated with density (R_d = -0.435).

Effective fertilizer management is essential for maintaining balanced nutrient availability, which is critical for saffron production, especially in arid and semi-arid regions (Amiri, 2008). Our study indicates that the choice of fertilization regime, dosage, type and planting density should be aligned with the specific objectives of saffron cultivation to achieve optimal results. Research shows that applying macronutrient fertilizers increases corm size (Dewir *et al*., 2022) and corm fresh weight (Salas *et al*., 2020). However, regardless of fertilizer treatments, increasing planting density leads to a significant reduction in the number and weight of medium and large daughter corms per plant (Seyyedi *et al*., 2018). This reduction may be due to heightened competition among corms for nutrients. Koocheki and Seyyedi (2016) observed a negative correlation between the number of corms and corm dry weight, suggesting that higher planting densities make mother corms more susceptible to nutrient limitations (Seyyedi *et al*., 2018). Other studies have highlighted the importance of mother corm nutrition for promoting growth and nutrient uptake in daughter corms (Amiri, 2008; Koocheki and Seyyedi, 2015b).
The previous observations are consistent with our findings. Our results regarding the corm multiplication factor and individual weight indicate that higher initial planting densities lead to fewer and smaller final corms compared to lower densities. Although the final number of corms is influenced by planting density and fertilization, this pattern is primarily due to competition for space and nutrients, as well as the characteristics of the initial corm. Additionally, Cardone *et al*. (2019), observed that corm behavior is also affected by its initial size and geographical origin.

Table 2. Pearson Correlation Analysis of corm parameter, chlorophyll content and flower weight

	FW3	CW3	F nb C	C_{mult} F	$_{Ind}$CW	Ch C
Treat	0.097	.398**	.757**	-.354*	-.514**	-0.1
	0.51	0.005	<.001	0.013	<.001	0.497
d	0.278	.386**	.715**	-.435**	-.469**	-0.077
	0.056	0.007	<.001	0.002	<.001	0.602

FW: Flower weight; CW: Corm weight; F nb C: Final number of corm; C_{mult} F: corm multiplication factor; $_{Ind}$CW: Individual corm weight; Ch C: Chlorophyll content. ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

Conclusion

Based on the obtained results, we can conclude that achieving optimal saffron yield, both in terms of stigma and corms, requires specific agricultural practices, including customized fertilization regimes and appropriate corm densities. Additionally, the initial corm parameters and weather conditions are as crucial as the fertilization regime and planting density in influencing production outcomes.

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IMPACT OF CLIMATE CHANGE ON SUNFLOWER PRODUCTION

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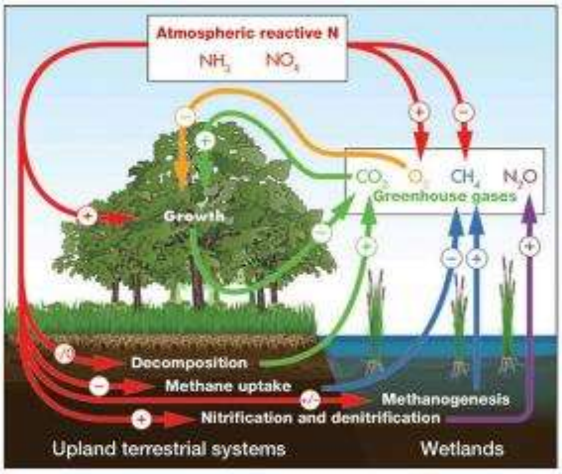
Abstract

Current population growth trends suggest that global food production is unlikely to meet future demand. However, according to projected climate change scenarios, this trend could be mitigated through the use of new drought-tolerant hybrids and sustainable management of crops and natural resources. Climate change is currently one of the main factors affecting agricultural production. These negative impacts are already evident, and projections indicate that this will only intensify in the coming years. This growing concern has sparked interest among domestic and international scientists in conducting detailed analyses of global warming and its effects on agricultural production. Agricultural producers will increasingly face the challenge of finding alternatives to reduce the negative effects of climate change. Agricultural production, particularly crop production, is essentially an 'open-air factory,' carried out in fields and thus highly susceptible to external environmental factors. The aim of this research was to examine the impact of climate parameters on sunflower yields from 2015 to 2023 in the Republic of Serbia. The study also assessed the effects of climate conditions on the economic outcomes of sunflower production, both under optimal growing conditions and in conditions where the effects of climate change were evident.

Keywords: *sunflower, climate change, yield, crops, economic results.*

Introduction

Agriculture represents a very important activity in the Republic of Serbia. Sunflower (Helianthus annuus) is a significant crop in both the Republic of Serbia and the world, with a tendency toward increasing cultivation areas and market importance. Sunflowers are cultivated in Serbia on just over 250,000 hectares (FAO, 2022). The success of stable production largely depends on the sowing time, applied agricultural techniques, hybrid selection, and weather conditions (Stojković et al., 2023). Given that agricultural production is mostly conducted in open fields, environmental conditions significantly affect its success. For the Serbian region, analyses of observed climate changes indicate a clear trend of rising air temperatures across the entire country, while precipitation changes show seasonal and spatial variability (Luković et al., 2014; Milovanović et al., 2017). It is expected that rising air temperatures, changes in the amount and distribution of precipitation, and increased frequency and intensity of drought will lead to higher crop water needs, both in Europe (Rio et al., 2018) and in Serbia (Gregorić et al., 2020). In recent years, we have witnessed frequent occurrences of severe droughts, as well as frequent weather hazards such as strong winds, storms, hail, and similar events. All these occurrences indicate that Serbia is among the countries highly susceptible to the negative effects of climate change (European Environment Agency, EEA, 2017). The increase in air temperature and changes in the distribution and amount of precipitation negatively impact the agricultural production of European countries, water resources, and consequently socio-economic changes due to the frequent changes in the supply of agricultural raw materials, which affect the uncertainty of operations in the food industry, trade, and other associated sectors of the economy and society as a whole (Fronzek et al., 2019). All of this raises concerns that, in the future, agricultural production may not be possible without the use of irrigation. Irrigation is considered the most reliable measure for adapting to climate change (Agovino et al., 2019). However, it has its limitations, primarily due to the scarcity of water resources, which will become increasingly limited and/or highly variable in terms of available water quantities (Idrizović et al., 2020). Research on the impact of climate change in Serbia indicates that the climate will remain favorable for agricultural production (Mihailović et al., 2015). Agriculture in southeastern Europe will be affected by climate change, as confirmed by numerous studies (Webber et al., 2018).



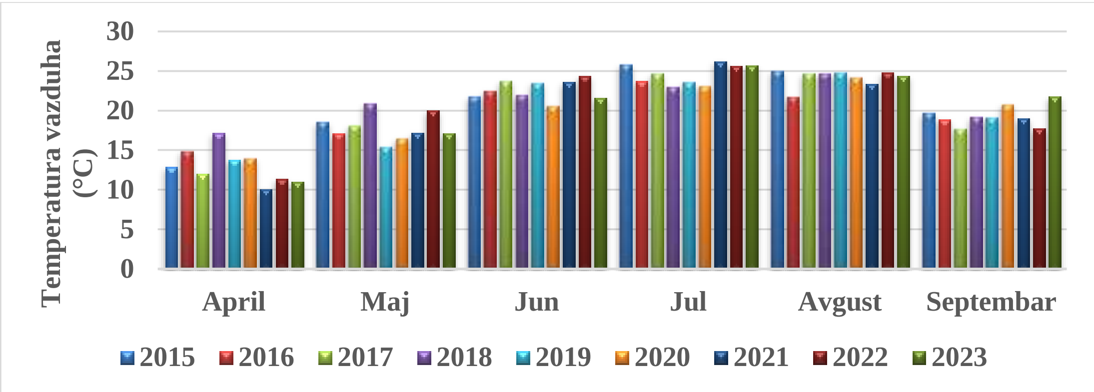
Picture 1. Gases and processes involved in the greenhouse effect. Sources from (Templer et al. 2012).

Material and methods

In order to assess the overall sunflower production in the Republic of Serbia from 2015 to 2023, data from the Republic Hydrometeorological Institute portal was used to gather information on average monthly air temperatures and total monthly air temperatures over the past 9 years (RHMZ, 2023). The average yield of the studied crops for the mentioned period was obtained from the portal of the Republic Statistical Office (RZS, 2023). Based on the data collected from these portals, an assessment was made of the impact of air temperature and total precipitation during the growing season on sunflower yield. Additionally, based on the data from variable cost calculations, the gross margin amounts for sunflower production were determined, and an analysis of the sensitivity of yield and price changes on the gross margin of sunflower production was conducted.

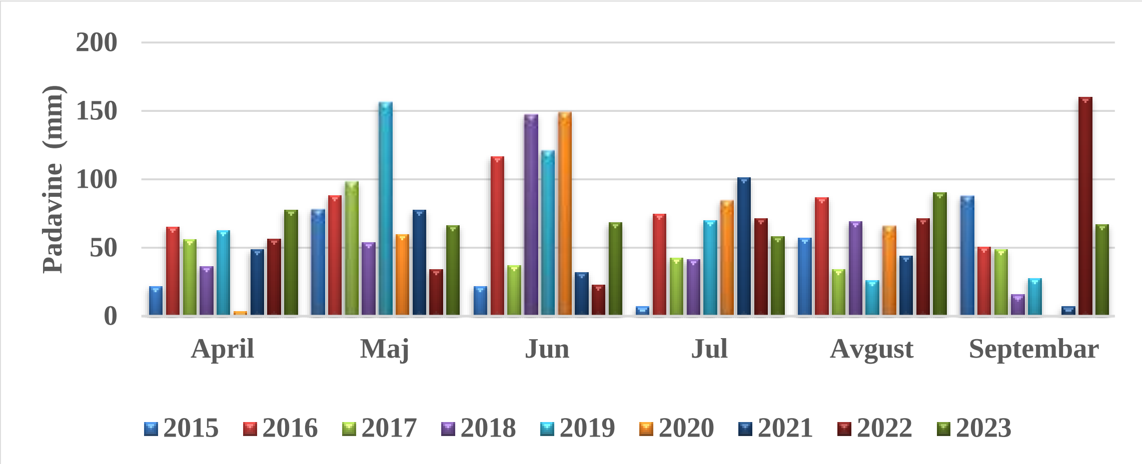
Results and discussion

Crop production is often referred to as a "factory under the open sky," which is why environmental conditions have a significant impact on production success. Climate change, based on observed climate parameters, indicates negative consequences, but it is also possible to expect some positive effects on agriculture (Stričević et al., 2019). In Serbia, an increase in the average daily temperature of 1.2°C, exceeding global averages, has been recorded. This increase is particularly notable during the summer period, along with a lack of precipitation, which is one of the limiting factors for the growth of the most important crops (Lalić et al., 2013). Studies have shown that the water requirements for certain crops in Serbia (orchards, grasses, sugar beets, soybeans) will increase by the end of the century (Stričević et al., 2019). The average long-term air temperatures in Serbia during the period from 1921-1940 were 10.2°C, while in the period from 1961-1990, they were 10.1°C. Precipitation amounts were approximately similar, ranging from 721 to 734 mm. Each crop type has a critical period for water and heat, with some having longer, and others shorter durations. For sunflowers, these critical phases are during the phenological stages of intensive growth, flowering, and grain filling. Sunflower is a significant oilseed crop grown worldwide due to its diverse uses in the food, industrial, and pharmaceutical sectors. It is known for its high oil content, nutritional value, adaptability to various climate conditions, and environmental benefits. Sunflower is an annual plant with a growing period of 90-130 days, during which it undergoes both vegetative and generative phases of growth and development: germination and emergence, leaf development, appearance of flower buds (buttoning), the stage where the flower bud elongates above the top leaf, bud opening, flowering phase, and maturation phase. Sunflower is a heavy consumer of water. In addition to 250 mm of winter reserves (in our region), it requires about 300 mm of rainfall during the growing season. Its greatest water needs are during the periods of intense growth and flowering (about 65% of the total water absorbed). In many years, insufficient rainfall leads to poorer flowering and reduced grain yield. The sum of active temperatures (above 10°C) required for sunflower growth and development ranges between 1,600 and 2,800°C. Newly emerged plants can withstand frosts down to -6°C. The optimal temperatures for flowering, seed formation, and filling are between 20-25°C. Very high temperatures (> 35°C), especially combined with a lack of rainfall from flowering to maturation, negatively affect seed and oil yield.



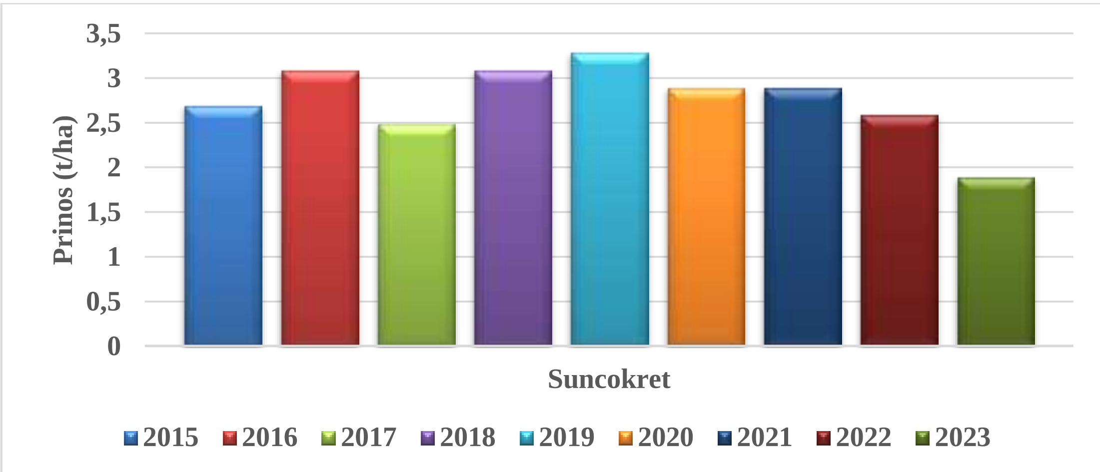
Graph 1. Average monthly air temperatures during the growing season of spring crops (RHMZ, 2023).

Based on the data from Graph 1, we can conclude that average air temperatures during the growing season of spring crops did not vary significantly over the years. The greatest variation was observed in April, and a similar trend was seen in May, but with smaller temperature fluctuations. The summer months of June, July, and August exhibited fairly consistent average monthly air temperatures over the past 9 years, with a slight increase in the last 3 years, except for June 2023, when temperatures were slightly lower than in 2021 and 2022. The highest average monthly air temperature during the observed period was recorded in July 2021, at 26.3ºC, while the lowest temperature was recorded in April of the same year, at 10.2ºC. The highest air temperature during the sunflower growing season occurred in 2018, averaging 21.27°C, while the lowest was in 2016, at 19.90°C. In the vegetative growth stages of sunflower, from 12-16 BBCH to 18-32 BBCH, average temperatures in April and May did not deviate much from the optimal range, which was favorable for proper germination and plant growth. The greatest temperature variations occurred in April, where a decrease in average daily temperatures has been observed in the last three years. The optimal temperature for the stages of seed formation, filling, and maturation is between 22-23°C. Average temperatures from June to August were higher than the optimal range: June by 2.73°C, July by 1.70°C, and August by 1.29°C.



Graph 2. Total monthly precipitation during the growing season of spring crops (RHMZ, 2023)

Based on the data from Graph 2, we can say that the total monthly precipitation amounts do not exhibit a clear pattern and vary significantly from year to year. The highest average precipitation was recorded in September 2022, measuring 160.8 mm, while the lowest amount during the studied period was measured in September 2020, with only 1.2 mm of rain on average for the month. The year 2020 was particularly unfavorable for crop production because insufficient rainfall in April slowed and unevenly affected the germination and emergence of spring crops.



Graph 3. Sunflower yield in the period 2015-2023 (RZS, 2023).

The sunflower yield during the observed period was significantly more uniform (with the exception of 2023), ranging from 1.9 to 3.3 t/ha. Although 2023 was not an extremely dry year, high air temperatures during June, July, and August led to a decrease in sunflower yields. In 2017, a satisfactory amount of rainfall occurred in May, June, and July, which coincided with the critical water period for sunflowers, resulting in a high yield that year. Research is being conducted to identify alternative preventive measures, including changes in sowing dates, variety selection, and the application of breeding methods (Popović et al., 2014), as well as various plant cultivation systems aimed at preserving soil moisture.

Table 1. Analysis of the sensitivity of the gross margin of sunflower production to changes in yield and price (average in the period 2015-2023)

		Price (din/kg)				
		-20%	-10%	Average	10%	20%
Yield (kg/ha)		**34.58**	**38.91**	**43.23**	**47.55**	**51.88**
-20%	**2,136.00**	16,398.07	25,632.00	34,865.93	44,099.86	53,333.79
-10%	**2,403.00**	25,632.00	36,020.17	46,408.34	56,796.51	67,184.68
Average	**2,670.00**	34,865.93	46,408.34	**57,950.75**	69,493.16	81,035.57
10%	**2,937.00**	44,099.86	56,796.51	69,493.16	82,189.81	94,886.46
20%	**3,204.00**	53,333.79	67,184.68	81,035.57	94,886.46	108,737.35

Source: Editing by the author

In Table 1, the results of the sensitivity analysis of the gross margin from sunflower production to changes in yield and price for both 10% and 20% variations (for both increases and decreases) are presented. The average price of sunflower during the period from 2015 to 2023 was 43.23 din/kg, while the average yield was 2,670.00 kg/ha. The average gross margin from sunflower production during the analyzed period was 57,950.75 din/ha. The sensitivity analysis of the gross margin from sunflower production established that even with a decrease in price and yield by 20%, the gross margin remains positive, amounting to 16,398.07 din/ha. Even in 2023, when the price of sunflower was 37.5 din/kg and the yield was 1,900.00 kg/ha, the gross margin would still remain positive at 13,776.65 din/ha. Based on this, it can be concluded that although affected by climate change, specifically drought, sunflower yields in certain years with pronounced drought can be significantly lower (by up to 30%), and with prices approximately 15% lower than the averages during the analyzed period of 2015-2023, the gross margin from sunflower production remained positive.

Conclusion

Based on the analysis of climate parameters and sunflower yields, a constant increase in average monthly temperatures in June, July, and August has been observed, which adversely affects the development of sunflowers and other spring crops. The total monthly amounts of precipitation show significant irregularity; insufficient rainfall in June, July, and August—when most crops are in their generative growth stages, representing a critical period for water—can lead to stunted growth, lack of fertilization, and reduced yields. It can be concluded that it is essential to work on developing new genotypes that would be adapted to the emerging conditions, to conduct research aimed at expanding the irrigation network, and to implement alternative agronomic methods to mitigate stress conditions with fewer consequences. Although the impact of climate change, specifically drought, is significantly expressed through reduced sunflower yields, the gross margin during the analyzed period from 2015 to 2023 remained positive. Specifically, agricultural producers who cultivated sunflowers did not incur losses and achieved positive economic results.

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ENSEMBLE EVALUATION AND MEMBER SELECTION OF REGIONAL CLIMATE MODELS FOR CROP MODEL IMPACT ASSESSMENT

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Abstract

Climate change poses a significant challenge to Greek agriculture, impacting crop productivity and sustainability. Wheat is a crucial crop, primarily grown in regions with favorable climatic conditions and fertile soils. Climate change negatively affects wheat production through increased temperatures, extreme weather events, and changes in precipitation, leading to reduced yield and seed quality. The impact varies by region, with high-altitude areas experiencing temperature fluctuations and reduced snow cover, while coastal areas face sea-level rise and coastal erosion. Practices such as cultivating resilient wheat varieties, adjusting sowing dates, and improving water management are essential. Climate change impacts increasingly rely on ensembles, which combine multiple climate models to balance individual model errors. Careful selection and evaluation of ensemble members is essential for accurate predictions. In this study, a region in central Greece was selected for its diverse elevation and climate conditions. Data from eight meteorological stations for the period 2006 to 2023 and 11 EURO-CORDEX regional climate model simulations for three emission scenarios (RCP2.6, RCP4.5, RCP8.5) were used. The selection methodology ranked models based on the sRPI index, which evaluates temperature deviation performance. Preliminary results from the calibrated CERES-Wheat model in Greek conditions showed that selecting only the best simulation scenarios provided more reliable predictions for flowering, maturity, and potential yield compared to using the full ensemble. Careful selection of ensemble members based on climate is expected to ensure more reliable agronomic model predictions, helping farmers better adapt to climate change and maintain crop sustainability and productivity.

Keywords: *ensemble model evaluation, climate change, wheat production, CERES-Wheat, simulation scenarios, Greece.*

Introduction

Climate change significantly impacts crop productivity and sustainability, posing a challenge for Greek agriculture (IPCC, 2023). Among the various crops cultivated in Greece, wheat holds particular importance. It is mainly grown in Thessaly, Macedonia, Thrace, and central Greece, areas known for their good climate and fertile soil (Koutsika et al., 2010). However, the effects of climate change (rising temperatures, extreme weather events, and alterations in precipitation patterns, etc.) are increasingly threatening wheat production, leading to reduced yields and compromised seed quality (Farooq et al., 2023; Jägermeyr et al., 2021).
The impact of climate change on wheat production differs among various regions in Greece. High-altitude areas experience significant variations in temperature and reduced snow cover,

while coastal areas face the difficulties posed by rising sea levels and coastal erosion. To mitigate these adverse effects, modern agricultural practices, such as cultivating resilient wheat varieties, adjusting sowing dates, and enhancing water management strategies, are crucial (Collins & Chenu, 2021).

Climate data analysis indicated a general trend of increasing temperatures and changing precipitation patterns across Central Greece. Average temperatures are projected to rise by 1.5°C to 2.5°C by mid-century, with more pronounced increases during the summer months (Georgoulias et al., 2022). Precipitation patterns are also expected to vary, with some models predicting drier conditions and others indicating increased winter rainfall. These changes are expected to impact the wheat growing season, affecting crop development stages and yields.

To mitigate the adverse effects of climate change, several adaptation strategies are recommended. These include cultivating resilient wheat varieties with enhanced tolerance to heat and drought, adjusting sowing dates to avoid peak temperature periods and align with favorable climatic windows, and improving water management through efficient irrigation practices and water conservation techniques (Collins & Chenu, 2021). The adaptation strategies proposed here are essential for sustaining wheat production in central Greece amid climate change, supporting farmers in making informed decisions to enhance crop resilience and ensure food security.

Accurate climate analysis is fundamental for developing effective adaptation measures. Such analysis often relies on ensembles, which combine multiple climate models to mitigate individual model errors and provide a comprehensive picture of future climate scenarios. Reliable predictions necessitate thorough selection and evaluation of ensemble members. The targeted selection of ensemble members, based on climate variables and geomorphological characteristics, is anticipated to ensure more accurate agronomic model predictions (Minaei et al., 2022; Ruane & McDermid, 2017; Wada et al., 2023). These advances help farmers adapt to climate change, thereby ensuring the sustainability of Greece's agricultural sector and maintaining crop productivity (Brunner et al., 2020; Knutti et al., 2017; Merrifield et al., 2020). In this study, a region in central Greece was chosen due to its varying elevation and climatic conditions. The research utilized data from eight meteorological stations for the period 2006 to 2023 and 11 EURO-CORDEX regional climate model (RCM) simulations across three emission scenarios (RCP2.6, RCP4.5, RCP8.5). Models were ranked using the sRPI index, which assesses temperature deviation performance of RCMs.

Materials and methods

The study focuses on a region in central Greece, due to its diverse elevation and climatic conditions, providing a comprehensive landscape for studying the impacts of climate change on wheat production. Data were collected from eight meteorological stations within this region (Fig. 1). These stations provided daily observations of precipitation (mm), maximum and minimum temperatures (^{o}C), while solar radiation data were provided from the Copernicus Atmosphere Monitoring Service (CAMS) (Guevara et al., 2021) for the period from December to June (i.e., the growing season) over 17 years (2006-2023). These stations are located at altitudes ranging from 98 to 760 meters above sea level.

Additionally, simulated climate data were obtained from 11 Regional Climate Models (RCMs) of the EURO-CORDEX project, with a spatial resolution of approximately 12.5 km. The RCMs simulated climate conditions under three emission scenarios: RCP2.6, RCP4.5, and RCP8.5 (Georgoulias et al., 2022). The consistency between the simulated and observed data was guaranteed by utilizing data from the grid cells that contained the meteorological stations (Figure 1).

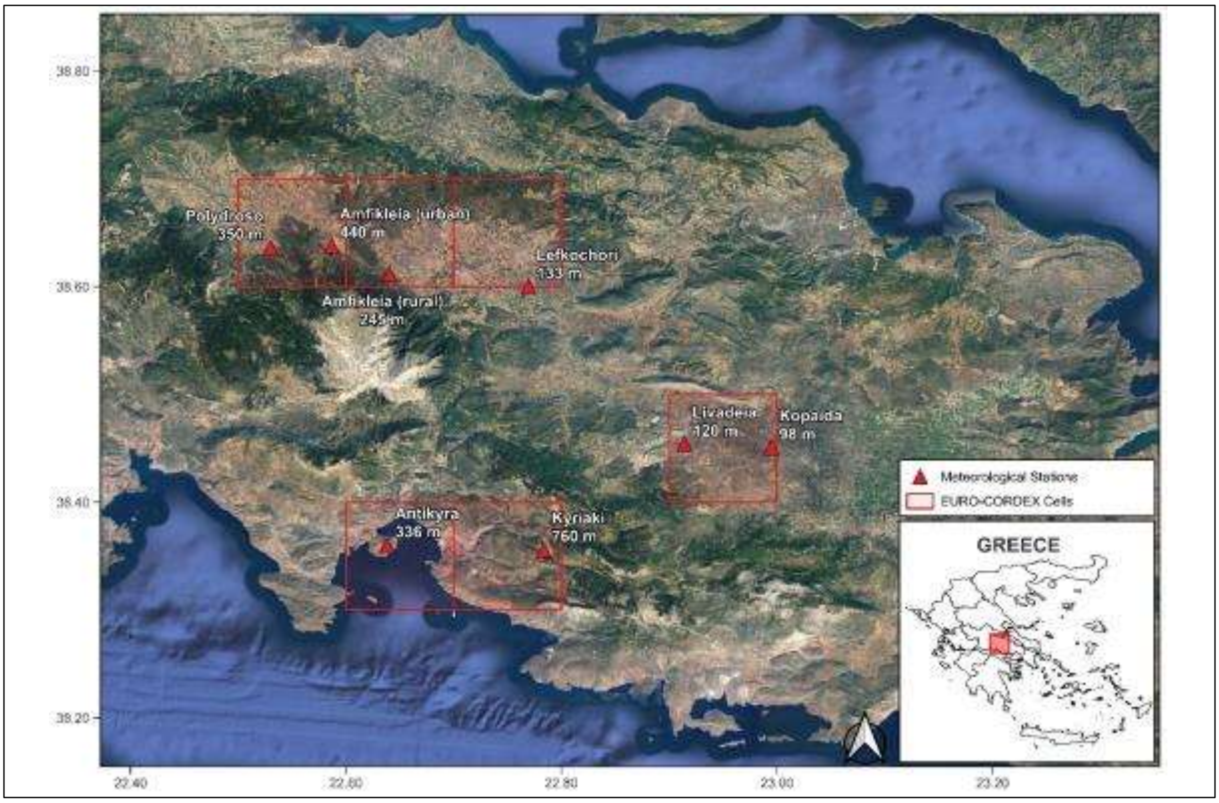

Figure 1. Study area with meteorological stations (red triangles). Their elevations and the respective EURO-CORDEX cells (red rectangles) are also shown

To ensure the selection of suitable climate model ensemble members, the methodology proposed by Aschonitis et al. (2019) was adopted. This method involves evaluating each of the 11 climate models using the standardized Root Mean Square Error index (sRPI) for each emission scenario. The sRPI index assesses model performance based on 12 statistical criteria that measure the deviation of temperature simulations from observations (Table 1). Models were ranked accordingly, with the models being categorized into top-performing models (sRPI > 50%) and low-performing models (sRPI < 50%) (Table 2).

Table 1. Statistical Criteria Used to Evaluate Climate Models

Abbreviation	Statistical Criterion
d	Index of Agreement
NSE	Coefficient of Efficiency (Nash-Sutcliffe efficiency)
KGE	Kling-Gupta efficiency
VE	Volumetric Efficiency
AME	Absolute Maximum Error
MAE	Mean Absolute Error
MBE	Mean Bias Error
RMSE	Root Mean Squared Error
R4MS4E	Fourth Root of the Mean Quadrupled Error
RAE	Relative Absolute Error
RVE	Relative Volume Error
SSE	Sum square error

Regional agronomic data from greek conditions were used to calibrate the CERES-Wheat model (Nikou and Mavromatis 2023), a crop model of the Decision Support System for Agrotechnology Transfer (DSSAT) (Hoogenboom et al. 2019). Crop simulated flowering dates, maturity dates, and potential yield were compared between the top-performing models, low-performing models, and the full ensemble to determine if there are any advantages of selective model inclusion. The performance of climate models and the CERES-Wheat model was assessed using common statistical metrics.
Based on crop model predictions, three sowing dates (November 15, December 1, and December 16) were tested to evaluate climate models and to determine if changing the sowing date could effectively mitigate the adverse impacts of climate change on wheat production. Accurate climate predictions combined with agronomic models are intended to provide practical insights that could assist Greek farmers adjust to changing climate conditions and maintain sustainable wheat production and food security in the region.

Results and discussion

The evaluation of the 11 climate models using the standardized Root Mean Square Error index (sRPI) revealed significant variability in their performance. Models, which had the highest sRPI scores, consistently showed lower deviations from observed temperature data, indicating their suitability for simulating local climate conditions. Conversely, models with lower sRPI scores exhibited higher errors and were deemed less reliable (not shown).

Table 2. Performance of Regional Climate Model Simulations

Category	Model Simulation
Top-Performing	CCLM4-8-17_ICHEC-EC-EARTH
	HIRHAM5_ICHEC-EC-EARTH
	RCA4_MPI-M-MPI-ESM-LR
	REMO2009_MPI-M-MPI-ESM-LR_r1
	REMO2009_MPI-M-MPI-ESM-LR_r2
Low-Performing	ALADIN63_CNRM-CERFACS-CNRM-CM5
	RACMO22E_CNRM-CERFACS-CNRM-CM5
	RACMO22E_ICHEC-EC-EARTH
	RACMO22E_MOHC-HadGEM2-ES
	RCA4_ICHEC-EC-EARTH
	RCA4_MOHC-HadGEM2-ES

The CERES-Wheat model simulations provided insights into how different climate scenarios would impact wheat phenology and yield. The results for the three sowing dates revealed several key findings. For anthesis dates, the best-performing models predicted dates within a range of 4-6 days (based on means and medians of Fig 2) earlier than observations, while the low-performing models 8-11days later and the full ensemble within a range of 1-4 days later. There is agreement between the three emission scenarios.
For maturity dates, the best simulation runs provided more accurate predictions (within 3-4 days earlier than observations), whereas low-performing models and the full ensemble predictions resulted in later (11 to 12 days and 3 to 5 days accordingly) predictions. An agreement was also found between the three emission scenarios.

Yield predictions from the top-performing models showed a variability in deviation ranging from -8% to +6% depending on the emission scenario compared to the low-performing models that showed an increase in the range of 23% to 28% and the full ensemble indicating also an increase by 16% to 20%. These results demonstrate the value of evaluating the climate model simulations before its use in impact assessment studies.

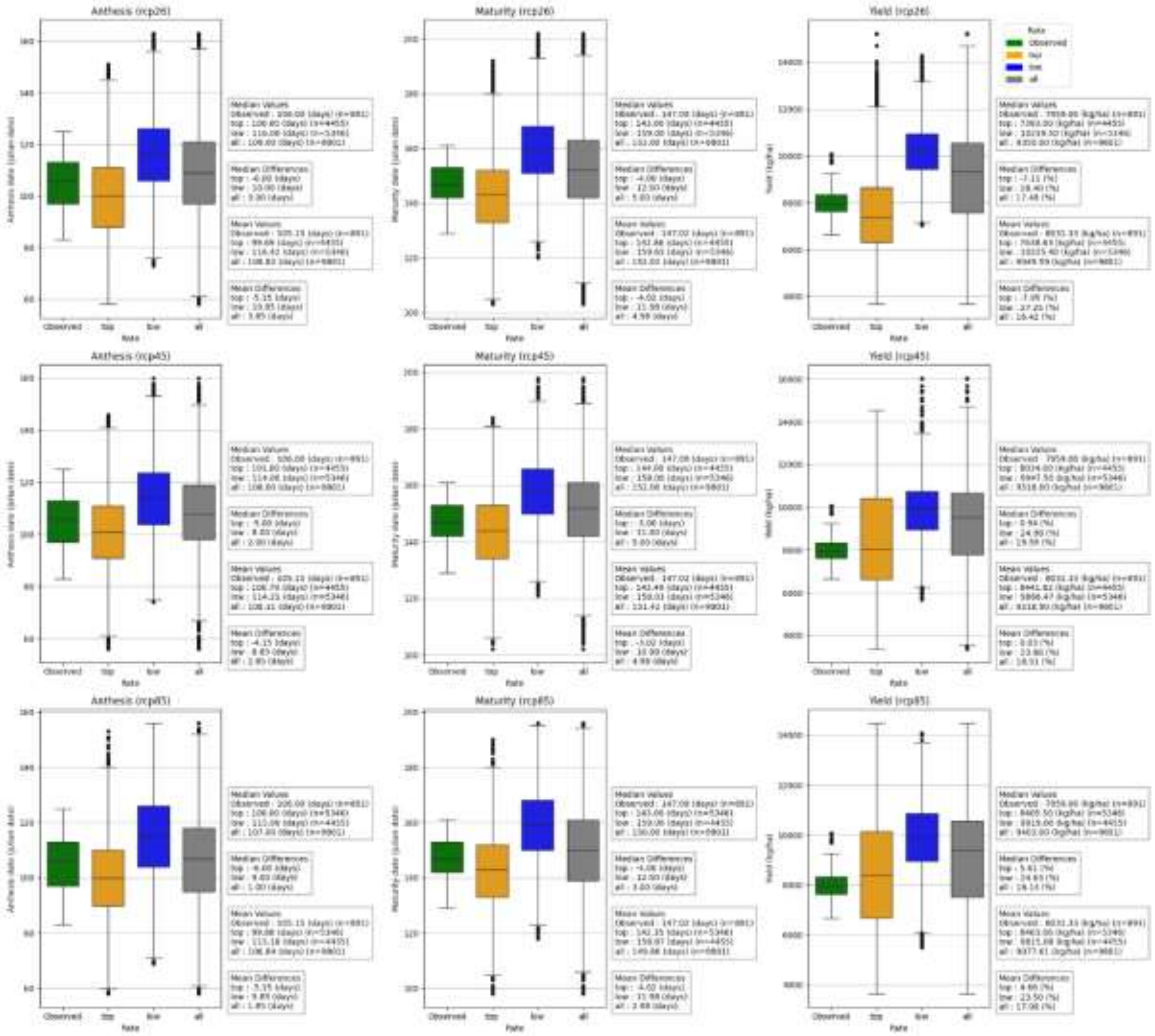

Figure 2. Comparison of anthesis days, maturity days (Julian dates), and yield (kg/ha), as derived from CERES-Wheat, for three different planting dates (15 November, 1 and 16 December), for the rcp26, rcp45 and rcp85 scenarios, for the observations (Observed), the total (all), the best (top: sRPI>50%) and worst (low: sRPI<50%) simulation scenarios. The sample size (total n of growing seasons) for each category is also presented.

Conclusion

This study highlights the critical role of climate model selection in enhancing the accuracy of crop model predictions. The study’s findings underscore the importance of selecting appropriate climate models for reliable agronomic predictions. The calibrated CERES-Wheat model, when driven by the best-performing climate models, provided more accurate predictions of wheat anthesis, maturity and potential yield, enabling better adaptation planning. Further research should continue to refine model calibration and explore additional adaptation measures to address the evolving challenges posed by climate change.

Acknowledgement

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THE EFFECT OF CHANGES IN ENVIRONMENTAL CONDITIONS ON THE GROWTH AND YIELD OF BELL PEPPER

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Abstract

Pepper (*Capsicum annuum* L.) is an economically important vegetable species in the Balkan region. Production of pepper, in recent years, has become very challenging, where abiotic stresses (extreme temperatures, drought) can reduce crop yield by more than 50%. In our region, bell pepper is one of the most popular types of pepper on the market. This experiment was conducted on 14 genotypes of bell peppers (different geographical origin) during two vegetative seasons (2020, 2021) in an open field and in the greenhouse (semi-controlled conditions) in Smederevska Palanka. Nine characteristics related to plant development and yield were observed. The aim of this study was to estimate the impact of abiotic stress conditions on pepper plant development, fruit quality and yield. The maximum recorded temperature during the observed growing seasons was 40.7 (flowering and fruiting period). The total amount of precipitation was below the optimum in both observed years: 540.7 mm (2020) and 380.1 mm (2021). The average yield recorded in greenhouse trials was higher by 56% (2020) and 78% (2021) in comparison to the trials in the open field. The results from the open field trial showed that genotype CA11 (USA origin) had the highest yield (2020 and 2021). A lower amount of precipitation in the open field had a negative effect on all observed traits (plant height, flowering time, fruit setting time, fruit size). Obtained results are essential in overcoming the problems caused by environmental changes and for improving the response of pepper plant resistance to abiotic stress.

Keywords: *Capsicum annuum L., drought, abiotic stress, high temperature, climate change.*

Introduction

Pepper (*Capsicum annuum* L.; n = 12) is an annual crop, belonging to family *Solanaceae*. It is a widely produced crop, whose area of cultivation is in the mid-latitudes (Erickson and Markhart, 2002). Based on FAO data (FAOStat. 2022), pepper is grown on a total of 2.02 million hectares, and the largest producers are China (0.757 mha), Indonesia (0.334 mha) and Mexico (0.156 mha). In the last 50 years, the area with pepper production has increased by 170% in the world (657% in China).
The origin of the genus *Capsicum* (perennial wild form) is from the tropical South American region - central and southern Bolivia (Gonzales-Perez et al., 2014). The species *Capsicum annuum* originated in the more humid areas of the Amazon river, and the further evolution of this species continued in Mexico and the northern part of Central America (Zou and Zhu, 2022). *Capsicum annuum* L. is characterized by great fruit diversity in size (1-25 cm), shape, color, pungency, pericarp thickness (Palma et al., 2020). The shape of the fruit is a very important trait, and basic characteristic for classification genotypes in groups (Zhigila et al., 2014). Genotypes of bell pepper can be very different according to the shape of the fruit: elongated, blocky, almost round, square (Sood et al., 2023). In the last few years, the

production of bell pepper has increased significantly (Anaya-Esparza et al., 2021). Besides the nutritional value of pepper fruits, it is an important source of various vitamins, phenols, carotenoids, antioxidants (Nadeem et al., 2011). The success of bell pepper production depends on various conditions, including environmental conditions (primarily temperature and precipitation), genetic diversity, as well as the resistance to diseases and pests. Changes in environmental factors (extreme temperatures and humidity) can lead to changes in the length of the vegetative season in pepper cultivation due to changes in physiological processes in plants (Bisbis et al., 2018). The optimum temperature for bell pepper cultivation is 21-33°C (Thuy and Kenji, 2015). Bell pepper is sensitive to high temperature (Erickson and Markhart, 2002), and temperature below 18°C and above 27°C for extended period slow down the development and ripening of fruits (Berke et al., 2003) and lead to a decrease in yield and fruit quality (Bisbis et al., 2018).
Climate change and its impact on agriculture is one of the most important topics today. Changes in climate parameters (extreme temperatures, rising atmospheric CO2 levels, drought) have significant impacts on agricultural production (Skendžić et al., 2021). There is evidence that the growing seasons of certain plant species have prolonged, and that the beginnings of the vegetative seasons of certain plant species have shifted due to milder winters (Lavalle et al., 2009). The consequences of climate change are also evident in the increase of insect populations (change of location, increased survival rate, number of generations, reduced number of natural enemies).
The aim of this study was to determine the effect of abiotic stress conditions on plant growth, fruit quality, and yield of 14 bell pepper genotypes.

Materials and methods

This experiment was conducted during two growing seasons (2020-2021) on the experimental fields of the Institute for vegetable crops in Smederevska Palanka, Serbia (latitude 44.354933”N, longitude 20.947183”E, elevation 101 m). A parallel trial was set up in an open field and in a greenhouse (semi-controlled conditions). Fourteen bell pepper genotypes of various geographical origins were chosen from the Institute of Vegetable Crops' gene bank collection and were used as material in this experiment. In the first decade of April, the seeds of each genotype were sown in styrofoam containers (ϕ 35 mm) and stored in a greenhouse with appropriate nourishment, irrigation and fertilization until planting. Half of the produced plants were planted in an open field (spacing between plants in row 25 cm, row spacing 70 cm). The experiment was organized in three repetitions using a complete randomized block design with 14 plots in one block and 10 plants in one plot. The experiment in the open field was conducted on the vertisol. Crop care consisted of inter-row cultivation, fertilization, protection against plant diseases and pests. Irrigation was conducted as needed, with a typhoon, several times during the growing season. In the greenhouse, the plants were planted in plastic pots (ϕ 28 cm). The temperature in the greenhouse was regulated by shading, regular nursing of the plants was carried out, and watering was carried out daily.
Nine traits related to plant development and yield were observed (1. Length of stem to first flowering brunch (cm) – 100 days from sowing; 2. Plant height (cm) – 100 days from sowing; 3. Fruit pericarp thickness (cm); 4. Fruit weight (g); 5. Sugar content (°Bx); 6. Fruit width (cm); 7. Fruit length (cm); 8. Time of fruit maturity – days from planting; 9. Flowering time - days from planting). The obtained results were analyzed using AMMI analysis (Gauch, 2006). Statistical analysis was performed using R software, version 4.4.0 (R Core Team, 2024). Based on the results of the AMMI analysis, ASV coefficient was calculated (Purchase et al., 2000) and genotypes and environments were ranked by stability.

In Figure 1 (a and b) are shown meteorological data in Smederevska Palanka for the periods 1949-2009 and 2010-2022. These data were collected from meteorological yearbooks of Republic hydrometeorological service of Serbia (RHMZ, 1946-2022). In addition, the image also shows meteorological data for the two observed years - 2020 and 2021.

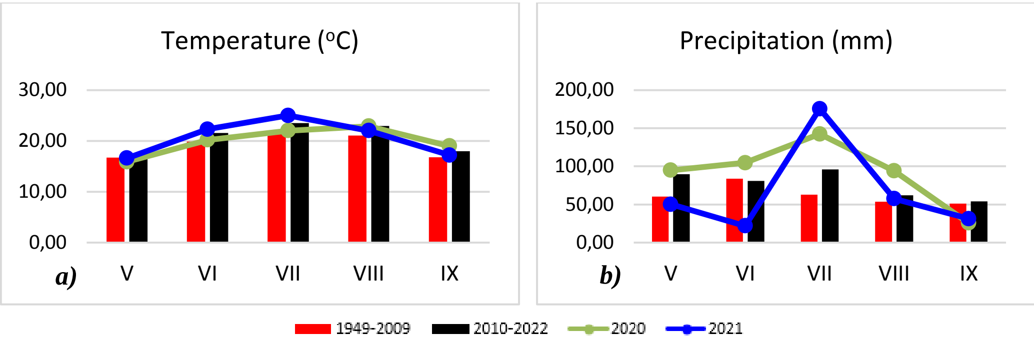

Figure 1. Meteorological data for Smederevska Palanka - a) average monthly temperatures (°C), b) total monthly precipitation (mm)

Based on the results shown in Figure 1, we can conclude that there is certain evidence of climate change in Smederevska Palanka. When compared to meteorological data recorded in a period of 50 years (1949-2009), the average monthly temperature in Smederevska Palanka in the last decade is higher by 1.49% (May) to 9.10% (July). The number of days where the maximum daily temperature is above 25°C has increased by 2-5 days (15.88% (August) to 35.89% (September)) in the last decade compared to the period 1949-2009. In 2020, the average monthly temperature was higher than the fifty-year average from June to September (by 0.36°C, 0.44°C, 1.82°C, 2.23°C, respectively). In May, the average temperature was 0.82°C lower than in the observed fifty-year period. In 2021, the number of days with a maximum daily temperature > 30°C was higher in all months of cultivation, compared to the multi-year average. The maximum recorded temperature during the observed growing seasons was 40.7°C (June 2021 - flowering and fruiting period).
The total amount of precipitation was below the optimum in both observed years: 540.7 mm (2020) and 380.1 mm (2021).The number of days with more than 10 mm of precipitation in the period of intensive plant development, flowering and fruit setting (June) decreased in the last decade compared to the fifty-year average by 11.7%, while the difference compared to the experimental years is even greater (2020 - 24 %, 2021 – 220%).

Results and discussion

Based on the AMMI analysis results (Table 1), it was determined that the genotype and the environment have a statistically significant effect on the variability of the nine observed pepper characteristics. The total sum of squares of the characteristics: length of stem and plant height were explained with the main effects of environment (66.33% and 59.04%, respectively). This means that these two observed traits were significantly influenced by the environment and that the obtained results differed depending on the environment in which the plants were grown. The variability of the trait fruit pericarp thickness was affected predominantly by the environment, although it was also affected by the GxE interaction. The variability of the remaining 6 observed traits was significantly affected by the divergence of genotypes. The total sum of squares for the characteristics fruit weight and fruit width were explained with the sum of squares of the environment with 38.27% and 40.39%, respectively. Based on this, we can conclude that the variability of these two traits was also significantly influenced by the environment in which the genotypes were grown.

Table 1. Percentage of SS in total SS of 9 observed traits (ANOVA for the AMMI model)

Source of variation	*T1	T2	T3	T4	T5	T6	T7	T8	T9
G	22.69**	28.33**	14.16**	50.98**	47.09**	42.15**	49.67**	48.47**	69.64**
REP (ENV)	0.13ns	0.14ns	1.72ns	0.09ns	1.62**	0.33ns	0.10ns	0.29ns	1.00ns
E	66.33**	59.04**	49.31**	38.27**	29.26**	40.39**	32.70**	28.94**	1.68*
GxE	9.86**	11.08**	23.24**	10.01**	18.01**	13.16**	16.85**	19.38**	18.94**
Error	0.99	1.40	11.56	0.65	4.02	3.97	0.38	2.92	8.74

**T1 - Length of stem; T2 - Plant height; T3 - Fruit pericarp thickness; T4 - Fruit weight; T5 - Sugar content; T6 - Fruit width; T7 - Fruit length; T8 - Time of fruit maturity; T9 - Flowering time; G – Genotype; REP – repetition; E – environment; SS – Sum of square*

Based on the obtained results, it was established that cultivation in semi-controlled environments provided more stable results compared to open-field pepper cultivation. However, the results also showed that the trait fruit weight had the most stable results recorded in open field production in 2020. The trait length of stem to the first fruit branch had a greater stability in 2021. During the year 2020, higher amounts of precipitation were recorded compared to the year 2021, while extreme temperatures during flowering and fruit setting were only observed for 5 days in 2020 (June). Except for the CA10 genotype, for the genotypes with the lowest variability of the trait length of stem to the first fruit branch (CA1, CA5, CA8, CA13) were also recorded below-average values for this trait, which means a lower number of fruits per plant and therefore a lower yield for the producers. The characteristic the flowering time is specific. The share of the sum of squares of the environment in the total sum of squares for this trait is only 1.68%, while the share of the sum of squares of the GxE interaction is 11 times higher.

It has been confirmed that over 80% of the potential yield of agricultural crops is lost annually due to abiotic stresses: extreme temperatures, drought, salinity, etc. (Hirt and Shinozaki, 2004). Crop sowing is often delayed due to extreme conditions during the period of optimal sowing dates (April and May). Plants enter the generative phase faster due to high temperatures, physiological processes are disrupted, pollen is sterile, flowers and fruits fall (Lee and Choi, 2013). The lack of moisture during production leads to the formation of fruits of poorer quality. For this reason, one of the most important steps in planning pepper production is to determine a good sowing date (Saqib and Anjum, 2021). It is recommended that production, especially during the period of initial development (up to the 10-leaf stage), be transferred to controlled conditions. It is predicted that by 2050 the number of inhabitants in the world will reach 9 billion. As a result of climate change and extreme abiotic conditions, many cultivated areas have been destroyed, we are facing drought and a lack of water for irrigation. To ensure safe food, it is necessary to work on plant genetic potential, resistance to abiotic stress and the introduction of new agrotechnical measures in the production of peppers.

The origin of all observed genotypes is from the northern hemisphere, from 19.39° to 52.69° latitude (CA12 and CA9, respectively). The genotypes that stood out with the highest stability, when considering all traits, are CA4, CA6, CA9, CA10 and CA13 (Hungary, Japan, France, Canada and Serbia). We can conclude that under the conditions of our country, the best adaptability still have genotypes originating from approximately the same latitude (around 45°). The highest fruit weight was recorded in genotype CA12 (originating from the United States); however, except for earliness and plant height, this genotype presented significant variability in all other parameters. The average yield recorded in greenhouse trials was higher by 56% (2020) and 78% (2021) in comparison to the trials in the open field.

Table 2. Ranking orders of AMMI stability values

Genotype	Origin	*T1	T2	T3	T4	T5	T6	T7	T8	T9	M
CA1	China	3	10	2	2	3	14	13	6	12	7
CA2	USA	11	11	9	5	11	13	5	12	6	9
CA3	Germany	14	9	14	7	9	1	3	5	5	7
CA4	Hungary	9	7	3	10	2	8	4	1	14	6
CA5	Netherland	5	8	7	12	1	12	14	4	9	8
CA6	Japan	7	12	5	1	5	9	8	7	1	6
CA7	Italy	10	14	10	9	7	3	6	3	2	7
CA8	Netherland	4	6	12	4	12	2	11	11	13	8
CA9	France	6	1	1	3	6	11	9	14	7	6
CA10	Canada	2	2	6	8	13	4	2	8	10	6
CA11	USA	13	13	11	6	14	5	7	10	8	10
CA12	Mexico	12	3	13	14	8	6	12	2	4	8
CA13	Serbia	1	5	8	13	4	7	1	9	3	6
CA14	Serbia	8	4	4	11	10	10	10	13	11	9
GH-20		4	3	1	3	1	2	3	3	1	2
GH-21		2	1	2	2	2	1	2	1	2	2
OF-20		3	4	3	1	4	3	1	4	3	3
OF-21		1	2	4	4	3	4	4	2	4	3

**T1 - Length of stem; T2 - Plant height; T3 - Fruit pericarp thickness; T4 - Fruit weight; T5 - Sugar content; T6 - Fruit width; T7 - Fruit length; T8 - Time of fruit maturity; T9 - Flowering time; GH – greenhouse; OF – open field; M – mean value*

Conclusion

The genotypes (14 bell peppers of different origin) and the environments (open field and greenhouse, observed for two years) have a statistically significant effect on the variability of the nine observed pepper characteristics. Based on the obtained results, there is evidence of certain climate changes in Smederevska Palanka. An increase in average monthly temperatures was recorded in the last decade (2010-2022) compared to the fifty-year average (1949-2009). Total precipitation decreased in both observed years: 540.7 mm (2020) and 380.1 mm (2021). Despite the above, appropriate measures such as on time sowing, shading, proper irrigation, seedling production under controlled conditions, and the good genetic potential of seed material, can significantly reduce side effects of climate change. Finally, it was determined that genotypes originating between latitudes 40° and 50° are stable enough to achieve satisfactory results in the climate conditions of our region.

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PLANT PROTECTION AND FOOD SAFETY

MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF ECTOPARASITIC NEMATODES ASSOCIATED WITH THE GENUS XIPHINEMA SPECIES IN KOSOVO VINEYARDS

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Abstract

In Kosovo, grapevine production has been extended in recent years. Its main production region is in Rahovec and neighboring municipalities. Due to the favorable weather conditions of the country, important grapevine pests and diseases have been developed. Therefore, the aim of this study was to investigate the genus *Xiphinema* species in symptomatic and damaged vineyards. Total number of seventy-one soil samples were collected in early autumn of the year 2022 and early spring of the year 2023. Soil samples were taken and transferred to the laboratory for nematode extraction and further analysis. The extraction of nematodes was carried out using Flegg modified Cobb technique. Each sample was subjected to morphological and microscopic identification. Of seventy-one soil samples, thirty-two samples were morphologically identified as *Xiphinema* spp. Furthermore, single nematodes from the positive samples were subjected to DNA extraction with commercial kits. In order to confirm morphological diagnosis, molecular analysis using the D2-D3 expansion segments of the 28S rDNA and the ITS containing region were undertaken using universal primer sets D2A-D3B and ITS1-ITS2, respectively. D2-D3 expansion domains of the ITS region and 28S rDNA gene region produced single fragments of 730 bp and 980 bp, respectively. Two years of monitoring survey conducted in two different seasons confirmed the occurrence the genus *Xiphinema* species in Kosovo vineyards.

Keywords: *Xiphinema* species, vineyards, ecto parasitic nematodes.

Introduction

In terms of production, grapevine is one of the largest cultivated crops worldwide, both for wine and table grapes production (Li *et al*. 2021; Pertot *et al.* 2017). In terms of cultivation, Europe incorporates around 40% of the vineyards worldwide (Fraga, 2019). Even though it has largest production area, grapevine faces many challenges like pests and diseases. In particular, plant parasitic nematodes pose a major threat to grapevine production. These nematodes usually damage below ground of its parts. Moreover, the injuries caused by plant parasitic nematodes on roots become prone to infections of other pathogens like bacteria, fungi or viruses leading to secondary infections (Malik *et al.* 2022). It is well known that the genus *Xiphinema* commonly called dagger nematode is characterized by the presence of a stylet used for root tissue penetration. Recent studies revealed that annual crop losses caused by this genus are estimated between 8 to 15% of total crop production worldwide (Archidona-Yuste *et al.* 2020). Particular species of this genus are vectors of several important plant viruses that cause significant damage to grapevine production (Decraemer *et al*. 2007). Among dagger nematodes, *Xiphinema index* and *Xiphinema americanum* are pathogenic in vineyards and cause economic damage by feeding on the roots (Xu *et al*. 2018; Taylor and Brown, 1997).

In Kosovo, the grapevine production area has been extended in recent years. Its main production area is in the Municipality of Rahovec and its neighboring municipalities. According to the *Green Report 2023*, total vineyard area in Kosovo has reached 3472 ha (MAFRD, 2023), however, many problems due to pests and diseases have also raised. It is well known that Kosovo has favorable weather conditions for the development of many important pests and diseases affecting grapevine production. Despite common fungal diseases, interesting studies were conducted in previous years. The presence of many important virus diseases, phytoplasma and insects affecting grapevine production have been previously recorded in Kosovo (Gjinovci *et al*. 2022; Bunjaku *et al*. 2022; Dida *et al*. 2017; 2018). Meanwhile, no data have been found concerning the presence of nematodes in affected vineyards. Apparently, no information is available in Kosovo regarding the occurrence and distribution of the genus *Xiphinema* species.

Therefore, to know the current situation of grapevine about the presence and distribution of parasitic nematodes in Kosovo, an intensive survey was conducted in symptomatic and damaged vineyards. Our study has investigated the genus *Xiphinema* species only in affected vineyards particularly, aimed to investigate the importance of *X. index* as a vector of Grapevine fan-leaf virus (GFLV).

Materials and Methods

This study was carried out in the Municipality of Rahovec which is considered the most important vineyard region in Kosovo (Fig. 1).

Fig. 1: Map of Kosovo focusing the main vineyards area of Rahovec

A total number of seventy-one soil samples were collected in early autumn of the year 2022 and early spring of the year 2023. Each sample consisted of 2 kg of soil collected from the rhizosphere with 30-60 cm depth and transferred to the laboratory with a portable cooler for nematode extraction. Each soil sample was extracted according to the EPPO protocol PM7/119 (EPPO, 2013) using Flegg modified Cobb technique. Extracted samples were then subjected to morphological and microscopic identification using Olympus CX 43 Microscope (Tokyo, Japan) with 40-fold magnification. Furthermore, a single nematode from each morphologically positive sample was subjected to DNA extraction using NucleoSpin® Tissue

XS Kit (Macherey-Nagel, Duren, Germany) strictly following manufactures protocol. The DNA isolated from each individual nematode was directly amplified by using two sets of universal primers. The ITS containing region was amplified using the forward primer ITS1 (5-TTGATTACGTCCCTGCCCTTT-3) and the reverse primer ITS2 (5-TTTCACTCGCCGTTA CTAAGG-3) (Vrain et al. 1992), while the D2A (5-ACAAGTACCGTGAGGGAAAGTTG-3) and D3B (5-TCGGAAGGAAC CAGCTACTA-3) were used to amplify the D2–D3 fragments of the 28S rDNA gene (Nunn et al. 1992). PCR amplification reactions were performed in a final volume of 25 μl containing 13.7 μl of MGW, 2.5 μl of PCR buffer 10X (Qiagen), 2.5 μl of $MgCl_2$ 25mM (Qiagen), 2 μl of dNTPs 10mM (Qiagen) 2 μl of extracted DNA and 0.3 μl of TapTaq 5U (Qiagen). In each PCR cycle 1 μl of each primer set (ITS1-ITS2) and 1 μl of each primer set (D2–D3) were added. The thermal cycling program employed an initial denaturation at 95°C for 3 min, followed by 39 cycles with a denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min (Wang *et al*. 2003). Amplification success was evaluated electrophoretically on 1.5 % agarose gel. The remaining PCR product was prepared for sequencing.

Results and discussion

In the present study, the determination of *Xiphinema* species was performed using an integrated approach based on morphological analysis combined with molecular-based techniques. Out of seventy-one soil samples included in our study, thirty-two of them were distinguished as assumed *Xiphinema* species. Morphological identification of these findings about the genus *Xiphinema* were carried out using the key described by Coomans *et al.* 2001. *Xiphinema* (Cobb, 1913) is among the largest genera in the family Longidoridae and has a body length of 1.2–7.3 mm, habitus straight to spiral with a stylet composed of needle-like (Fig. 2).

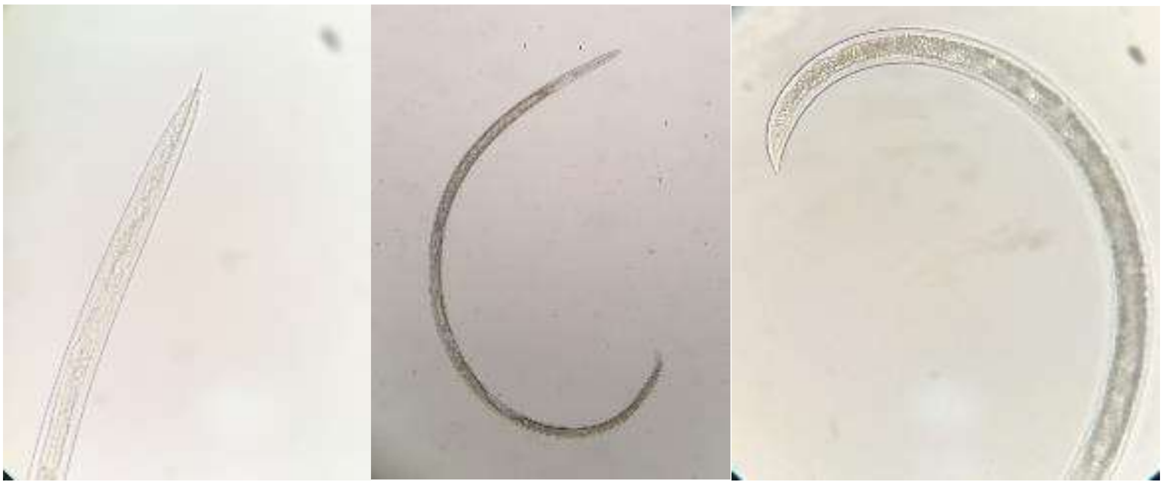

Fig. 2: Anterior part with stylet (left), habitus of *Xiphinema* spp. (middle), and posterior part (right)

In order to confirm morphological findings, single nematodes from each positive sample were subjected to molecular analysis. Two sets of universal primers were used to amplify the ITS containing region using ITS1–ITS2 primer set according to Vrain *et al*. (1992) and the D2A and D3B primer set were used to amplify the D2–D3 fragments of the 28S rDNA gene as described by Orlando *et al*. (2016). In each analyzed sample, the amplified product of the ITS1-ITS2 and D2-D3 expansion domains of the ITS region and 28S rDNA gene region produced single fragments of 730 bp and 980 bp, respectively (Fig. 3). Furthermore, ten selected samples were chosen based on different locations and delivered for multi-locus sequence analysis.

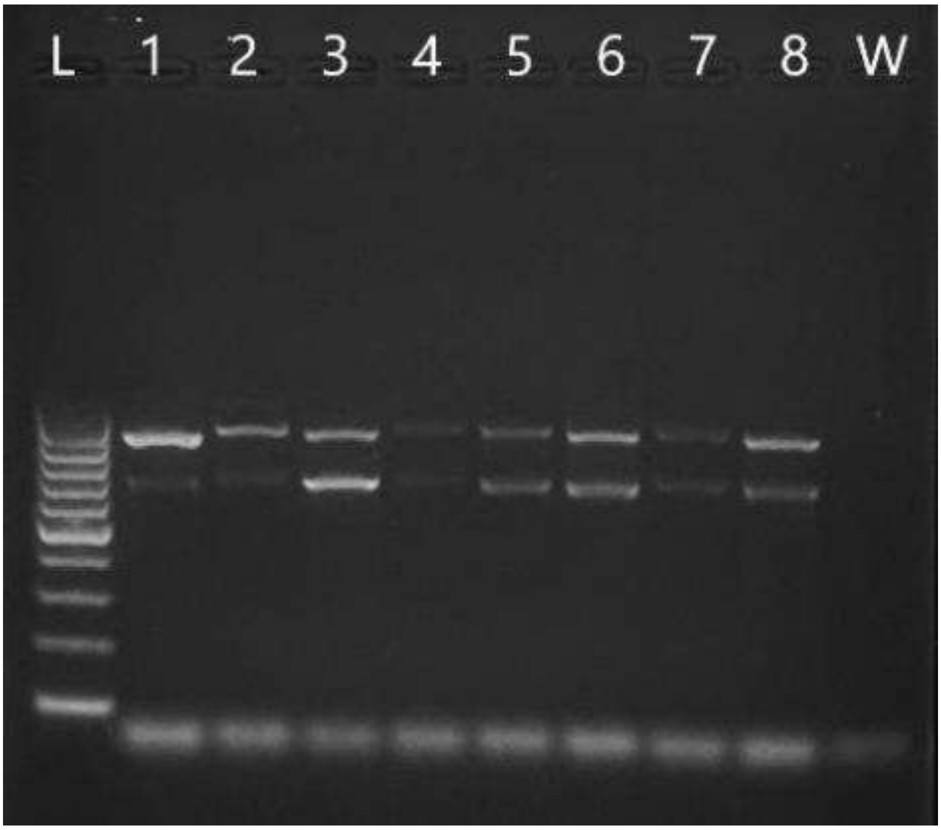

Fig. 3: Electrophoresis gel showing results of the ITS1-ITS2 and D2-D3 expansion domains producing single fragments of 730 bp and 980 bp, respectively.

Conclusion

To our best knowledge, this is the first evidence about the investigation and preliminary data about the genus *Xiphinema* species in Kosovo vineyards. These results highlighted the occurrence and distribution of *Xiphinema* species in the surveyed area. The collected data indicated that regulated dagger nematode of the genus *Xiphinema* species were distributed mainly in confined GFLV infected area. Indeed, our findings were significant in correlation with the recent study described by Singh *et al*. (2020). However, precautions and appropriate sanitation practices should be adopted by nurseries and farmers to avoid the spread of this species in proven healthy and protected vineyards of the country. The presence of ectoparasitic nematodes could encourage farmers to monitor their grapevine fields and prevent expanding their vineyards in infested areas. This study will help farmers to avoid planting close to GFLV symptomatic vineyards and possibly to use any cultural practice to maintain healthy infested vineyards. The first evidence of *Xiphinema* species in Kosovo vineyards shall be an alert for deeply investigating and evaluation of the impact of these ectoparasitic nematodes and their damage to the grapevine yield production.

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PHYTOTOXIC EFFECTS OF *THYMUS VULGARIS* AND *CITRUS SINENSIS* ESSENTIAL OILS ON SEED GERMINATION OF SEVEN CEREAL VARIETIES

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Abstract

In this study, we evaluated the allelopathic effects of essential oils of *Thymus vulgaris* and *Citrus sinensis* on the seeds (seed germination and length of roots) of 7 varieties of cereals (durum wheat : VITRON, SIMETO, CHEN'S, OUED BARED; soft wheat : MAWNA, ARZ ; and barley: SAIDA). The chemical composition of the essential oils was determined using gas chromatography/mass spectrometry (GC/MS). The main constituents of essential oil were carvacrol (48.56%) for *Thymus vulgaris* and β - pinene (30%) for *Citrus sinensis*. The results of seed bioassay showed that essential oils of *Thymus vulgaris* and *Citrus sinensis* exhibited an phytotoxic effect at high concentrations (1 and 0.1 mg/ml), The essential oils suppressed the seed germination and length of roots. Germination of seeds was inhibited 100 % with concentration 1 and 0.1 mg/ml for both essential oils. A similar inhibitory effect was observed regarding the length of roots, *Thymus vulgaris* essential reduced length of roots at 1 ;0.1 and 0.01 mg/ml. The data suggest a greater effect of *Thymus vulgaris* essential oils compared to *Citrus sinensis* essential oils. Our study confirmed the phytotoxic effect of *Thymus vulgaris* and *Citrus sinensis* essential oils. further studies are necessary for essential oils of *Thymus vulgaris* and *Citrus sinensis* to be used as bioherbicides. The research needs to focus on determining the herbicidal effect on cereal weeds and the minimum inhibitory dose that cannot cause damage to host plants under field conditions.
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Key words: *Citrus siensis, Thymus vulgaris, Essential oil, Germination, cereals.*

Introduction

The losses caused by weeds are one of the main limiting factors for staple crops worldwide. Interest in pesticides of natural origin (particularly plant-based) as an alternative to chemical pesticides has grown, as they are environmentally friendly, biodegradable, non-toxic and have a specific action, thus attracting considerable attention (Tripathi and Dubey, 2004 ; Isman ,2000; Ishii,2006; Ito and al.,2023). The yield of major crops, as well as the quality of harvested products, are severely compromised by weed infestation (Đorđević and al.,2022). In recent years, research has focused on exploring new ecological compounds with herbicidal activity for crop protection and weed management (Araniti and al., 2014). Particularly on cereals, Đurđić et al.(2016) considers, The wheat is the most important grainy plant used in human nutrition and it is the second on the ladder of overall grain production, after the corn Essential oils represent alternatives to chemical herbicides and have been widely studied as valuable ecological compounds with herbicidal activity for weed management (Araniti and al, 2018, Sarić-Krsmanović and al, 2023). The phytotoxicity of certain essential oils against weed seed germination has been proven (Tworkoski, 2002 ; Ibanez and Blazquez , 2020). However, the phytotoxic activity of essential oils must be selective, so that they do not harm crops. Therefore, besides the study of the herbicidal activity of essential oils, it is also necessary to know their potential effect on crops. Among essential oils, many have been identified as allelochemicals and tested for phytotoxicity(Kostina-Bednarz and al., 2023). The herbicidal effect of *Citrus sinensis* and *Thymus vulgaris* essential oils has been reported by Nepomuceno and Salgueiro, (2012) and Alexa and al, (2018). Zheljazko and al, (2021), *Thymus vulgaris* essential oils showed promise as a potential suppressor of seed germination and radicle growth of the weed species Raphanus sativus, Lactuca sativa, and Lepidium sativum. Our objective was to evaluate the phytotoxic effect of *Citrus sinensis* and *Thymus vulgaris* essential oils on cereal seeds considered as strategic crops in Algeria.

Materials and methods

Cereal seeds

Mature, healthy seeds of 7 cereal varieties donated by the Chlef cereals and pulses cooperative (CCLS) were used: 4 durum wheat varieties (VITRON, SIMETO, CHEN'S, OUED BARED), 2 soft wheat varieties (MAWNA, ARZ) and one barley variety (SAIDA).

Plant essential oils

Fresh *Citrus sinensis* and *Thymus vulgaris* fresh leaves were used for isolation of the essential oil, air-dried plant material was submitted to water distillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia method. The essential oils are stored in opaque bottles in the refrigerator at a temperature of 4°C and protected from light.

Gas chromatography (GC) analysis

The chemical composition of the essential oils was determined using gas chromatography/mass spectrometry (GC/MS). The operating conditions were as follows: the carrier gas was helium at a flow rate of 1 ml/min, the injector temperature was 250°C and 0.1 µl was injected in splitless mode for 0.1 min onto an HP5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm); the column temperature was programmed from 60°C to 260°C at a rate of 5°C/min with a final hold time of 10 min. For the Agilent 5973 model mass spectrometer, the temperature is: interface (280°C), source (230°C), quadrupole (150°C); ionization energy is 70 eV. Kovats indices of the compounds were calculated using the retention time of a series of n-alkanes.

Biological test

Using bioassays (% seed germination and root growth) we investigated the phytotoxic effect of the essential oils of *Thymus vulgaris* and *Citrus sinensis* on the seeds of 7 varieties of cereals. A total of 20 seeds were placed in 90mm diameter Petri dishes, containing a layer of filter paper (Whatman N01). The essential oil was emulsified with Tween20 (v/v 0.05%) and dissolved in distilled water. to obtained the final concentration: 0.0001, 0.001, 0.01, and 1 mg/ml of *Citrus sinensis* and *Thymus vulgaris* essential oils . 4 ml of the solution obtained was used to impregnate the germinating seeds. Distilled water mixed with Tween 20 (v/v 0.05 %) served as a control. The petri dishes were placed in a growth chamber at 25°C ± 2°C in dark for 10 days. The seed germination and elongation of radicel was observed in Petri dishes every 24 hours.

Statistical analyses

The results were subjected to an analysis of variance (ANOVA). Mean comparison tests were performed using the Fisher method at the 5% level and the software used to process the data was XLSTAT software

Results and discussion

Chemical composition of *Citrus sinensis* and *Thymus vulgaris* essential oils.
Essential oils were isolated from the leaves of *Citrus sinensis* and *Thymus vulgaris* by hydro distillation. Qualitative and quantitative analysis of essential oil chemical profiles is reported in Table 1. GC/MS analysis identified and characterized 26 compounds representing 95.72% of the *Citrus sinensis* essential oil and 22 compounds representing 91.75 % of the total *Thymus vulgaris* essential oil. The essential oil of *Citrus sinensis* was characterized by high levels of β - pinene (30%) while content of other components ranged from 9.37% to 0.07%. 15 components were present at less than 1%. Chi et al, (2020) and Mohammed et al, (2024) reported a similar number of constituents in *Citrus sinensis* essential oil, but with a different percentage of main compounds; in particular, a higher content of limonene. The essential oil of *Thymus vulgaris* was characterized by high levels of carvacrol (48.56%) and thymol (17.54%) and other components ranging from 8.70 to 0.09%. Five components were present at less than 1%. These results are in accordance with those previously reported. by Alexa and al., (2018) who reported that the major component of thyme essential oil was thymol.Vinciguerra et al. (2019) reported that p-cymene (36.36%) and thymol (24.35%) were the main components of *Thymus vulgaris* essential oil.

Phytotoxicity of *Citrus sinensis* and *Thymus vulgaris* essential oils
The results of seed bioassay showed that seed germination and length of roots of durum wheat, common wheat and barley varieties was inhibited at high concentrations (1 and 0.1 mg/ml) of *Thymus vulgaris* and *Citrus sinensis* essential oils. *Thymus vulgaris* essential oil show a strong inhibitory power (100%) at concentrations of 1, 0.1 and 0.01 mg/ml with VITRON, SIMETO and MAWNA varieties and 1 and 0.1 mg/ml for CHEN'S, OUED BARED, ARZ and SAIDA varieties (Figure 1). Sarić-Krsmanović and al, (2023) report the inhibiting effect that essential oils can have on seed germination. A comparable effect was observed on length of roots at levels of 1; 0.1 and 0.01 mg/ml, resulting in a 50% reduction in length of roots compared to the control (Table 2). The inhibitory effect was confirmed by statistically significant differences between all test concentrations (for seed germination and length of roots) and the control across the seven cereal varieties. However, no statistically significant difference was found for the low concentrations (0.001, 0.0001, and 0.00001 mg/ml). The effect of the high concentration used on the inhibition of seed germination was reported by Dorđević and al., (2022) for *S. glauca*, the allelopathic effect expressed through the inhibition of seed germination was more pronounced only after treatments with the highest used concentrations of extracts. Similar effect was observed with *Citrus sinensis* essential oils at the concentration 1 mg/ml (seed germination and length of roots) (Figure 2) (Table 3). The data suggest a greater effect of *Thymus vulgaris* essential oils compared to *Citrus sinensis* essential oils. The literature highlights a good phytotoxic capacity for *Thymus vulgaris* essential oil (Rolli et al. ,2014). The high phytotoxicity recorded for the essential oil of *Thymus vulgaris* compared with that of *Citrus sinensis* may be related to its high thymol and carvacrol content. Effectively Synowiec et al., (2017) report that the most phytotoxic group consisted of four essential oils, namely *Carum carvi*, *Thymus vulgaris*, *Mentha piperita* and *Salvia.officinalis* principally composed of oxygenated monoterpenes, while the second least phytotoxic group consisted of *S. canadensis* essential oil, rich in mono- and sesquiterpene hydrocarbons. Abd-ElGawad and al ,(2020) have mentioned that α- and β-pinene, 1,8 cineole, linalool, and carvacrol were the most effective allelopathic monoterpene compound.It has been suggested that the effect of essential oils can disrupt the permeability of the cell membrane structure. Such a phenomenon is due to the penetration of monoterpenes through the cell wall and cell membrane, or causes a leakage of cellular potassium that inhibits glucose-dependent respiration (Mutlu et al., 2011). Varietal sensitivity to essential

oils was different, with the VITRON variety being the most sensitive to *Thymus vulgaris* and *Citrus sinensis* and essential oils, followed by CHEN'S, OUED BARED, MAWNA, ARZ and SAIDA, in contrast to SIMETO, which recorded the highest percentage of seed germination. Abrahim et al. (2000) and Batish et al. (2004), reported that reduction of a plant's respiratory activities by monoterpenes reduces the amount of photosynthesis and disrupts germination as well as radicle growth.

Table 1. Chemical composition of *Citrus sinensis* and *Thymus vulgaris* essential oils

Components	*RI*	*Citrus sinensis* Contents (%)	*Thymus vulgaris* Contents (%)
α-thujene	930	1.49	1.0
α -pinene	939	2.04	5.69
camphene	953	-	0.31
sabinene	976	-	tr
β - pinene	979	30	0.4
myrcene	991	4.52	1.40
α phellandrene	1008	0.68	0.21
3 carene	1012	6.41	0.15
(+)-4-carene	1035	2.77	-
limonene	1035	9.37	0.30
β -ocimene	1070	0.74	0.1
1,3,6-octatriene,3,7-dimethyl-	1070	8.59	-
γ- terpinene	1080	4.42	8.70
cis sabinene hydrate	1094	0.21	-
terpinolene	1096	2.53	0.10
α -campholenal	1124	0.42	
linalool	1128	1.24	6.20
camphor	1143	-	-
borneol	1165	-	0.1
terpineol-4	1177	2.14	-
α terpineol	1189	0.07	0.1
citronellol	1226	1.05	-
carvacrol	1300	-	48.56
thymol	1309	-	17.54
(Z) β-elemene	1393	0.44	**-**
(E) β - elemene	1398	8.97	**-**
Caryophyllene	1417	3.48	**-**
trans- caryophyllene	1417	-	**0.50**
***α* -humulene**	1474	1.33	**0.10**
γ-cadinène	1513	0.56	**0.09**
caryophyllene oxide	1606	0.23	**-**
β sinensal	1706	0.72	**-**
α sinensal	1750	0.3	-
Total identified (%)		95.72	91.7

RI : retention index ; Tr: Trace (value less than 0.01%)

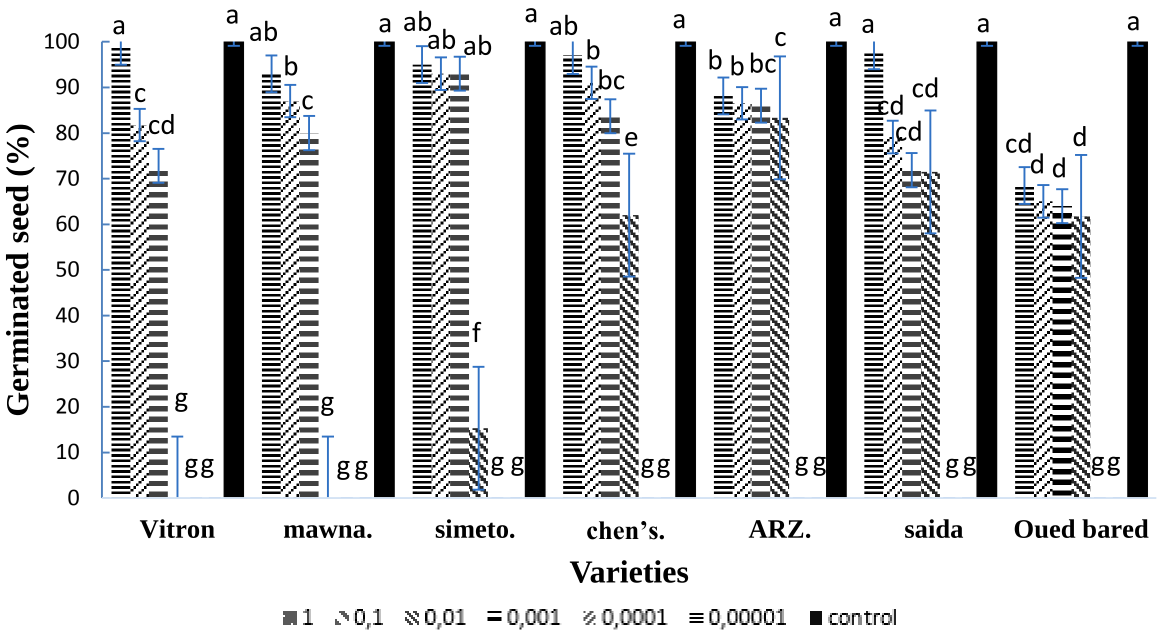

Figure 1. Effects of different concentrations of *Thymus vulgaris* essential oil on seed germination of 7 varieties of cereals. Means sharing the same letter are not significantly different at the 5% level of significance

Table 2. Effects of different concentrations of *Thymus vulgaris* essential oil on radicle length (mm) of 7 varieties of cereals

Concentration of essential oil (1mg/ml)	Vitron	Mawna	Simeto	Chen's	ARZ	Saida	Oued bared
1	0±0.00^{d*}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}
0,1	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}
0,01	0±0.00^{d}	0±0.00^{d}	4,05±0.09^{c}	4,15±1.06^{c}	6±0.10^{c}	5,88±0.89^{c}	5,7±0.06^{c}
0,001	0±0.00^{d}	5,17±0.24^{c}	4,57±0.16^{c}	6,55±2.03^{bc}	6,85±1.06^{bc}	6±0.56^{b}	6,15±0.36^{b}
0,0001	4,3 ± 0.25^{c}	5,62±0.06^{b}	6,6±1.26^{b}	7,37±1.07^{b}	7,37±0.8^{b}	6,23±0.17^{b}	6,38±2.12^{b}
0,00001	5,25 ± 0.30^{b}	6±0.21^{b}	8,1±1.13^{ab}	7,4±0.06^{b}	8,43±0.14^{ab}	6,35±1.08^{b}	6,48±0.04^{b}
Control	6,02 ± 0.31^{a}	8,05±0.06^{a}	9,7±0.05^{a}	9,55±0.15^{a}	10,05±0.24^{a}	9,7±0.44^{a}	9,37±1.05^{a}

* Within each column, means sharing the same letter were not significantly different at the 5% level of significance

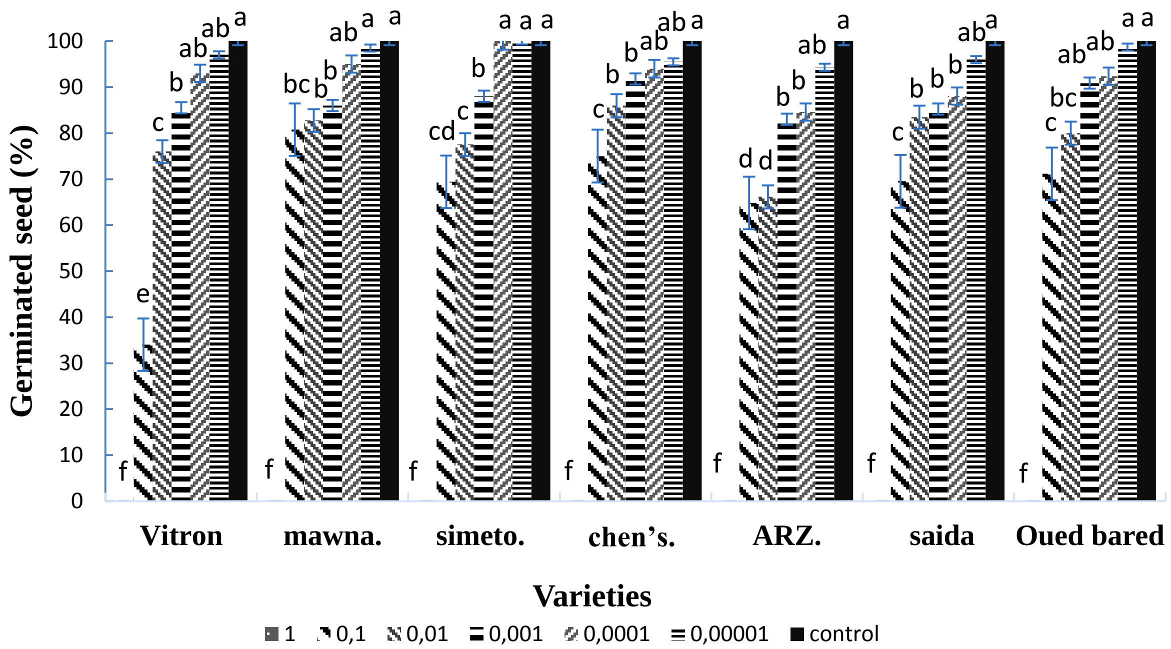

Figure 2. Effects of different concentrations of *Citrus sinensis* essential oil on seed germination of 7 varieties of cereals. Means sharing the same letter are not significantly different at the 5% level of significance

Table 3. Effects of different concentrations of *Citrus sinensis* essential oil on radicle length (mm) of 7 varieties of cereals. .

Concentration of essential oil (1mg/ml)	**Vitron**	**Mawna**	**Simeto**	**Chen's**	**ARZ**	**Saida**	**Oued bared**
1	0,05±0.24^{d*}	0,05±0.03^{d}	0,12±0.05^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{c}
0,1	1,5±0.08^{c}	6,62±1.65^{c}	5,23±0.99^{c}	6,52±0.07^{c}	4,6±1.92^{c}	5,72±0.89^{c}	6,6±0.14^{bc}
0,01	4,05±1.35^{b}	6,87±0.55^{c}	6,5±0.47^{c}	6,57±0.15^{c}	5,72±0.35^{c}	6,45±1.24^{bc}	6,75±0.88^{bc}
0,001	4,3±0.07^{b}	7,52±0.78^{b}	7,62±0.21^{b}	7,35±0.06^{bc}	6,7±0.70^{bc}	6,87±0.58^{b}	8,12±0.45^{b}
0,0001	4,57±0.89^{b}	7,42±1.13^{b}	7,82±0.34^{b}	8,52±0.75^{b}	7,95±1.87^{b}	7,25±0.27^{b}	8,57±2.13^{ab}
0,00001	5,77±1.54^{a}	8±0.84^{a}	8,57±3.12^{ab}	8,95±2.47^{a}	9,52±0.03^{ab}	7,9±0.46^{ab}	9,04±2.76^{a}
Control	6,02 ± 0.31^{a}	8,05±0.06^{a}	9,7±0.05^{a}	9,55±0.15^{a}	10,05±0.24^{a}	9,7±0.44^{a}	9,37±1.05^{a}

* Within each column, means sharing the same letter were not significantly different at the 5% level of significance

Conclusion

Our study confirmed the phytotoxic effect of Thymus vulgaris and Citrus sinensis essential oils used in higher doses on seed germination and length of roots in vitro. further studies are necessary for essential oils of Thymus vulgaris and Citrus sinensis to be used as bioherbicides. The research needs to focus on determining the herbicidal effect on cereal weeds and the minimum inhibitory dose that cannot cause damage to host plants under field conditions.

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Conflicts of Interest: The authors declare no conflict of interest.

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MICROBIOLOGY OF WATER IN PRIMARY FOOD PRODUCTION

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Abstract

Microbiologically acceptable water is one of the basic preconditions for animal health and food safety. The experiment used drinking water originating from primary food production, including water from animal farms and meat, milk and fish production, sampled in 2021-2022 from Republic of Srpska (Bosnia and Herzegovina). A total of 757 samples were examined, of which 55.48% originated from the water supply system and 44.52% from wells. The aim of the study is to determine the microbiological status of drinking water from primary food production, using standard methods BAS EN ISO 6222, BAS EN ISO 7899-2 and BAS EN ISO 9308-1. The research determined 70.54% satisfactory samples and 29.46% unsatisfactory samples. Analyzing the results of unsatisfactory samples in relation to the origin, 31.39% of the samples were from the water supply system, and 68.61% were from wells. In relation to the tested parameters, 17.83% of samples were unsatisfactory due to an increased total count of bacteria cultivated at 22°C, and 20.21% of samples were unsatisfactory due to an increased total count of bacteria cultivated at 37°C. When it comes to pathogens, 13.47% unsatisfactory samples were due to the presence of intestinal enterococci, 15.59% due to the presence of *Escherichia coli* and 15.98% due to the presence of coliform. The microbiological status of water from primary food production is of concern, as almost a third of the samples are unsatisfactory especially considering the presence of intestinal enterococci, coliforms and *Escherichia coli*.

Keywords: *water, food, animal, production, microbiology.*

Introduction

Water is used in various ways in milk production and dairy industry, thereby becoming part of the food intentionally, inevitably or accidentally. No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and may be subject to natural or manufactured sources of pollution that may result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination. Zero-probability level of microbiological contamination of drinking water does not exist (Directive, 2020).

The presence of bacteria in drinking water *per se* is not an issue, as long as no pathogenic organisms are present: there are bacteria in drinking water, even in relatively high numbers (10^3 to 10^6 cells/mL), without consequences on human health (Hoefel *et al*., 2005; Hammes *et al*., 2008).

Water temperature is an essential factor influencing bacterial growth kinetics and competition processes. Drinking water temperatures typically range between 3 and 25°C in European countries (Niquette *et al*., 2001), and fluctuate seasonally within this temperature range even within a single drinking water distribution system. Elevated water temperatures have often been associated with increased bacterial abundance in drinking water distribution systems (Francisque *et al*., 2009; Liu *et al*., 2013), and with higher numbers in indicator organisms.

The microbiological quality of water is commonly defined as a maximum acceptable number or concentration of bacteria that do not constitute a health hazard (Directive, 2020).
Minimum requirements for parametric values used to assess the quality of water intended for human consumption is 0/100 ml for *Escherichia coli* and intestinal enterococci. In Republic of Srpska (Bosnia and Herzegovina) the limit value for total count on 22°C is 100 CFU/ml and for total count on 37°C the limit is 20 CFU/ml. Also, coliform bacteria, *Escherichia coli* and intestinal enterococci must not be detectable in 100 ml sample of water (Official Gazette, 2017).
The production and distribution of biological stable drinking water should be a non-negotiable goal for water utilities with the perspective of providing the same water quality to consumers than produced at the treatment facility. This can only be achieved by adequate monitoring and control of microbial processes during water treatment and distribution (Prest *et al*., 2016).
The presence of *Escherichia coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *Escherichia coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *Escherichia coli* is a coliform bacteria and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water (Edberg, 2000; WHO, 2008).
The water used during handling and processing of milk products can be potential sources of microbial contamination with possible negative consequences on food safety. Especially, the water used in keeping the hygiene of milking and milk storage utensils is crucial to keep the quality and safety of the products. *Escherichia coli* was isolated in 39.20% of samples of water (Amenu *et al*., 2016).
Use of contaminated water in the handling and processing of milk products can cause a higher potential health risk than the risk through direct drinking. This is due to the fact that multiplication of pathogenic micro-organisms can occur in milk and milk products with amplification of the load of the pathogens (Amenu, 2013).
Some studies revealed that wash water can be source of bacterial contamination for milk and further compromise the quality and safety of milk or milk products (Kivaria *et al*., 2006; Perkins *et al*., 2009).
In a study by Habes *et al.* (2015) it has been founded that all water samples used in the production of milk and milk products were microbiologically safe.
Kalaba *et al*. (2020) found that in 2015-2017 of the 584 water samples, 26.20% were unsatisfactory.
In the study of testing water at milk collection points, 20.40% of unsatisfactory samples were found, of which 10.20% was detected *Escherichia coli* and coliforms, and faecal streptococci in 12.24% (Denžić *et al*., 2013).
Analysis of water from milk collection points, originating from wells, it was found 63.90% of unsatisfactory samples (Jaki *et al*., 2010).
The aim of the study is to determine the microbiological status of drinking water from primary food production.

Material and methods

The experiment used drinking water originating from primary food production, including water from animal farms and meat, milk and fish production, sampled in 2021-2022 from Republic of Srpska (Bosnia and Herzegovina). A total of 757 samples were examined (311 in 2021, and 446 in 2022).
Laboratory testing of samples was performed at the Public Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka.

Microbiological examination was carried out according to the Official Gazette (2017). This included enumeration of colony forming units (CFU) expressed as total count of bacteria cultivated at 22°C, and 37°C according to BAS EN ISO 6222 (ISBIH, 2003a), coliform bacteria and *Escherichia coli* according to BAS EN ISO 9308-1/A1 (ISBIH, 2018) and intestinal enterococci according to BAS EN ISO 7899-2 (ISBIH, 2003b).

In our research and in the statistical analysis of the obtained results, we used, as basic statistical methods, descriptive statistical parameters. The research results are presented in tables and figures.

Results and discussion

Water supplies within food production premises should be subject to risk and hazard assessment to ensure that appropriate water quality is maintained throughout the production process (Dawson, 2000).

The presence of pathogenic bacteria from water supply system is a particularly worrying fact given that water must be microbiologically correct, which means that it must not contain pathogens (Official Gazette, 2017). A possible explanation for this is dilapidation and damage to water supply installations leading to water contamination.

Many infectious diseases of animals and humans are transmitted by water contaminated with human and animal excrement, which becomes a source of pathogenic bacteria, viruses and parasites (protozoa, parasite eggs) capable of surviving for different periods, and raise the health risk for many people throughout the world. In order to eliminate the risk related to disease transfer, water intended for mass consumption is treated and disinfected before use (Sasakova *et al*., 2013; Fridrich *et al*., 2014). On the basis of the results, adequate measures can be taken that include prevention of contamination and systemic disinfection.

The research determined 70.54% satisfactory samples and 29.46% unsatisfactory samples. The obtained results are similar to the results of Kalaba *et al.* (2020) and Golić *et al.* (2023).

When it comes to the representation of samples in relation to the category, 55.48% of samples was from water supply system and 44.52% was from wells. Analyzing the results of unsatisfactory samples in relation to the origin, 31.39% of the samples were from the water supply system, and 68.61% were from wells. Comparing the results of water testing in relation to the category, it can be noticed that there is a significantly higher number of unsatisfactory samples of well water in relation to water supply sistem, which is expected considering that the public water supply system is under daily control with regular chlorination. In contrast, well waters do not flow, stagnate and are not under constant control or very rarely, usually once a year as an official control.

Indicator organisms are used to assess the microbiological quality of water. Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms (Edberg, 2000).

The use of indicator bacteria, in particular *Escherichia coli* and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health (Hijnen *et al*., 2000).

Enterococci include a number of species that occur in the faeces of humans and warmblooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *Escherichia coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as secondary indicators of faecal pollution (WHO, 2008).

According to the WHO (2008), colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or

vegetation. Two incubation temperatures and times are used for total count, 37°C for 48 h to encourage the growth of bacteria of mammalian origin, and 22°C for 72 h to enumerate bacteria that are derived principally from environmental sources.
In relation to the tested parameters, 17.83% of samples were unsatisfactory due to an increased total count of bacteria cultivated at 22°C and 20.21% of samples were unsatisfactory due to an increased total count of bacteria cultivated at 37°C. When it comes to pathogens, 13.47% unsatisfactory samples were due to the presence of intestinal enterococci, 15.59% due to the presence of *Escherichia coli* and 15.98% due to the presence of coliform. The results obtained differ significantly from the results Golić *et al.* (2023).

Conclusion

The microbiological status of water from primary food production is of concern, as almost a third of the samples are unsatisfactory, especially considering the presence of intestinal enterococci, coliforms and *Escherichia coli*. This is of particular concern given the significant participation of well water in the primary food industry, given that it is not under constant monitoring. For these reasons, it is recommended to take additional measures in order to improve the microbiological quality of water in primary food production, as well as regular and more frequent monitoring.

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HARMFUL ENTOMOFAUNA ON STRAWBERRY IN BIJELJINA AREA (BOSNIA AND HERZEGOVINA)

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Abstract

During the cultivation, strawberry is attacked by numerous harmful insect species that cause direct and indirect damage, thus significantly reducing quality and yield. Harmful entomofauna on strawberry in Bijeljina area (entity of Republic of Srpska, Bosnia and Herzegovina) was examined in 2024, in the location Mala Obarska). Using entomological methods such as visual examination of plants, using an entomological catcher and collecting insects using an exhauster, their presence, abundance and symptoms of damage were determined. Small insects (shield moths, cicadas, etc.) were collected directly from the plants using an exhauster. All collected larvae and pupa of collected insects, were reared in the lab to adult stadium. Total number of determined insect species was 18, from five orders and 16 families. The largest number of species from the order of Hemiptera have been determined, nine species from seven families (*Aphis forbesi* Weed, *Chaetosiphon fragaefolii* (Cockerell), *Graphosoma lineatum* (L.), *Dolycoris baccarum* (L.), *Lygus pratensis* (L.), *Coreus marginatus* (L.), *Pyrrhocoris apterus* (L.), *Cercopis vulnerata* (Rossi) and *Trialeurodes vaporariorum* Westwood. From the order of Coleoptera, five species from five families have been determined (*Phyllotreta undulata* Kutschera, *Anthonomus rubi* (Herbst), *Agriotes obscurus* (L.), *Stelidota geminata* Say and *Cetonia aurata* (L.). The species *Tettigonia viridissima* L. and *Omocestus* spp. from the order of Orthoptera were determined. From the order of Thysanoptera, *Frankliniella occidentalis* (Pergande) was determined and from the order of Lepidoptera, it was the species *Acleris comariana* (Lienig & Zeller).

Keywords: *harmful insects, strawberry, Bijeljina.*

Introduction

Strawberry production in the world is constantly increasing. According to FAO (Food and Agriculture Organization of the United Nations, 2024), the largest strawberry producer in the world is China with 3,336,690 million tons per hectare, followed by the USA with 1,055,963 million tons per hectare, and then Egypt, Mexico, Turkey, Spain, Brazil and Russia. In recent years, strawberry production in Bosnia and Herzegovina has also tended to grow, driven by significant investments, primarily in the fruit processing process (Kurtović et al., 2012). Strawberries are grown in BiH on an area of 1332 ha, with an average yield of 7.7 t/ha (Maličević et al., 2015). One of the most important strawberry production areas in Bosnia and Herzegovina is the area of the city of Bijeljina.

Strawberry is a fruit species that is inhabited by a large number of harmful insect species that can cause economically significant damage. Most of them are polyphagous and they attack the other plant species, especially from the Rosaceae family (Sarajlić et al., 2018). According to Alford (1984), about 90 species of strawberry pests have been recorded, ten of which cause economically significant damage. The most important harmful insects of strawberries worldwide are *Lygus rugulipennis* Poppius, *Anthonomus rubi* (Herbst), *Otiorhynchus sulcatus* (Fabricius), *Frankliniella occidentalis* (Pergande) and the other species (Cross et al., 2001; Gotlin Čuljak, 2015.a.). As more important pests of strawberries, Radman et al. (1981)

mention the next species: *Otiorhynchus rugosostriatus* Goeze, *Aphis forbesi* Weed., *Pentatrichopus fragaefolii* Cockerell, *Philaenus spumarius* L., *Melolontha melolontha* L., *Anthonomus rubi* Herbst., *Coenorrhynchus germanicus* Hb., *Priophorus padi* L., *Argyroploce lacunana* Denis & Schiff, *Cnephasia incertana* Tr., *Cnephasia communana* Tr., and species from families Noctuidae, Cicadellidae and Halticinae. According to Ciglar (1989) the most important harmful insects species on strawberry are *Anthonomus rubi* Herbst, *Acleris comariana* (Lienig&Zeller) and *Rhynchites germanicus* Herbst. In our region, in the early 70s of the last century, the appearance of insects species from the genus *Lepyrus* was mentioned as a pest of strawberries, while in Western Europe and Russia, species from the genus *Polydrosus* were mentioned (Petrova et al., 2013). In the territory of Bosnia and Herzegovina, the harmful entomofauna of strawberries has been studied very little. Thus, in this area as a harmful insects, Festić (1996) mentioned the next species: *Philaenus spumarius* L., *Aphis forbesi* Weed, *Anthonomus rubi* Herbst, *Eudia pavonia* L., *Glyptina fallax* Weise and *Haltica pusilla* Duft. Most of them are polyphagous, while a significantly smaller number of species are associated only with strawberries. During a three-year study (2009-2011) in the territory of Bosnia and Herzegovina, Zovko (2021) identified 70 species of harmful insects from seven orders. The most numerous order was Hemiptera with a total of 29 species, followed by the Coleoptera order with 15 species. The lowest number of species belonged to the orders Hymenoptera and Thysanoptera. Certain species of insects damage the root system, while many inhabit the above-ground part of the plant, causing various damage symptoms. Thus, the larvae of the following species cause damage to the root system: *Agriotes* spp., *Tipula* spp., *Scutigerella* spp., *Agrotis* spp., *Melolontha melolontha, Phyllopertha horticola* L., *Hoplia philanthus* Füessly and *Serica brunnea* L. (Cross et al., 2001). In Croatia, Maceljski (2002) mentioned three species from the genus *Otiorhynchus* (*O. ovatus* (L.), *O. mastix* (Olivier) and *O. rugosostriatus* (Goeze)) whose larvae gnaw the roots of strawberries, and in case of large numbers, they cause wilting and decay of plants (Cross et al., 2001; Gotlin Čuljak, 2015.a.). According Radman et al., (1981), *Otiorhynchus rugosostriatus* is a very important species in the area of Prijedor and Sarajevo. Occasional damage to the root system is caused by polyphagous species *Melolontha melolontha* L., *Phyllopertha horticola* (L.), *Amphimallon solstitiale* (L)., *Hoplia philanthus* (Füessly) and *Serica brunnea* (L.), especially if the strawberry plants were there was a pasture (Cross et al., 2001). A large number of aphids species occur on strawberry, but few species cause significant damage worldwide, such as *Aphis forbesi* Weed, *Chaetosiphon fragaefolii* (Cockerell), *Aphis gossypii* Glover, *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulzer) and *Myzus ascalonicus* Doncaster (Cross et al., 2001; Maceljski, 2002). Blackman and Eastop (2000) were mentioned 30 aphids species of strawberry in the USA. Among them the most common species are *Aphis forbesi*, *Aphis gossypii* and *Chaetosiphon fragaefolii*, while they occur occasionally *Aphis ruborum* (Börner), *Aulacorthum solani* (Kaltenbach), *Chaetosiphon jacobi* (Hille Ris Lambers), *Ericaphis fimbriata* (Richards), *Macrosiphum euphorbiae* (Thomas), *Macrosiphum rosae* (L.), *Myzus ornatus* Laing and *Sitobion fragariae* (Walker). One of the most important harmful aphid species of strawberry in the world is *Chaetosiphon fragaefolii* (Cross et al., 2001; Rondon et al., 2004). In the Bosnia and Herzegovina, the fauna aphids species on strawberry has been studied very little. Radman et al. (1981) were mentioned two species, *Aphis forbesi* and *Chaetosiphon fragaefolii*. Both species the sap sucks from leaves and buds and, thus, causing direct damages such as chlorosis, curling and deformation (Rondon and Cantliffe, 2004; Gotlin Čuljak, 2015.b.). Also, these species cause indirect damage because they make large amounts of honeydew which covers the plant and represents a favourable base for developing the fungus black mould. All this causes a decrease in yield and reduced quality of fruits (Cédola and Greco, 2010). In addition, *Chaetosiphon fragaefolii* transmits several very important strawberry viruses such as strawberry mild yellow edge virus (SYEV), strawberry crinkle virus (SCV), strawberry mottle virus (SMV) (Blackman and Eastop, 2000; Cross et al., 2001; Rondon and Cantcliffe, 2005). A serious problem, especially in greenhouses can be the polyphagous species *Aphis gossypii* Glover (Lecant and Deguine, 1994), which causes great damage, especially in warmer production areas.

In addition to the mentioned species, thrips are also important pests of strawberries. Thus, during two years of research in Turkey, Atakan (2008) found 14 different species of thrips. Among them, *Frankliniella occidentalis* Pergande was the dominant species. In many countries this species causes significant damage to strawberries grown in greenhouses and the open fields (Atakan, 2008; Pinent et al., 2011). By sucking the sap from leaves and floral organs, this species prevents fruit set. Also feeding on strawberry fruits it causes deformation, which significantly reduces their quality (Atakan, 2011).

Bedbugs also cause significant damage to strawberries by feeding on parts of the flower, seeds or young fruits. In the area of Central and Northern Europe, the following species cause damage to strawberries: *Lygus rugulipennis* (Poppius), *Plagiognathus arbustorum* (Fabr.), *Plagiognathus chrysanthemi* (Wolff), *Closterotomus norwegicus* (Gmelin), *Closterotomus fulvomaculatus* (De Geer) and *Lygocoris pabulinus* L. (Cross et al., 2001; Alford, 2014). A very widespread species throughout Europe and an important pest of strawberry in the southern and western parts of the USA is *Lygus hesperus* Knight (Swezey et al., 2007; Zalom et al., 2014). The polyphagous species *Lygus rugulipennis* often occurs in large numbers and is highly adaptable to different weather conditions (Accinelli et al., 2002). The damage is caused by larvae that feed on flower buds, flowers and fruits. As a result of their feeding, the fruits remain small, deformed and lose marketability (Easterbrook, 2000; Cross et al., 2001; Cross, 2004; Łabanowska, 2007b). Significant damages from *Lygus hesperus* and *Lygus rugulipennis* were recorded on strawberries grown in greenhouses (Mešić et al., 2016). One of the most important pests that, in addition to strawberry, attacks raspberry, blackberry and wild rose is *Anthonomus rubi* (Herbst) (Alford, 2014). Thus, in Poland this species causes 10-30% damage every year (Łabanowska and Chlebowska, 1999; Łabanowska, 2002), and in some cases over 80% (Popov, 1996). Damage is caused by adults that feed on strawberry leaves during the supplementary feeding period. After oviposition in the flower buds, the female bites the stalk of the bud that doesn’t open, withers and bends downwards. It’s a characteristic symptom of the presence of this species. This significantly increases the intensity of damages (Aasen et al., 2004). Greater damage from this species in one location can be expected in the second and third year of strawberry cultivation (Aasen et al., 2004; Krauß et al., 2014). The intensity of the damage depends on the cultivars characteristics, the weather conditions during the oviposition and the length of the flowering period (Popov, 1996). Thus, significantly less damage was present on strawberry cultivars that have a shorter flowering period (Cross and Easterbrook, 1998; Cross et al., 2006). In the Northern and Central Europe, damage to strawberries is also caused by several species tortrix moths (Tortricidae, Lepidoptera). Among them, the most common are *Acleris comariana* (Lienig & Zeller), *Clepsis spectrana* (Treitschke), *Olethreutes lacunana* (Denis & Schiff.), *Cacoecimorpha pronubana* (Hübner) and *Cnephasia asseclana* (Denis & Schiff.) (Gratwick, 1992; Cross et al., 2001).

According to survey by Radman et al. (1981) the most widespread tortrix moths species of strawberry in Bosnia and Herzegovina are *Argyroploce lacunana* (Denis & Schiff.), *Cnephasia incertana* (Treitschke) and *Cnephasia communana* (Herrich-Schäffer). The larvae of these species gnaw the leaves, and often feed on parts of the flower. Such damaged flowers don’t to fructify or develop deformed fruits. The larvae of *Cnephasia asseclana* and *Cnephasia incertana*, in addition to damaging the leaves, often bore into the fruits, usually in the region of the sepals (Alford, 2014). Although tortrix moths larvae are often present in commercial strawberry plantations, economically significant damage are very rare (Cross et al., 2001). In our region, the most abundant species is *Acleris comariana*, which is widely distributed in Europe, North America, China and Japan (Gratwick, 1992; Alford, 2014). Damage is caused by larvae that gnaw and feed on young leaves, but sometimes also feed on flowers (Barić, 2015). An increase of tortrix moths population can also be caused by the excessive application of pesticides, since the population of natural enemies is significantly reduced in such plantations (Gratwick, 1992).

Materials and Methods

The survey was completed in 2024, in the field, in Bijeljina area (location Mala Obarska) and in the laboratory of the Faculty of Agriculture in East Sarajevo. The area under strawberries was 2000 m^2, there were 8,000 seedlings. The time of planting was August 10th, 2023. The distance between the rows is 140 cm, while the distance between plants in a row is 20 cm.

Entomological methods were used to determine insect presence, abundance, and damage symptoms. These methods included visual plant examination, 'mowing' with an entomological catcher, and insect collection using an exhauster. The visual method consists in examining plant organs (buds, leaves, flowers, whole plants). By randomly selection, ten plants at ten places were examined. The visual examination was carried out from April to the beginning of June. The number of aphids was evaluated according to the Banks scale (Maceljski and Igrc-Barčić, 1999). The scale is from 0-4 (0-without the presence of aphids, 1-very weak attack (individual aphids or very small colonies); 2-weak attack (small number of colonies on plants); 3-medium attack (mass appearance of aphids, large number colonies that aren’t yet connected); 4-strong attack (plants are covered by aphids, colonies are very numerous, large and connected and cover plant organs). The walking diagonally across the field, 100-200 plants were examined. The intensity of damage caused by *A. rubi* was determined by randomly selecting ten strawberry bushes at each visual examination and determining the number of damaged flower buds in relation to the total number of flower buds on the selected strawberry plants (Kikas and Libek, 2002).

Using the entomological catcher and the "mowing" method were used to pass over the plants, so that all the insects that were on the surface of the plant organs fell into the catcher. This method is used to collect and determine the presence of winged insects (cicadas, flea beetles, bed bugs) (Dimić et al., 2013). Small insects (shield moths, cicadas) were collected directly from the plants using an exhauster. All sampled infested plant matherial was observed in laboratory. All collected larvae and pupa of collected insects, were reared in the lab to adult stadium. In this purpose was used plastic containers and Petri dishes. In the bottom placed a layer of cellulose wadding occasionally misted in order to maintain the freshness of the leaf. The collected insects were fixed in 70% alcohol; some of them were prepared, making microscopic preparations or the entomological collection. The determination of collected insects was done in the laboratory using appropriate keys (Blackman and Eastop, 1994; Blackman and Eastop, 2000; Dietrich and Eastop, 2006; Jelinek and Audisio, 2010).

Results and Discussions

A one-year survey of the entomofauna affecting strawberries in the Bijeljina area identified 18 species from five orders and 16 families. From the Orthoptera order, *Tettigonia viridissima* (Tettigoniidae) and *Omocestus* spp. (Acrididae) were found. The Hemiptera order was the most diverse, with nine species from seven families. These included *Graphosoma lineatum* and *Dolycoris baccarum* (Pentatomidae), *Aphis forbesi* and *Chaetosiphon fragaefolii* (Aphididae), *Lygus pratensis* (Miridae), *Coreus marginatus* (Coreidae), *Pyrrhocoris apterus* (Pyrrhocoridae), *Cercopis vulnerata* (Cercopidae), and *Trialeurodes vaporariorum* (Aleurodidae). Five species from five families were identified in the Coleoptera order:

Anthonomus rubi (Curculionidae), *Phyllotreta undulata* (Bruchidae), *Agriotes obscurus* (Elateridae), *Stelidota geminata* (Nitidulidae), and *Cetonia aurata* (Scarabaeidae).
From the order Thysanoptera, *Frankliniella occidentalis* (Pergande) (Thripidae) was determined, and from the order Lepidoptera *Acleris comariana* (Lienig & Zeller) (Tortricidae) was determined (Tab. 1).

Table 1. The presence of insects on strawberry in the Bijeljina area in 2024 year

	Ordo	**Family**	**Species /date of collection**	April 20	May 9	May 15	May 26	June 5	June 17
1.	Orthoptera	Tettigoniidae	*Tettigonia viridissima*	-	-	1	2	2	3
2.		Acrididae	*Omocestus* spp.		-	1	3	2	3
3.	Thysanoptera	Thripidae	*Frankliniella occidentalis*	-	-	-	1	2	3
4.	Hemiptera	Miridae	*Lygus pratensis*	-	1	-	2	3	2
5.		Pentatomidae	*Graphosoma lineatum*	-	-	-	1	3	3
6.			*Dolycoris baccarum*	-	-	1	3	2	-
7.		Coreidae	*Coreus marginatus*			2	4	3	-
8.		Pyrrhocoridae	*Pyrrhocoris apterus*	-	-	1	3	1	-
9.		Cercopidae	*Cercopis vulnerata*	-	-	2	3	2	-
10.		Aleyrodidae	*Trialeurodes vaporariorum*	-	-	-	1	3	-
11.		Aphididae	*Aphis forbesi*	1*	1*	2*	3*	2*	1*
12.			*Chaetosiphon fragaefolii*	1*	2*	3*	3*	2*	1*
13.	Coleoptera	Bruchidae	*Phyllotreta undulata*	-	-	-	1	2	-
14.		Curculionidae	*Anthonomus rubi*	5	4	-	-	2	4
15.		Elateridae	*Agriotes obscurus*	-	-	-	1	2	-
16.		Nitidulidae	*Stelidota geminata*	-	-	-	2	3	-
17.		Scarabaeidae	*Cetonia aurata*	-	1	2	4	3	2
18.	Lepidoptera	Tortricidae	*Acleris comariana*	-	1	2	2	1	-

*Presence and abundance of aphids according to the Banks scale (Maceljski and Igrc-Barčić, 1999).

The species from the order Orthoptera (*T. viridissima* and *Omocestus* spp.) during May and June by visual examination and using an entomological catcher were determined. These species fed on strawberry leaves in the form of larger or smaller irregular bites on young leaves. However, their number was not high, so they didn't cause significant damage. According to Rotim (2019), damage from the species from the families Tettigoniidae and Acrididae is greater in years with a dry spring. Literature data about damage on strawberry caused by species from the order Orthoptera are very scarce. Petrova et al. (2013) mention the

species *Omocestus rufipes* (Zetterstedt) as a common species in strawberry plantations in Latvia.
During the survey, five species of bedbugs by visual examination and using an entomological catcher during May and June were identified (*L. pratensis, G. lineatum, D. baccarum, P. apterus* and *C. marginatus*). The mentioned species didn’t cause significant damage to strawberries. In the world, several species of these insects are reported as important pests of strawberries. In Europe, damage to strawberries is caused by species *Lygus rugulipennis* Poppius, *Lygus hesperus* Knight, *Plagiognathus arbustorum* (Fabricius), *Plagiognathus chrysanthemi* Wolff, *Calocoris norwegicus* (Gmelin), *Calocoris fulvomaculatus* (De Geer) and *Lygocoris pabulinus* (L.) (Easterbrook, 2000; Cross et al., 2001; Özgen, 2012; Alford, 2014; Mešić et al., 2016). Damage from these species varies depending from environmental conditions, cultivar sensitivity, length of flowering period and fruit development (Łabanowska, 2007b). According to Cross (2004), due to the feeding of bedbugs, losses can be up to 80%. By sucking on flower parts, seeds or young fruits, these species cause fruit deformation. Damage in the form of necrotic changes is visible on the leaf, flower and leaf petioles. The species often feed on the seeds of the strawberry fruit, so the fruits remain deformed and lose their market value (Cross et al., 2001). Among of them, the highest number of individuals was in *C. marginatus* (Tab. 1). Younger larvae of this species suck juices from leaves, and older larvae and adults feed on generative parts of plants. It prefers to feed on plants from the families Polygonaceae, Asteraceae and Rosaceae. It’s very often found on various berry fruit trees, especially in blackberry and raspberry plantations (Fitzgerald and Jay, 2011). During the survey, *P. apterus* was the least numerous. It is an omnivorous species that mostly feeds on fruits and seeds of plants from the Malvaceae and Tilaceae families. Similar damage is caused by the species *Dolycoris baccarum*, which by feeding on larvae and adults can cause deformation of strawberry fruits. It’s considered a minor pest of strawberries (Alford, 2007), although it can be considered potentially harmful (Petrova et al., 2013). The total number of individuals the species *G. lineatum* during May and June was seven (Tab. 1). This species is extremely polyphagous and is widespread in southern Europe. It prefers plants from the Apiaceae family, and is often present on *Rumex* plant species. It’s not listed as a strawberry damage insects pest.
The studying the strawberry entomofauna in Bosnia and Herzegovina, Radman et al. (1981) stated that cicadas were sometimes present in greater abundance in strawberry plantations. During the survey, in May and June, the species *C. vulnerata* (total of seven individuals) by visual examination and using an entomological catcher was also determined. In addition, strawberries are regularly inhabited by aphids. According to literature data, several species of aphids occur on strawberries. That are *A. forbesi, C. fragaefolii, M. persicae, A. gossypii, M. euphorbiae, M. ascalonicus* and the other species. However, the most important and common species are *A. forbesi* and *C. fragaefolii,* which is in accordance with results of our research. These species most often inhabited young strawberry shoots and the reverse side of young leaves mostly along the main leaf nerve (Fig. 1), which is in accordance with literature data (Cross et al., 2001; Maceljski, 2002). During the survey, the abundance of these species was assessed as a very weak attack at the end of April because as single individuals or very small colonies were found in this period. However, during May, their number increased, and the attack was assessed as medium strong. In this period a large number of colonies were found that were not yet connected. Furthermore, at the beginning of June, their number gradually decreased.

Figure 1. C. fragaefolii (https://influentialpoints.com)

Figure 2. T. vaporariorum (orig.)

At the end of May and beginning of June, visual examination individuals of *T. vaporariorum* were determined (Fig. 2). This species has a cosmopolitan distribution. It’s a polyphagous species that feeds on many cultivated plants, primarily vegetable plants and flowers, but also weeds (Sekulić et al., 2008). The presence of this species on strawberry is most likely accidental, because there were plants in the immediate vicinity that serve as food for them, so they were present there at the time of coolection.
According to numerous literature data, *Anthonomus rubi* is one of the most important pests of strawberries. Adult is black colour, body length 2-4 mm (Fig. 3). During our survey, at the end of April and beginning of May adults on strawberry leaves were found. The damage to flower stems was recorded during May, according to literature data about the harmfulness of this species (Berglund, 2007; Lindsey, 2009). According to the results of our survey, the intensity of damage to flower buds determined during visual examination was 1,7% (May 9), then 2,3% (May 15) and 2,9% (May 26). Newly hatched adults were determines in the first decade of June and mid-June, which is in agreement with literature data about the bionomy of this species (Sarajlić et al., 2018; Graora, 2022). According to Łabanowska and Chlebowska (1999), the percentage of damaged strawberry flower buds varies significantly depending on the earliness of the cultivar, weather conditions during the flowering period and the oviposition period. Thus, significantly greater damage from this species was recorded on cultivars that bloom earlier (Kikas and Libek, 2002; Alford, 2014; Krauß et al., 2014).

Fig. 3. Anthonomus rubi (orig.)

Fig. 4. Fruit damage from S. geminata (orig.)

In addition, *C. aurata* is also a very common species on strawberries. The adult appears from April to September, especially during May and June, which is in agreement with the results of our research. The damage from adults of this species hasn’t been determined. According to Maceljski (2002), this species causes the greatest damage to soft fruits such as pears, peaches and figs. At the end of May and the beginning of June, the *S. geminata* was determined. In the last few years, the presence of the species has been confirmed in many European countries (Köhler, 2009; Merkl et al., 2009; Spasić et al., 2011; Stan, 2019). The main damage were caused by adults feeding on ripe strawberry fruits (Fig. 4). After copulation, the female lays her eggs on the fruits, most often on already bitten places. The larvae feed on the surface and inside the fruit. Fruits damaged by adults and larvae, as well as pathogens that inhabit damaged fruits, deteriorate very quickly and aren’t suitable for harvesting. According to Spasić et al. (2011), this species caused significant damage in several locations in Serbia, while Nikolić and Nježić (2019) report significant damages of this species in the west part of the Republic of Srpska. The presence of *A. comariana* larvae on strawberry at the beginning of May and until mid-June were determined. In that period, the larvae fed on leaves and parts of the flower, which is in accordance with literature data about the bionomy and harmfulness of this species (Sigsgaard et al., 2014; Barić, 2015).

Conclusion

The harmful insects of strawberry causes significant damage, reducing the yield and quality of the fruit. It’s necessary to determine the composition of the entomofauna, the development cycle of insect species in order to take appropriate protection measures. During our survey, the total number of determined species was 18, from five orders and 16 families. The largest number of species from the order Hemiptera was determined which indicates the importance of monitoring them in the following growing seasons, in order to take plant protection measures in time.

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EFFECT OF CEREAL-LEGUME INTERCROPPING AND MARIGOLD *TAGETES* SP. ON SOIL PLANT PARASITIC NEMATODES

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Abstract

Intercropping increases biodiversity and helps farming systems cope with changes in cultivation conditions. Growing different crops together reduces pest damage and the need for chemical crop protection. The aim of the present work was to determine the quantitative changes in soil plant parasitic nematodes during mixed cultivation of cereal and leguminous crops with marigold in the context of sustainable agricultural production and biodiversity. The research was carried out in 2021-2023, under field conditions in training trial field Vrazhdebna of the Forestry University, Sofia (42°70'76.1"N, 23°43'73.1"E). The intercropping system included winter barley, malting barley and common winter wheat, sown in rows of strips, between which the legumes chickpea and vetch were successively sown in the spring and marigold was added. The results showed that intercropping of cereal and leguminous crops led to a significant reduction of nematodes, the lowest value of Pf/Pi was 0.41, compared to growing them alone. The inclusion of marigolds was also found to further reduce nematode populations, the Pf/Pi value was 0.35. The performance of the intercropping systems was also evaluated on the basis of the intercropping index land equivalent ratio (LER). The LER values for economic yield were highest in the variants winter barley + legumes /vetch and chickpea/ + marigold (2.31) and malting barley + legumes /vetch and chickpea/ + marigold (2.94), which showed that these systems had an advantage in using the resources of the environment. Intercropping of these crops can be used by farmers to control plant parasitic nematodes to reduce the use of synthetic pesticides.

Keywords: *intercropping, soil plant parasitic nematodes*.

Introduction

The plant-parasitic nematodes cause delays in the development of crops by damaging the plants mechanically and disrupting the water and nutrient balance. Furthermore, the damage caused by these pathogens also serves as an entrance for numerous other soilborne phytopathogens. It is frequently observed that nematodes, in conjunction with pathogenic microorganisms such as bacteria and fungi, can give rise to complex diseases (Powell, 2012; Ravichandra, 2014). This can result in what is termed 'synergistic damage' to cultivated plants, including possibly overcoming existing plant resistance against certain pathogens. In conventional agriculture, plant-parasitic nematodes are primarily controlled by chemical nematicides (Haydock *et al*., 2006). However, chemical products are typically only capable of achieving a partial and transient reduction in the populations of harmful nematodes (Kapagianni *et al.*, 2010; Timper, 2014). In recent years, a number of alternative strategies have been developed to suppress plant-parasitic nematodes in different crop groups. These include the use of biological control, breeding of resistant/tolerant varieties, cultural practices and others (Raina *et al.*, 2020; Reddy and Reddy, 2021). Nevertheless, there has been only limited progress in implementing them. Concurrently, the emergence of nematode resistance to commonly used nematicides (Oka, 2020) is increasing the necessity for the development of alternative control options.

The practice of growing two or more crops simultaneously in the same place for a period of time can be regarded as a cultural phenomenon that enhances biodiversity within the agroecosystem, maintains ecological equilibrium, and facilitates more efficient utilisation of natural resources (Reddy and Reddy, 2017). The cereal-legume intercropping is an approach that employs the mechanisms of competition and natural regulation, thereby enhancing soil fertility, reducing the necessity for fertilisers and the application of herbicides (Hauggaard-Nielsen et al., 2008). Intercropping may be regarded as an alternative control measure within the framework of Integrated Pest Management (IPM), with the potential to reduce damage from pest populations while reducing the use of synthetic pesticides and limiting their negative impacts on the environment and human health (Mir *et al.*, 2022). Furthermore, intercropping offers enhanced lodging resistance for lodging-susceptible cereals, reduces the risk of yield loss, improves the quality and quantity of production, and provides greater financial stability for farmers (Reddy and Reddy, 2017). The emergence of novel and expanding trends in agriculture necessitates the conduct of research to facilitate the development of cost-effective and readily implementable alternatives to conventional synthetic pesticides or to identify measures compatible with integrated programmes to safeguard and minimise the utilisation of chemical agents. The existing literature on the impact of intercropping cereals, legumes and Tagetes on plant parasitic nematodes control is insufficient and inconsistent.

The objective of the present study was to quantify the changes in soil nematodes that parasitize plants during the cultivation of cereal and leguminous crops in conjunction with marigold in the context of sustainable agricultural production and biodiversity.

Materials and methods

The experiment was carried out under field conditions at the Vrazhdebna training field of the University of forestry in Sofia (42°70'76.1"N, 23°43'73.1"E) in Bulgaria, during the 2021-2023 growing season. The area exhibits a temperate continental climate characterised by an average annual precipitation of 620.8 mm, an average annual duration of sunshine of 500 h to 1,750 h, and an average annual temperature of 12.02 °C. The soil of the area is classified as slightly stony Fluvisols according to the classification system of the Food and Agriculture Organization (FAO).

Experimental design

The trial intercropping system included barley (*Hordeum vulgare* L.), cv. Obzor winter barley and cv. Veslets malting barley. It also included common winter wheat (*Triticum aestivum* L.), cv. Enola. The cereal crops were sown in autumn. The sowing depth was 5 cm, with an inter-row distance of 13 cm and an intra-row distance of 6 cm. In the spring, vetch (*Vicia sativa* L., cv. Obrasets 666) and chickpea (*Cicer arietinum* L., cv. Plovdiv 8) were sown successively between the strips, with a sowing depth of 5 cm, a row spacing of 20 cm, and an inter-row distance of 20 cm. Simultaneously with the sowing of leguminous crops, marigold (*Tagetes patula* nana Brocade Spanish) was sown between the rows of wheat, vetch and chickpea. The sowing depth was 1 cm, with 30 plants in a row. Plant protection measures were not applied, except application of herbicide after sowing the crops. The total area of the experimental block was 600 m², with each experimental plot comprising 6 rows of crops each with a length of 5 m.

The experiment was laid out in complete randomized block design (RCBD) with 10 variants and 3 replicates. The following variants were tested: WB: winter barley: 6 rows; MB: malting barley: 6 rows; CWW: common winter wheat: 6 rows LVC: legumes: 6 rows /3 rows vetch и 3 rows chickpea/; WB + LVC: 6 rows /2 rows winter barley + 1 row vetch и 1 row chickpea +2 rows winter barley/; MB + LVC: 6 rows /2 rows malting barley + 1 row vetch и 1 row chickpea + 2 rows malting barley/; CWW + LVC: 6 rows /2 rows common winter wheat + 1 row vetch и 1 row chickpea + 2 rows common winter wheat/; WB + LVC + M: 6 rows /2 реда winter barley + 1 row vetch и 1 row chickpea +2 rows winter barley/+ marigold; MB + LVC + M: 6 rows /2 rows malting barley + 1 row vetch и 1 row chickpea + 2 rows malting barley/; CWW + LVC + M: 6 rows /2 rows common winter wheat + 1ред vetch и 1 ред chickpea + 2 rows common winter wheat/

The impact of intercropping was determined using the quantitative parameter land equivalent ratio (LER), which compared the economic yields obtained by intercropping the specified crops with the yields obtained by growing the same crops as monocultures (Chapagain and Riseman, 2014).

Nematological analysis

All plots in the 10 variants were sampled after sowing of cereals for initial nematode population densities (Pi) and once before harvest for final nematode densities (Pf).

In order to ascertain the presence of free-living soil nematodes, soil samples were collected from 10 distinct target points for each variant. These samples were placed in plastic bags and stored in a refrigerator at 4°C. They were then processed within 10 days. Following the mixing of the soil samples, a representative sample of 100 cm^3 was extracted using a measuring cylinder. The data obtained pertains to this volume. The nematodes were extracted from the soil by the centrifugation method, killed and fixed in accordance with the methodology outlined by Van Bezooijen (2006). To ascertain the total number of individuals isolated, the liquid containing the nematodes was refilled to a volume of 100ml with water. The suspension was then stirred with a magnetic stirrer and 5 times the volume of 1 ml was transferred to the Bogorov Modified Counting Chamber. A stereomicroscope with a magnification of 40× was used to enumerate all nematodes initially. On re-enumeration, plant-parasitic nematodes were separated out if present. The number of dead and living nematodes was quantified following the sowing of the tested crops and prior to the harvesting of the crops. The mean number of nematodes extracted was determined by Peters (2013).

Due to the laboriousness of the process of determining the nematode species affiliation and the multivariate experiment, the individuals of the nematode communities were analysed only quantitatively.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and differences of means (3 replicates × 2 years, $n = 6$) were determined by Duncan's multiple range test ($P \leq 0.05$) using the statistical software package SYSTAT 13.

Results and discussion

The initial population density (Pi) of the plant-parasitic nematodes within each variant exhibited a high degree of similarity, with no statistically proven differences (Table 1).

At the conclusion of the growing season, an increase in the population of plant-parasitic nematodes was observed in the monocultures of winter barley, malting barley, and common winter wheat in comparison to the period following sowing. The density of plant-parasitic nematodes was found to decrease when cereals were grown in conjunction with leguminous crops. The final population density (Pf) in the variant malting barley and legumes (chickpea and vetch) had the lowest value of 118 per100 cm^3 soil, followed by the density in winter barley and legumes (chickpea and vetch) of 199 per 100 cm^3 soil, and the highest value was found in common winter wheat legumes (chickpea and vetch) of 209 per 100 cm^3 soil. The reduction in nematode numbers was statistically validated in the malting barley and legumes and winter barley and legumes combinations. The final nematode density (Pf) data indicates that the addition of marigold had a significant impact on the population. Prior to harvest of malting barley from the legume and marigold co-cropping variant, the nematode population density showed a significant reduction in soil free-living plant-parasitic nematodes, with a Pf value of 75 per 100 cm3 soil.

Table 1. Effect of intercropping systems on soil plant-parasitic nematodes

Intercropping crops	Plant parasitic nematodes per 100 cm^3 of soil (PPNs/100cm^3 soil)		Proportion of final and initial population of nematodes (Pf/Pi)
	Initial population (Pi) after sowing of cereals	Final population (Pf) before final harvest	
WB	281a	343a	1.22a
MB	291a	331a	1.14a
CWW	286a	407	1,42a
LVC	259a	271a	1.05ab
WB + LVC	261a	199b	0.76b
MB + LVC	286a	118b	0.41c
CWW + LVC	269a	209b	0.78b
WB + LVC + M	257a	98c	0.38c
MB + LVC + M	215a	75c	0.35c
CWW + LVC + M	249a	123b	0.49c

Mean ± SE; different letters in column = stat. difference; Duncan's multiple range test ($P \leq 0.05$);
WB-wheat barley, MB-malting barley, CWW-common winter wheat, LVC- legumes vetch and chickpea, M-marigold

The incorporation of marigold into the variant winter barley with legumes (Pf = 98) demonstrated a proven quantifiable impact on soil free-living nematodes. Although the addition of marigold had a smaller effect on nematode density in the variant with wheat (Pf = 123), the nematode densities in combinations with marigold were statistically lower compared to monoculture and intercropping with legumes only.

The design of the experiment precluded a direct comparison of nematode densities across crops. However, using Pf/Pi ratios, it was possible to gain insight into the impact of crops in the intercropping system on plant-parasitic nematode densities. (Table 1).

The intercropping of malting barley and legumes (chickpea and vetch) resulted in a significant reduction of nematodes, with a Pf/Pi value of 0.41, which was statistically significantly lower than that observed in single cropping and the variants winter barley with legumes and wheat with legumes. The effect of mixed crops of winter barley with legumes and wheat with legumes was almost similar. The Pf/Pi ratio values were 0.76 and 0.78, respectively. The inclusion of marigold in the variants resulted in a further reduction in nematode population density, with the lowest Pf/Pi value of 0.35 being observed in the variant malting barley, legumes and marigold, and the highest Pf/Pi value of 0.49 being observed in common winter wheat, legumes and marigold. The intermediate position was occupied by the variant winter barley, legumes and marigold, with a value of Pf/Pi =0.38.

The ratio Pf/Pi is a quantity that depends on the density of Pi (McSorley and Gallaher 1995), and changes in the abundance of nematode populations depend on environmental factors that can cause rapid and short-term changes in nematode populations in soil (Thoden *et al.*, 2011). Therefore, further research is needed to establish the relationships between Pf and Pi within the intercropping of these crops. A substantial body of research has been conducted on the efficacy of *Tagetes* in the control of plant parasitic nematodes (Koleva and Mitova, 2021; Munif *et al.*, 2021; Fourie, 2024). It is known that the Tagetes species are highly toxic to plant parasitic nematodes, suppressing them through a biochemical interaction known as allelopathy. Tagetes plants secrete root exudates containing toxic bioactive substances (such as α-terthienyl and others) that have nematicidal, insecticidal, fungicidal, antiviral and cytotoxic activities (Bhattacharyya, 2017). In addition to the previously discussed mechanisms, Tagetes also suppresses plant-parasitic nematodes through the following means: it is an unsuitable host for nematodes, activates microorganisms that are antagonistic to nematodes, and acts as traps for nematodes (Grubišić *et al.*, 2018). The control of plant-parasitic nematodes by antagonistic plants provides several benefits, including biodegradability, selective toxicity to target organisms, safety to non-target organisms and the environment, and renewability in nature (Bhattacharyya, 2017). It is established that marigold can be employed as an alternative to synthetic nematicides. In certain instances, *Tagetes* sp. has been demonstrated to reduce nematode populations at greater depths within the soil than soil fumigation (Karakas and Bolukbasi, 2019). The successful incorporation of *Tagetes* sp. into an integrated nematode management programme necessitates the undertaking of research to ascertain its effectiveness against locally occurring nematode populations and in different farming systems.

To ascertain the impact of competition between the species utilised in the intercropping system, the Land Equivalent Ratio (LER) was calculated. The highest total LER was observed in the variant comprising malting barley, legumes and marigold (2.78), while the lowest LER was observed in the variant comprising winter wheat and legumes without marigold (2.07). The mean LER values of the other intercropping variants were greater than 1.0 (Figure 1), indicating superior crop development (Dhima *et al.*, 2007).

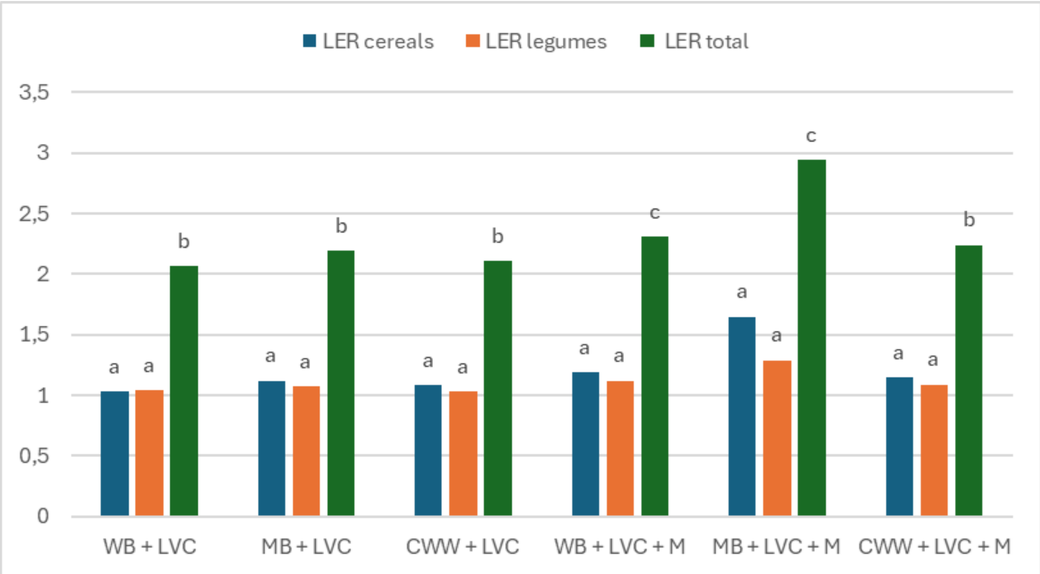

Figure 1. Means of LER of cereal-legume intercropping and marigold

Different letters within the same LER (bars with the same color) indicate statistically significant differences, Duncan's multiple range test ($P \leq 0.05$); WB-wheat barley, MB-malting barley, CWW-common winter wheat, LVC- legumes vetch and chickpea, M- marigold

The higher yield obtained serves to demonstrate the advantage of intercropping over monoculture cultivation. The temporal and spatial differences of crops in the intercropping system facilitate the more efficient utilisation of nutrients in agroecosystems (Duchene *et al*, 2017; Yang *et al.*, 2021). An advantage of intercropping systems of cereals and leguminous crops has been previously reported for crops such as barley and lentil (Dahmardeh, 2013), field beans and wheat (Kamalongo and Cannon, 2020), barley and faba bean (Tang *et al.*, 2023). In general, the quantitative parameters nematode density and the Land Equivalent Ratio (LER) index were significantly affected by marigold intercropping, indicating that intercropping is an effective method of utilising environmental resources compared to monocultures.

The data obtained here may be pertinent to the selection of certain crops for co-cropping in agro-ecosystems where inter-crop competition can assist in overcoming biotic stress factors.

Conclusions

The addition of *Tagetes patula* into the cereal-legume intercropping system was found to have a proven effect on soil free-living plant-parasitic nematodes.

The final nematode density (Pf) was lowest in the *Tagetes* variants, followed by the legume variants, and the highest values were observed in the cereal monoculture.

The LER values demonstrate that yields were significantly higher in the cereals-legume intercrop and *T. patula* than in the monoculture crops.

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THE EFFECT OF ESSENTIAL OILS ON MYCELIAL GROWTH AND SPORULATION OF *BOTRYTIS CINEREA*

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Abstract

In vitro study of the vapor antifungal activity of 10 essential oils (EO’s): *Ilicum verum* (anise), *Pinus mugo* (pine), *Cinnamomum zeylanicum* (cinnamon), C*upressus sempervirens* (cypress), *Eucalyptus globulus* (eucalyptus), *Lavandula angustifolia* (lavender), *Laurus nobilis* (laurel), *Mentha piperita* (mint), *Rosmarinus officinalis* (rosemary) and *Thymus vulgaris* (thyme) on the mycelial growth and sporulation of plant pathogen *Botrytis cinerea* was performed. The EO’s were applied in five concentrations of 0.02, 0.04, 0.1, 0.15 and 0.2% of air phaser. At all concentrations, the EO’s of thyme and cinnamon showed complete inhibition of the mycelial growth of *B. cinerea* after 168 hours. Mint oil completely inhibited mycelial growth in all treatments except in treatment with the lowest concentration of oil (0.02% of air phase) while lavender oil completely inhibited mycelial growth in treatments with the three highest concentrations of oil. For treatments in which mycelial growth was determined, sporulation was also measured. The effect of EO’s on sporulation was expressed as a percentage in relation to the number of conidia in the control, which was marked as 100%. There was no sporulation in treatments with pine oil (0.15 and 0.2% of air phase), while sporulation less than 50% compared to the control was found in treatments with lavender oil (0.02% of air phase), eucalyptus oil (0.02 and 0.04%), anise oil (0.02 and 0.04%), laurel oil (0.02, 0.04 and 0.1%), rosemary oil (0.02, 0.1, 0.15 and 0.2%) and pine oil (0.04%).

Key words: *essential oils, antifungal activity, Botrytis cinerea.*

Introduction

The causal agent of gray mold, *Botrytis cinerea* Pers., is aggressive polyphagous non-specific plant pathogenic fungus. This pathogen causes devastating diseases on more than 1400 cultivated and wild plant species including fiber, protein, oil and horticultural crops (Elad *et al*., 2016; Cheung *et al*., 2020). It is estimated that this pathogen causes annual losses of up to USD 100 billion all over the world.
The use of synthetic fungicides is still the most effective protection against many plant pathogens including *B. cinerea*. In spite of their efficiency, the widespread use of synthetic pesticides has significant drawbacks including handling hazards, pesticide residues in food, feed and soil, fungal resistance against synthetic compounds, ecosystem equilibrium disruption and threats to human health (Palfi, 2017). All these negative effects of pesticides have indicated the need for alternative non-chemical methods in plant protection. In order to find new and ecologically acceptable biofungicides, many studies related to antifungal effects of alternative products are being conducted (Combrinck *et al*., 2011; Duduk *et a*l., 2015; Sharma *et al*., 2017, Nguyen *et al*., 2017). Biological compounds extracted from plants could be one of the most important alternatives which do not have any hazardous effects on human

health and environment. Essential oils reduce the growth of fungal hyphae and promote the degradation of the fungal cell membrane. Also, they cause changes in the composition of the cell wall, plasma membrane disorders, disorganization of the mitochondrial structure and disruption of the enzymatic reaction in mitochondrial membrane such as electron transport, proton transport and affects phosphorylation (Knobloch, 1989).

Materials and methods

The in vitro antifungal properties of 10 EO’s: Ilicum verum (anise), Pinus mugo (pine), Cinnamomum zeylanicum (cinnamon), Cupressus sempervirens (cypress), Eucalyptus globulus (eucalyptus), Lavandula angustifolia (lavender), Laurus nobilis (laurel), Mentha piperita (mint), Rosmarinus officinalis (rosemary) and Thymus vulgaris (thyme) on the mycelial growth and sporulation of plant pathogen Botrytis cinerea were tested. The EO’s used in this study were commercial samples produced in Kemig Ltd., Sesvete – Soblinec, Croatia. The selected fungal strain was obtained from the culture collection of the Faculty of Agrobiotechnical Sciences in Osijek, Croatia. The pathogen was isolated 2020 from grapes and identified based on morphological characteristics, taking into account the cultural characteristics of the fungi as well.

For determination of vapor effect of EO’s, the modified method of Edris and Farrag (2003) was used. A piece of mycelium (diameter 4 mm) is placed in the middle of Petri dishes with 15 mL of PDA, and essential oil is applied to the dishes cover on sterile filter paper (diameter 7 mm). The EO’s were applied in five amounts (10 µL, 20 µL, 50 µL,75 µL and 100 µL) which corresponded to 0.1, 0.2, 0.5, 0.75 and 1% EO’s concentrations of air phase. Petri dishes are stored in a thermostat chamber at a temperature of 25°C and a light regime of 12 hours light / 12 hours darkness. In the control, filter paper soaked in an appropriate amount of sterile distilled water was used. The inhibition zone was measured after 168 hours. The effect of essential oils on the growth of B. cinerea mycelium was calculated according to the formula of Wu et al. (2013):

$$I (\%) = [(C-T)/(C-0.4)] \times 100$$

where is:

I (%) - percent inhibition of mycelial growth
C – mycelial growth in control
T - mycelial growth in the presence of essential oil

After measuring the mycelial growth, sporulation was determined by preparing a suspension of conidia in 100 ml of sterile distilled water, and the concentration of conidia of suspension was measured using a hemocytometer. The effect of essential oils on sporulation was expressed as a percentage in relation to the number of conidia in the control, which was marked as 100%.

In order to determine the germination of conidia, from the prepared suspension for each variant of the experiment, 20 µl was taken and spread on the PDA medium. Petri dishes are placed

at a temperature of 25 °C and a light regime of 12 hours light / 12 hours darkness. After 24 hours, the number of germinated conidia is determined on a sample of 3 x 20 conidia. Conidia were considered germinated if the germ tube is equal to or greater than the length of the conidia.

The reduction/inhibition of germination of conidia was calculated according to the formula of Youssef *et al.* (2019) and expressed as a percentage:

$$\text{Reduction/inhibition (\%) of germinating conidia} = \frac{\text{gc} - \text{gt}}{\text{gc}} * 100$$

where is:
gc - average germination of conidia of isolate in the control
gt - average germination of conidia isolate at a certain concentration of essential oil

Statistical analysis of experimental results was performed with the SAS 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) using analysis of variance ANOVA and the Fisher LSD test (P= 0.01).

Results and discussion

The vapor effect of essential oils on mycelial growth, sporulation and conidia germination of *B. cinerea* were tested. The results of the influence of essential oils on mycelial growth are shown in Table 1.

At all concentrations, the EO’s of thyme and cinnamon completely inhibit the mycelial growth of *B. cinerea*. It is worth mentioning the excellent inhibitory effect of mint oil, where a slight inhibition (39.01%) of mycelial growth was observed only at a concentration of 0.1% of air phase, but with no statistically significant difference compared to the control. Anise oil also had a very good inhibitory effect on the growth of the fungal mycelium, but only at higher concentrations of the EO. At concentrations of 0.5, 0.75 and 1% of air phase, anise oil showed statistically significant differences compared to the control with an inhibitory effect of 64.81 and 100%. The results confirmed other findings on fungistatic activity of thyme, cinnamon, anise and mint essential oils (Arras and Usai, 2001; Behnam *et al.*, 2006; Allagui *et al.*, 2023). Laurel, cypress, and eucalyptus oils had the weakest inhibitory effect. Allagui *et al.* (2023) states that cypress oil completely inhibited growth of *B. cinerea* mycelia, while Grgić *et al.* (2016) states that cypress oil weakly inhibited the growth of the same pathogen. Pine oil had a slightly better inhibitory effect than the previously mentioned oils. There were statistically significant differences compared to the control only at a concentration of 0.1% of air phase with an inhibitory effect of 35.26% and 32.51% at a concentration of 1% of air phase.

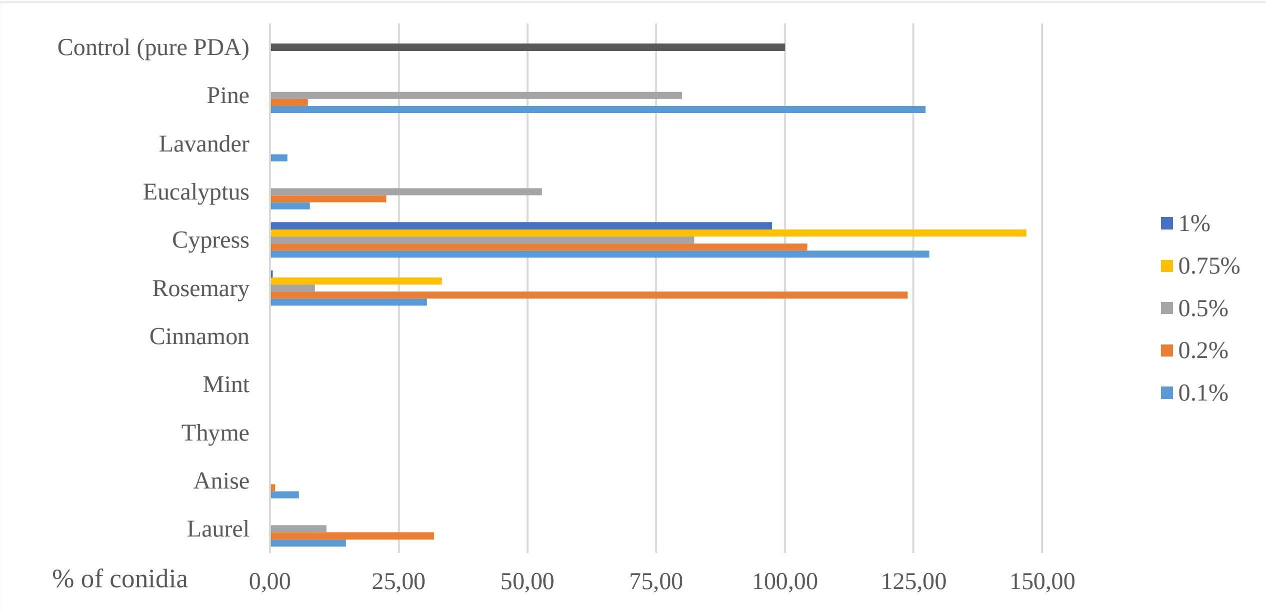
The effect of EO’s on sporulation was expressed as a percentage in relation to the number of conidia in the control, which was marked as 100% (Graph 1). There was no sporulation in treatments with pine oil (0.75 and 1% of air phase), while sporulation less than 50% compared to the control was found in treatments with lavender oil (0.1% of air phase), eucalyptus oil (0.1 and 0.2%), anise oil (0.1 and 0.2%), laurel oil (0.1, 0.2 and 0.5%), rosemary oil (0.1, 0.5, 0.75 and 1%) and pine oil (0.2%).

The inhibitory effect of essential oils on conidium germination has been reported by several authors (Wilson et al., 1997; Andrade and Vieira, 2016; Hou *et al.*, 2020). In our research, after 24 hours incubation the germination rate was decreased by 6.67 (0.1% of air phase for rosemary oil) to 71.67% (0.1 and 0.2% of air phase for anise oil) compared to control (Table 2).

Table 1. The effect of essential oils on mycelial growth of *B. cinerea* (%)

Essential oil	**0.1%**	**0.2%**	**0.5%**	**0.75%**	**1%**	**LSD 0,01**
Laurel	0.00 ± 0..00	0.00 ± 0..00	0.00 ± 0..00	0.00 ± 0..00	7.49 ± 3.50	7..02
Anise	0.00 ± 0.00	0.00 ± 0.00	64.81 ± 0.20	100.00 ± 00.00	100.00 ± 0.00	0.39
Thyme	100.00 ± 00.00	100.00 ± 0.00	100.00 ± 00.00	100.00 ± 00.00	100.00 ± 0.00	0.00
Mint	39.01 ± 19.10	100.00 ± 0.00	100.00 ± 00.00	100.00 ± 00.00	100.00 ± 0.00	38.28
Cinnamon	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 00.00	100.00 ± 00.00	100.00 ± 0.00	0.00
Rosemary	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.39 ± 0.39	12.02 ± 3.06	6.18
Cypress	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Eucalyptus	13.99 ± 10.64	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	21.33
Lavander	14.58 ± 14.58	18.32 ± 9.24	100.00 ± 0.00	100.00 ± 00.00	100.00 ± 00.00	34.60
Pine	1.18 ± 1.18	14.58 ± 4.79	9.85 ± 9.85	35.26 ± 12.36	32.51 ± 12.63	41.74
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
LSD 0,01	31.62	12.51	11.84	14.87	16.17	

Data are presented as the mean ± standard error of four repetitions. The individual columns show the differences between different essential oils at the same concentration. The rows show the difference between different concentrations within the same oil. Fisher's LSD test (P≤ 0.01).



Graph 1. Effect of essential oils on number of *B. cinerea* conidia (%) of conidia compared to control)

Table 2. Effect of essential oil on the reduction of *B. cinerea* conidia germination (%)

Essential oil	0.1%	0.2%	0.5%	0.75%	1%
Laurel	11.67 ± 1.67	13.33 ± 1.67	13.33 ± 4.41	-	-
Anise	71.67 ± 4.41	71.67 ± 1.67	-	-	-
Rosemary	6.67 ± 1.67	11.67 ± 1.67	11.67 ± 3.33	11.67 ± 1.67	15.00 ± 2.89
Cypress	8.33 ± 3.33	8.33 ± 4.41	16.67 ± 1.67	20.00 ± 5,00	35.00 ± 2.89
Eucalyptus	51.67 ± 4.41	56.67 ± 4.41	56.67 ± 4.41	-	-
Lavander	16.67 ± 4.41	-	-	-	-
Pine	13.33 ± 3.33	16.67 ± 3.33	16.67 ± 1.67	-	-
Control	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
LSD 0,01	11.33	9.40	9.18	7.50	5.30

Data are presented as the mean ± standard error of four repetitions. The individual columns show the differences between different essential oils at the same concentration. The rows show the difference between different concentrations within the same oil. Fisher's LSD test (P≤ 0.01).

Conclusion

The research examined the effect of ten essential oils on the suppression of the mycelium growth, sporulation and conidia germination of the plant pathogenic fungus *B. cinerea*. Thyme and cinnamon essential oils completely inhibit mycelial growth and sporulation of *B. cinerea* at all applied concentrations. Although mint oil did not completely inhibit mycelial growth at the lowest concentartion (0.1 % of air phase), sporulation was also not determined. Compared to control, sporulation decreased at least 50% in treatments with lavender oil (0.1% of air phase), eucalyptus oil (0.1 and 0.2%), anise oil (0.1 and 0.2%), laurel oil (0.1, 0.2 and 0.5%), rosemary oil (0.1, 0.5, 0.75 and 1%) and pine oil (0.2%), while germination rate was decreased by 6.67 (0.1 % of air phase for rosemary oil) to 71.67% (0.1 and 0.2% of air phase for anise oil).

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IN VIVO EVALUATION OF ANTIFUNGAL ACTIVITY OF NICOTINAMIDE COMPOUNDS AGAINST *BOTRYTIS CINEREA* IN TOMATOES

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Abstract

Botrytis cinerea is the most important and most frequently isolated pathogen within the genus *Botryotinia*. This necrotroph is considered to be one of the most destructive pathogens in agriculture in greenhouses and in the field. In tomatoes, *B. cinerea* causes grey mould, with symptoms appearing on ripe fruit, but can also be spread to leaves and stems. Pyridine derivatives are extremely important chemical compounds due to their physico-chemical properties and their diverse biological applications. Scientific studies indicate the antifungal effect of quaternary pyridinium compounds, especially nicotinamide derivatives. Most studies to date have focused on *in vitro* tests. Therefore, further investigation of the effects of these compounds on phytopathogenic fungi through *in vivo* experiments is crucial for the improvement and development of environmentally friendly fungicides. This study aimed to investigate the antifungal activity of four selected compounds, chosen based on previous *in vitro* antifungal results, on the growth of *B. cinerea* mycelia and their inhibitory effect on conidia germination. The *in vivo* experiments were carried out on ripe tomato fruits at concentrations of 100 µg/ml. Mycelial growth was measured 96, 120, and 144 hours after inoculation to determine possible differences in the antifungal activity of the nicotinamide compounds compared to the controls. All tested compounds showed a significantly weaker inhibitory effect regardless of the incubation time and positive controle (fenhexamide). Compound (12) Na-DBP showed significantly stronger antifungal activity compared to the other compounds tested, with the highest efficacy observed 120 and 144 hours after inoculation. All applied compounds used showed significantly higher inhibition of conidia germination compared to the control (pure PDA).

Keywords: *antifungal activity, nicotinamide compounds, B. cinerea, tomato.*

Introduction

Botryotinia fuckeliana (de Bary) Whetzel, also known as *B. cinerea* in the anamorphic stage, is the main and most frequently isolated pathogen within the genus *Botryotinia*. The adaptation of *B. cinerea* to different climatic conditions is reflected in its wide distribution in different geographical areas, ranging from tropical and subtropical areas to cold temperate and northern and southern areas. *B. cinerea* causes major losses to horticultural crops in the field and the greenhouse at all stages of plant development (Williamson et al., 2007). In his recent study, Singh (2024) lists 616 genera that serve as hosts for this pathogen. This necrotroph is considered one of the most destructive pathogens in agriculture, especially when it comes to fruit, vegetables, and flowers before and after harvest during storage and transport (Jarvis, 1977; Williamson et al., 2007). *B. cinerea* attacks various parts of the host plant at different stages of development, from germination to fruit ripening. It can attack the stem, flowers, leaves, and fruits and can also occur in the seeds (Yahaya et al., 2015). However, it causes the most damage to mature, older, weakened, or injured host tissue. Symptoms caused by this pathogen include fruit rot, soft fruit rot, yellow-brown lesions on leaves and stems that can spread in concentric rings, and wilting. A characteristic symptom of this pathogen is an accumulation of conidia in the form of a grey coating. The appearance of this symptom usually occurs under favourable conditions, such as cold to moderate temperatures (10 - 25°C) with high humidity (Williamson et al., 2007; Quesada-Ocampo, 2015). Different modes of infection, the ability to infect a wide range of plant hosts, a high reproductive potential, and the ability to survive for a long time under unfavourable conditions in the form of sclerotia form the basis for the success of *B. cinerea* as a pathogen (Williamson et al., 2007). Despite constant warnings about the negative consequences for human health and the environment (Zemmouri et al., 2022), using fungicides is still the predominant method of controlling this pathogen. Given the pronounced tendency to develop resistance to fungicides, this pathogen is becoming increasingly important in agriculture and scientific research (Amselem et al., 2011; Adnan et al., 2018; Adnan et al., 2019). Research is increasingly focusing on the search for new chemical compounds with antifungal activity, which should be less toxic and harmful to the environment than commercial fungicides. One of these is quaternary pyridinium compounds, which are derivatives of vitamin B3. They are considered important chemical compounds due to their properties and diverse biological applications (Altaf et al., 2015). Numerous scientific studies confirm their antifungal activity against important phytopathogenic fungi, including the pathogen *B. cinerea* (Wu et al., 2012; Wu et al., 2013, Siber et al., 2019; Bušić et al., 2022). Based on the preliminary results of the *in vitro* investigation of 12 newly synthesized nicotinamide pyridinium compounds, those exhibiting very effective antifungal activity against the growth of *B. cinerea* pathogen mycelia were selected. This study aims to investigate the selected nicotinamide compounds and their potential as more environmentally friendly fungicidal agents for protection against *B. cinerea* pathogens under *in vivo* conditions.

Material and Methods

The *in vivo* test of the effect of the synthesized compounds on the growth of mycelia of the pathogen *B. cinerea* was carried out with selected compounds that showed an antifungal effect in the *in vitro* test. The *in vivo* test was carried out using the modified method of Hao et al. (2020) on undamaged medium-sized tomato fruits. A *B. cinerea* isolate from the culture collection of the Department of Phytopathology of the Faculty of Agrobiotechnical Sciences Osijek was used for the experiment. The isolate was grown on a PDA (potato dextrose agar) medium enriched with antibiotics. An isolate that had been incubated for seven days was used for the artificial infection. Before artificial infection, tomatoes were sterilized with 1% NaClO solution (sodium hypochlorite) for 2 minutes, then washed with distilled water and dried at room temperature. A needle was used to puncture a wound in the dried and disinfected tomato fruit. A concentration of 100 µg/mL of the synthetic compound was injected into each wound using a micropipette. The injection of 1% DMSO into the tomato fruit served as a negative control, while the positive control was a tomato injected with a commercial fungicide at the recommended dose. Two to three hours after treatment, a piece of *B. cinerea* mycelium was removed from the PDA growing culture using a circular cutter with a diameter of 4 mm and placed on the wounds of the fruit with a sterile needle. Each treatment was carried out in 4 replicates with 4 tomatoes each. The tomato fruits were placed on moist, absorbent paper in plastic containers. Each container was placed in a plastic bag for 48 hours to maintain moisture for initial infection. The containers were incubated at room temperature. The diameter of mycelial growth was measured 48, 72, 96, 120, and 144 hours after inoculation.

The inhibitory effect of the tested synthesized compounds on the germination of the conidia was performed in 2 replicates for each compound at a concentration of 100 µg/ml. DMSO (1%) was used as a negative control, while a commercial fungicide at the recommended dosage was used as a positive control. A control variant with distilled water was also set up. Conidia older than 10 days were used for the *in vitro* germination test according to the modified method described by Cao et al. (2016). After harvesting the conidia from the PDA medium, they were filtered and suspended to a concentration of 1×10^8 spores per milliliter of sterile water. The spore concentration was determined using a Neubauer hemocytometer. The resulting conidial suspension and the treatments concentration of 100 μg/mL were pipetted onto 60 mm diameter Petri dishes containing clean PDA medium. The dishes were then incubated for 4 hours in a climate chamber at 25°C and 90% relative humidity. The same procedure was repeated for the positive and negative controls and control variant with distilled water. From 80 conidia per treatment (4 replicates of 20 conidia each), the number of germinated conidia was recorded and the length of the germ tubes was measured using an Olympus BX41 microscope. The conidia were considered germinated if the germ tube was equal to or larger than the diameter of the conidium (Qin et al., 2010). According to the formula given by Youssef et al. (2019), the percentage of inhibition of germinated conidia was calculated: Inhibition (%) of germinated conidia = (gc - gt) / (gc) * 100

Where:

gc - average germination of conidia of the isolate in the control

gt - average germination of conidia of the isolate in the synthesized compound

Results and Discussion

The *in vivo* study on the effect of selected nicotinamide pyridinium compounds (concentration 100 μg/ml) on the growth of *B. cinerea* mycelia is shown in Table 1 for different incubation times. ANOVA showed a statistically significant difference between the compounds for 96, 120 and 144 hours. At 48 hours after inoculation there was no increase, and at 72 hours after inoculation there was a slight increase in some treatments (data not showed).Tukey's multiple comparison tests were used to test which compounds showed statistically significant differences ($p \leq 0.05$).

Table 1. Effect of nicotinamide pyridinium compounds (concentration 100 μg/mL) on the growth of *B. cinerea* mycelia

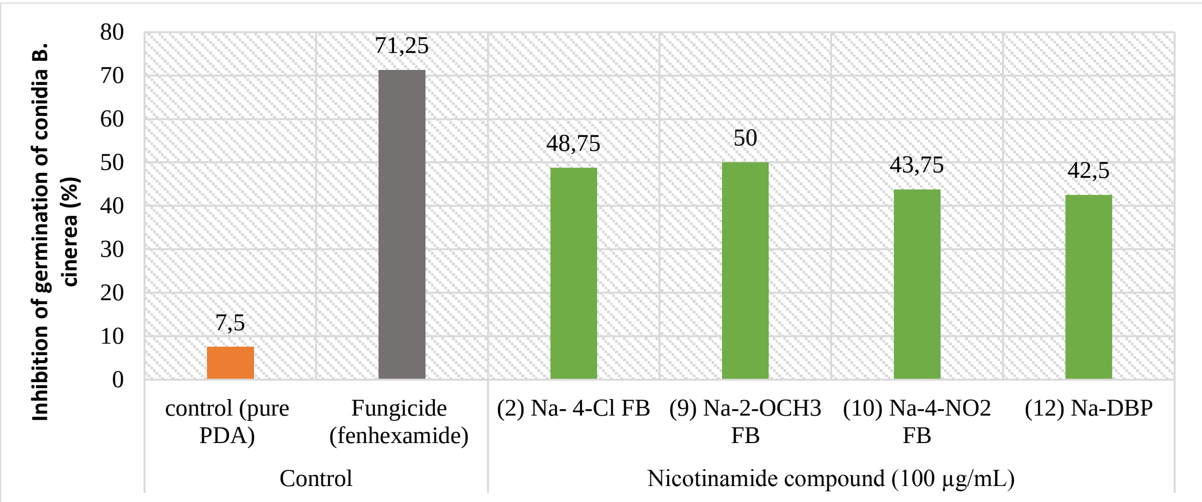
Nicotinamide compound / h from inoculation	96 h M ± SEM	120h M ± SEM	144h M ± SEM
(2) Na- 4-Cl FB	29.00 ± 1.47 A	37.25 ± 2.04 A	45.33 ± 1.70 Aa
(9) Na-2-OCH3 FB	26.67 ± 1.08 A	37.00 ± 1.83 A	43.86 ± 2.14 Aa
(10) Na-4-NO2 FB	27.21 ± 1.16 A	39.77 ± 2.04 A	46.62 ± 2.32 Aa
(12) Na-DBP	27.00 ± 0.94 A	34.71 ± 1.89 A	37.86 ± 2.39 A
control (pure PDA) a	25.58 ± 0.62	32.83 ± 1.25	35.08 ± 1.62
positive control (fenhexamide) A	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Comparison of nicotinamide compounds (Tukey's test)	—	—	10 > 12

The columns show the mean of four replicates (M) and the standard error of the arithmetic mean (SEM).

Significant differences between the compounds and the positive control are labelled with the letter A ($p \leq 0.05$), significant differences between the compounds and the control (pure PDA) with the letter a ($p \leq 0.05$).

Significant differences between the compounds, i.e. Tukey's test ($p \leq 0.05$), are labelled with numbers next to the corresponding compound

The results of the study show that no nicotinamide compound with an applied concentration of 100 µg/ml completely inhibited (100%) the growth of the pathogenic mycelium of B. cinerea. 96 and 120 hours after inoculation, the compounds did not differ statistically significantly in their antifungal activity compared to the control (pure PDA). In the incubation period, i.e. 144 hours after inoculation, the three compounds (2) Na-4-Cl FB, (9) Na-2-OCH3 FB and (10) Na-4-NO2 FB showed a significantly weaker effect on the growth of mycelia compared to the control ($p \leq 0.05$). A significant difference was also observed for compounds (10) Na-4-NO2 FB and (12) Na-DBP, with compound (12) Na-DBP having a significantly stronger antifungal effect. Similar results were obtained by Yan et al. (2022) in their study in which they synthesized 15 new pyridine derivatives. In vitro tests showed that all compounds exhibited moderate to good antifungal activity against B. cinerea, while in vivo tests showed that only one compound exhibited good antifungal activity. Overall, these results suggest that the inhibitory effect of nicotinamide pyridinium compounds decreases with the applied concentration of 100 µg/ml when the incubation period is delayed, except in the case of compound (12) Na-DBP. Finally, all pyridinium nicotinamide compounds showed a significantly weaker inhibitory effect than commercial fungicides regardless of the incubation time. On the other hand, Xu et al. (2023) investigated 19 newly synthesized pyridinium compounds on seven different pathogenic species, including B. cinerea. In this study, ten compounds showed an inhibitory effect of more than 90% on B. cinerea, with one compound exhibiting the highest antifungal activity, even better than commercial fungicides.
Bušić et al. (2022) also investigated the antifungal activity of 11 synthesized pyridine derivatives in vitro using a mycelial growth assay on four different pathogenic fungi, including B. cinerea. The results showed that 8 of the 11 compounds tested exhibited less than 50% antifungal activity against B. cinerea, suggesting that the activity of each compound depends on the type of pathogen. The inhibitory effect of selected compounds on the germination of the conidia of the B. cinerea pathogen is shown in Graph 1.



Graph 1. Inhibitory effect of nicotinamide pyridinium compounds on the germination of *B. cinerea* conidia (%)

All applied compounds showed a significantly higher inhibition of conidia germination compared to the control (pure PDA). Comparing the compounds with the control (pure PDA), the strongest inhibition of conidia germination was observed with compound (9) Na-4-Ph FB and compound (2) Na-4-Cl FB. However, none of the compounds showed stronger inhibition of conidia germination than the positive control. Among the compounds, the least inhibition of conidial germination was observed with compound (12) Na-DBP. In addition, Xuet al.

(2023) reported a significant inhibition of the germination of *B. cinerea* conidia. According to their studies, two pyridinium compounds showed the strongest inhibition of conidia germination with 64% and 76%, respectively.

Conclusion

Based on preliminary *in vitro* results of newly synthesized nicotinamide pyridinium compounds, four compounds were selected that showed good to very good antifungal effects on the growth of the mycelium of the pathogen *B. cinerea*. The antifungal activity of the selected compounds was tested in an *in vivo* test on tomato fruits. The results obtained show that the compounds exhibit good antifungal activity compared to the control only after a delay in the incubation period, i.e. 120 hours after inoculation, but without statistical significance. Numerous studies show that pyridinium compounds, thanks to their good antifungal effect, indicate a possible application as new, more environmentally friendly fungicides in plant protection. From these results we can conclude that some of the compounds tested *in vivo* can even stimulate the growth of the pathogen *B. cinerea*, which does not mean that these compounds could not have antifungal activity against other pathogens. On the other hand, the results show that compounds (2) Na-4-Cl FB and (9) Na-4-Ph FB, although they had lower antifungal activity on the growth of *B. cinerea* mycelium, had the most significant inhibitory effect on spore germination. Despite the insufficient antifungal effect of the synthesized compounds on the mycelial growth of the causal *B. cinerea*, further research with these compounds is needed, not only on the pathogen *B. cinerea* but also on other important species of phytopathogenic fungi.

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THE LIPOLYSIS LEVEL OF SOME GREEK ARTISANAL CHEESES: A REVIEW OF LAST YEAR’S DATA

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Abstract

In this review the lipolysis level of some Greek artisanal cheeses was studied. Researchers studied the lipolysis profile of the artisanal mountainous hard Kefalotyri cheese made traditionally in Pindos mountains from raw ewe’s milk without starter cultures, during summer. Free fatty acids (FFAs) were the most abundant volatile group of this cheese with butyric acid and 3-methyl butanoic acid to be the main. Cheese showed a rather satisfactory hygienic sanitary condition. Researchers studied the lipolysis level of Kashkaval Pindos cheese a semi-hard pasta-filata cheese made from raw ewe’s milk without starter addition or made by pasteurized milk with addition of a commercial cheese starter. Raw milk Kashkaval cheeses showed higher levels of lipolysis than pasteurized ones. Butyric, myristic, palmitic, stearic and oleic acids were the main FFAs. All cheeses were found to be safe. Artisanal Tsalafouti cheese resembles to fresh acid an acid rennet curd cheeses with spreadable texture and no gas openings. It is traditionally made from ‘boiled’ (90^0C) ewe’s milk in the mountains at the end of the lactation period. Researchers found a high level of hexanoic acid in a semi-industrial way made cheese. Xinotyri of Naxos Island is an artisanal fully ripened hard raw goat’s cheese. Researchers found that FFAs profile was similar to other goat cheeses with oleic, palmitic, myristic, stearic and capric acid to be the main. Soft Xinotyri Naxos cheese is an acid-curd goat’s milk cheese typically made from raw milk. Researchers found a low lipolysis level that was not affected by using raw or pasteurized milk.

Key words: *lipolysis, artisanal cheeses.*

Introduction

Lipolysis (lipid hydrolysis) is essential for the development of cheese flavour. Lipolysis results in the formation of FFA, which may directly contribute to cheese flavor, especially short and intermediate chain FFA or act as precursors for other flavour compounds such as esters, methyl ketones and secondary alcohols which are also of importance (Fox etal., 1993; Molimard & Spinnler, 1996; Collins et al., 2003).

Lipolysis level varies considerably among cheese varieties from slight in many internal, bacterially ripened varieties such as cheddar, gouda and Swiss cheeses to extensive in hard Italian varieties, surface bacterially ripened (smear) cheeses and blue mould cheeses (Woo et al., 1984 a, b; Fox et al., 1993; Fox & Wallace, 1997; McSweeney & Fox, 1993; Collins et al., 2003). For some cheese varieties, a specific group of volatile compounds is recognized as being the major contributor to its flavor. In hard Italian cheese varieties FFAs are important contributors to cheese flavor. However, in blue-mould ripened cheeses the impact of FFA to cheese flavour is less than that of Italian varieties. This was due to the dominant influence of methyl ketones to the flavour of these cheeses (Woo et al., 1984 a,b; Mollimard & Spinnler, 1996).

Artisanal cheeses are known to take recognition by consumers during the last years. These cheeses are frequently linked to certain local regions. They are made by traditional methods in dairy farms using a simple equipment by family members as a staff and without certification or label (Koutsoukis et al., 2017). Due to their recognition in the last years, efforts should be made to standardize their production process with a view to make safe cheeses of high and constant quality. In this way the local economy will be supported and export of these cheeses outside the region of their production should be done. With a view to meet consumers demands, artisanal cheeses should be produced in an industrial scale. The transition from the local small scale cheesemaking to the large industrial includes milk pasteurization, addition of starters, control of ripening conditions, packaging etc. to improve cheese quality and safety. The combination of traditional and industrial practices in cheese manufacture results in a standard cheese product that is greatly accepted by the consumers. This work is a review of last year's data on lipolysis profile of some Greek artisanal cheeses.

Materials and Methods

Data of this review were collected from research papers on the field of artisanal Greek cheeses. All methods used for lipolysis determination are reliable and known ones in literature.

Results and Discussion

Kashkaval cheese is a pasta filata cheese variety that is produced in many countries. In Greece, the traditional way of making Kashkaval cheese has been referred many years ago from the previous century. Kashkaval cheese is a local, hard crafted pasta filata cheese which is produced in specific Greece places such as the mountains of Pindos by using a traditional method. Its production has been transferred from generation to generation. Pappa et al (2019) studied the biochemical characteristics of the traditional type of Kashkaval cheese that was made by using raw or pasteurized ewe's milk, during ripening and storage. Generally, lipolysis was not intense in Kashkaval cheese. Pasteurization of milk significantly affected the total free fatty acids (TFFAs) content of Kashkaval cheese (Table 1). Pasteurized milk cheeses had lower amount of TFFAs than raw ones (Table 1), except for the 30 days cheeses where no differences were found (Table 1). Pappa et al. (2020) made a semi-industrial Kashkaval of Pindos cheese by using 100% sheep milk (KS) or with the addition of 10% goat milk (KG). The free fatty acid content of KS and KG Kashkaval of Pindos cheeses is shown in Table 1.

Table 1. Free fatty acids (peak area x 10^3) of semi-industrial produced Kashkaval of Pindos cheese made from 100% sheep milk (KS) or a mixture of 90% sheep milk-10% goat milk (KG) during ripening and storage.

Kashkaval cheese	60 Day		90 Day		180 Day	
Free fatty acids	KS	KG	KS	KG	KS	KG
Propanoic acid, 2 methyl	3636.63	2314.9	3558.8	1486.6	5052.8	2061.3
Butanoic acid, 2 methyl	7317.6	1667.7	9961.35	1880.55	24,456.6	2256.15
Butanoic acid, 3 methyl	6817.7	4928.25	7821.7	4091.3	26,031.7	3465.6
Hexanoic acid	3494.3	2094.93	3479.07	4079.5	5315.05	4191.97
Hexanoic acid, 2 ethyl	137.9		1114.6	1198.65		802.3
Butyric acid	7814.2				13,580.2	
Octanoic acid					1061.7	
Total free fatty acids	29,218.3	11,005.8	25,935.5	12736.6	75,498.05	12,777.3

*Source: Pappa et al. (2020)

Total free fatty acids increased from day 60 to day 180 in KS cheese, while in KG cheese they remained stable (Table 3). 3 methyl butanoic acid, 2 methyl butanoic acid and butanoic acid were among the most abundant volatiles found in KS cheese.

Soft Xinotyri cheese is an acid-curd goat’s milk cheese variety that is produced traditionally in Naxos Island of the Cycladic complex. Soft Xinotyri cheese is not shaped and is consumed as fresh or ripened and it is cold stored. Pappa *et al.* (2017) studied the lipolysis level of soft Xinotyri cheese that was made from raw or pasteurized milk without the addition of starter cultures, during storage at 4^0C for 60 days. The results showed no significant differences in individual as well as in total FFA content were found between raw and pasteurized milk soft Xinotyri cheese. This probably happens as the time of 60 days is short enough to arise important differences in lipolysis level between raw and pasteurized milk cheeses. Regardless the use of raw or pasteurized milk in soft Xinotyri cheese production, the most abundant free fatty acids determined were oleic (C18:1), palmitic (C16:0), myristic (C14:0), capric (C10:0) and caprylic (C8:0) acids (Table 1). Also, a low lipolysis level was found in both raw and pasteurized milk as well as in pasteurized milk cheeses (Table 2).
Xinotyri cheese is a hard short ripened and acid-curd farmhouse cheese that is made in the mountains of Naxos Island in the Cyclades complex from raw goat’s milk from indigenous breeds. Often goat’s milk is mixed with a small amount (5%) of cow’s milk. No starter cultures are added. However, sometimes a small amount of cheese whey from the previous day is used to avoid a long coagulation time (24 h). Free fatty acids content was found to increase significantly during Xinotyri cheese ripening (Table 2). Oleic, palmitic, myristic, stearic and capric acid were the most abundant free fatty acids determined in Xinotyri cheese (Table 1). Total free fatty acid content of 6-day Xinotyri cheese differ significantly of that of 45 days cheese and of cheeses of the following days (Table 2). Xinotyri cheese showed an intense lipolysis with total FFA content at the end of ripening to be 20,024 $mgkg^{-1}$ (Bontinis et al., 2012). A 15% percent of the total free fatty acids were short and medium chain acids (C4:0 to C12:0).

Table 2. Free fatty acids (mg kg^{-1}) of hard Xinotyri cheese, fresh soft Xinotyri cheese and Kashkaval cheese during ripening and storage.

Cheese type	Ripening time	Treatment	C2:0	C4:0	C4ISO	C5ISO	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Total	Reference
Hard Xinotyri cheese	6	1		71.0			75.4	90.7	113.3	83.0	161.7	363.0		219.8	676.5	138.8	114.0	2107.2	Bontinis et al. (2012)
	22	1		140.0			209.4	363.1	688.8	405.0	1220.2	2547.7		1261.6	4157.3	601.0	368.7	11962.9	
	45	1		145.5			268.3	504.3	873.2	492.0	1516.9	3992.0		1464.7	5144.2	714.0	452.7	15567.8	
	90	1		172.4			268.7	494.3	886.1	512.6	1609.0	4621.1		1499.8	5690.0	817.0	483.0	483.0	
	180	1		286.3			398.3	763.7	1228.6	643.0	1854.8	4357.7		1591.0	7256.0	1088.3	588.0	588.0	
Fresh soft acid-curd Xinotyri goat’s cheese	1	1	111.6				87.0	99.6	98.2	123.1	185.6	556.7		251.8	641.7	181.4	92.3	2429.1	
	1	2	102.7				94.9	85.7	87.2	70.6	118.3	201.5		143.1	312.0	125.7	77.6	1419.3	Pappa et al. (2017)
	15	1	119.2				91.7	102.3	107.5	109.3	182.1	561.9		238.1	623.3	163.6	87.6	2386.5	
	15	2	72.9				92.7	79.7	84.1	70.6	110.9	207.3		142.8	288.7	137.0	76.7	1363.4	
	30	1	74.7				107.7	196.6	186.8	152.8	220.8	668.2		266.0	1000.4	272.1	130.2	3276.3	
	30	2	62.1				94.4	86.8	90.2	72.9	116.7	229.6		144.9	437.6	163.7	79.2	1578.3	
	60	1	54.6				134.1	300.4	290.4	228.9	298.5	888.1		349.8	2559.6	417.0	175.0	5696.4	
	60	2	67.9				83.1	133.3	135.0	137.4	156.2	366.29		195.7	487.8	229.2	83.7	2075.7	
Kashkaval cheese	1	1	269.7	5.7	4.7	7.1	27.2	2.5	10.4	27.5	33.7	102.1	11.0	53.7	56.5	2.3		615.1	Pappa et al. (2019)
	1	2	142.7	3.9	1.8	4.4	27.7	1.9	7.3	17.5	14.9	80.8	5.4	31.2	42.4	0.5		351.9	
	30	1	561.3	28.8	8.0	14.5	30.6	6.4	24.2	28.2	122.9	276.9	13.4	99.4	175.1	8.2		1387.8	
	30	2	192.6	12.0	2.9	7.0	31.7	2.8	15.5	25.8	47.1	125.6	7.4	54.3	76.6	3.2		604.4	
	60	1	779.5	49.8	18.5	17.2	29.4	7.7	46.3	29.4	126.9	284.2	13.8	100.9	195.5	12.0		1707.0	
	60	2	188.0	26.5	10.7	8.4	29.6	3.5	20.1	34.7	65.2	163.2	10.3	54.0	113.6	4.6		650.0	
	90	1	856.9	55.8	50.1	18.0	31.7	11.2	44.0	52.9	187.5	397.9	20.2	138.9	280.6	15.1		2160.6	
	90	2	292.9	47.9	26.6	13.6	26.3	4.2	20.2	32.1	66.2	148.6	9.8	58.1	106.5	7.0		586.2	
	180	1	967.9	234.4	61.5	56.0	31.5	16.7	67.6	62.3	222.9	444.7	26.4	136.1	335.4	17.4		2680.5	
	180	2	330.7	98.4	33.5	20.1	30.7	4.7	31.5	30.3	89.1	201.7	12.5	74.3	147.1	8.4		887.0	

1: Cheese made from raw milk, 2: Cheese made from pasteurized milk

Kefalotyri is a traditional hard Greek cheese that is made from ewe’s or goat’s milk or mixtures of them. Sometimes cow’s milk can be used. Kefalotyri cheese is produced in various parts of Greece with technologies that vary from place to place. A very popular Kefalotyri cheese is the artisanal mountainous Kefalotyri cheese that is still traditionally manufactured in the mountains of Pindos, Greece. This artisanal cheese is made by using raw ewe’s milk without the addition of starter cultures, during summer. Pappa et al. (2021) studied the biochemical characteristics of mountainous Kefalotyri cheese during ripening and storage. Free fatty acids were the most abundant group of volatiles found. However, butyric acid and 3 methyl butanoic acid were the most abundant free fatty acids determined (Table 3).

Table 3. Free fatty acids (peak area x 10^3) in mountainous Kefalotyri cheese and Tsalafouti cheese during ripening.

		Ripening days		Reference
Cheese type	Free fatty acids	Day-90	Day-180	Pappa et al. (2021)
Mountainous Kefalotyri cheese	Acetic acid	225.5	270.2	
	Butyric acid	2238.3	1629.6	
	Butanoic acid 2 methyl	251.3	201.3	
	Butanoic acid 3 methyl	595.5	744.8	
	Hexanoic acid	262.9	247.5	
	Total free fatty acids	3573.7	3093.5	
		Ripening days		Reference
Cheese type	Free fatty acids	Day-30		Pappa et al. (2022)
Tsalafouti cheese	Hexanoic acid	2837.07		
	Octanoic acid	1960.85		
	Total free fatty acids	4797.92		
Tsalafouti cheese	Free fatty acids	Day-30		Pappa et al. (2022)
	Butanoic acid-2ethyl	435.2		
	Hexanoic acid	813.93		
	Octanoic acid	3833.5		
	Free fatty acids	Day-30		
Tsalafouti cheese	Acetic acid	1173.4		Pappa et al. (2023)
	Hexanoic acid	4287.68		
	Octanoic acid	2378.13		
	Total free fatty acids	7839.21		

Tsalafouti is a traditional Greek cheese that resembles to fresh acid and acid/rennet curd cheeses. It has a spreadable texture with no gas openings. Due to its high moisture level, Tsalafouti cheese has a relative low-fat content that makes it a healthy alternative for consumers that are on diet. Due to its pleasant organoleptic characteristics which are much appreciated by the consumers there is an increasing trend for making Tsalafouti cheese during the last years. Artisanal Tsalafouti cheese is traditionally made in the mountains from ewe’s milk at the end of the lactation period. The milk is ‘boiled’ (90^0C) and then is left to acidify. Milk ripens naturally by the action of diverse indigenous lactic acid bacteria (LAB), mainly mesophilic LAB which grow slowly at approximately 10^0C for 15-20 days. However, Pappa et al. (2023) made a Tsalafouti cheese with a semi-industrial way in to standardize its making technology by using mesophilic commercial starters that contain a mixture of *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* strains. Free fatty acids were not found in high amounts. However, hexonoic acid (C6:0) was the most abundant free fatty acid determined (Table 3).

Conclusions

This review describes the lipolysis level of some Greek artisanal cheeses. Raw milk Kashkaval of Pindos cheeses showed higher lipolysis levels than the pasteurized ones. In general no differences in lipolysis were observed between sheep milk and goats'(10%) Kashkaval cheeses. Lipolysis of soft Xinotyri cheese was not affected by using raw or pasteurized goat's milk. An intense lipolysis was observed in hard Xinotyri cheese. FFAs were the main volatiles of mountainous Kefalotyri cheese. FFAs of Tsalafouti cheese were not found in high amounts.

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INFLUENCE OF FEED FORM ON YELLOW MEALWORM ADULT PRODUCTIVITY: PRELIMINARY RESULTS

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Abstract

Insect farming is an opportunity for production of protein meals, pet food, novel food and oils (for feed and bioenergy). Industrial farms are now present in many countries and highlight the need for greater sustainability, safety for operators and feed efficiency. Like livestock sector, the pellet form of feed could also contribute to these needs in the breeding of Yellow Mealworm (*Tenebrio molitor* L.). In fact, the pellet has greater density, allowing for savings in transport costs; helps provide balanced and more efficient diets; reduces the dustiness, improving the healthiness of the working environment. Commonly, the production of *T. molitor* begins with the oviposition of adults directly in boxes with feed for the new larvae. This work evaluated the effect of the form of the feed on the productivity of adults. Egg-laying adults were used to compare bran of size <0.5 mm (A), size 0.5-2 mm (B), bran assembled into pellets (C) and into “cookie” (D). After one month of oviposition, results showed adult survival between 91% and 96%, without significant differences between forms. The number of larvae produced/female was significantly greater on A (65.7 larvae) and B (65.3 larvae) compared to the assembled forms C (33.9 larvae) and D (44.8 larvae). Among the latter, the number of larvae was significantly greater on bran assembled into cookie (D) compared to the pelleted form (C). In conclusion, the first results show lower productivity of adults on assembled feed (pellets and cookies), the causes of which (greater cannibalism or mortality of newborn larvae) should be investigated to obtain improvements. Furthermore, observations on growing larvae would complete the knowledge for the entire breeding cycle.

Keywords:*Rearing insect, Tenebrio molitor, Diet, Bran, Pellet.*

Introduction

Insect farming is a circular economy opportunity (Cadinu*et al*., 2020) for the production of feed (Gasco *et al*., 2023), novel food (Baek *et al*., 2017), and also oils for energy purposes (Manzano-Agugliaro *et al*., 2012). In this context, Yellow Mealworm larvae function as decomposers, exhibiting an impressive ability to transform organic inputs, such as by-products and former foods, into high-value proteins, fats, and chitin (Luciano *et al*., 2022; Lienhard *et al*., 2023). Furthermore, their rearing by-product (frass) is a recognized nutrient-rich fertilizer (Poveda *et al*., 2019). The breeding of Yellow Mealworm (*Tenebrio molitor* L.) on a large scale highlights the need for improved healthiness of the working environment and greater efficiency in the production cycle. However, mealworms are generally reared on bran or ground cereals, leading to workplace dustiness. Nebbia *et al*., (2019) have demonstrated a significant association between exposure to flour dust and the development of asthma-related respiratory allergies among workers involved in the breeding of *Tenebrio molito*r. As a potential solution to these health risks, using pelleted feed could reduce the dustiness and transport costs of bran. The pelleted form has been studied in other animal husbandry

(Abdollahi *et al*., 2013; Bonfante *et al*., 2016) but its use in *T. molitor* breeding is poorly documented. Good results have been obtained on growing mealworms fed on pelleted rabbit feed, with larvae’s growth, and nutritional profile remaining unaffected due to the change of feed form (Brai*et al*., 2023). Moreover, diets assembled in pellets or cookies have already been used in studies of the efficacy of insecticides and mycoinsecticides against Tenebrionidae (Ahmad *et al*., 2016, Rice *et al*., 2019). Mixtures of by-products in assembled form are often used in the evaluation of new diets for mealworms. This experimental methodology allows to reduce the self-selection of the food by the larvae (Morales-Ramos *et al*., 2020; Baldacchino *et al*., 2023). However, studies on the use of pelleted feed administered to egg-laying adults are lacking. This is particularly important since the production of mealworms begins with the oviposition of adults directly into boxes with feed for the new larvae. The aim of the work is to test the influence of the feed form given to adults in the phase of oviposition.

Materials and Methods

Feed in four different forms was tested on egg-laying adults of *Tenebrio molitor* L., at *insectarium* of CIHEAM-Bari (Apulia region – Italy) in 2023. Wheat bran pellets (CESAC s.c.a., Conselice, RA, Italy) were ground and sieved on 2 mm and 0.5 mm mesh sieves. Bran sizes <0.5 mm and 0.5-2 mm were used for feed forms A and B, respectively; unground pellets were used for feed form C. Finally, feed form D was obtained from the mixture of A and B (ratio 50:50 w/w), assembled into “cookies” according to the method described in a previous study (Baldacchino *et al*., 2023).
The insects were provided by the colony reared on bran and yeast (ratio 95:5 w/w), supplemented with pumpkin as a wet source. Pupae were sexed (Bhattacharya *et al*., 1970) under a stereoscope (mod. SMZ745T, Nikon Europe B.V.) and stored separately for sex until the beetles’ eclosion. Groups of five males and five females (per replicate) were placed in plastic cups measuring 13 x 7 cm (h x Ø) and containing the specific feed form to be tested. The trial included ten replications/feed form in a completely randomized design. Every ten days for a month, the living adults were counted and transferred to cup with new feed. Adult productivity was verified on the 30th day, by counting the number of live larvae/replicate. The data were subjected to homogeneity and normality test. The Kruskal-Wallis test and pairwise multiple comparisons with Bonferroni correction were applied to number of live females. The repeated measures ANOVA and Tukey-Kramer HDS test post-hoc were applied to number larvae/female.

Results and discussion

Adult mortality was negligible in all feed forms tested. In fact, after one month of oviposition, the percentage of live adults was between 91% and 96%, and not significantly different between feed forms (H=2.479; df=3; p=0.479). These values are in accordance with what has been recorded in previous studies of bran (Baldacchino *et al*., 2024) and ensure the stock of egg-laying females in the period of maximum fertility (Morales-Ramos *et al*., 2012).
The productivity of the adults (calculated as larvae produced/female) was similar between feed forms in the first 10 days of oviposition. Subsequently, the number of larvae was constant in the sieved feed (A and B) while it decreased in both feeds assembled in pellets and cookies (C and D) (Figure 1). However, the results derive from observations limited to the first month of oviposition; the trend of subsequent oviposition could provide more complete data. Nevertheless, these preliminary results are already transferable, as some breeders use egg-laying adults for a month or slightly more.

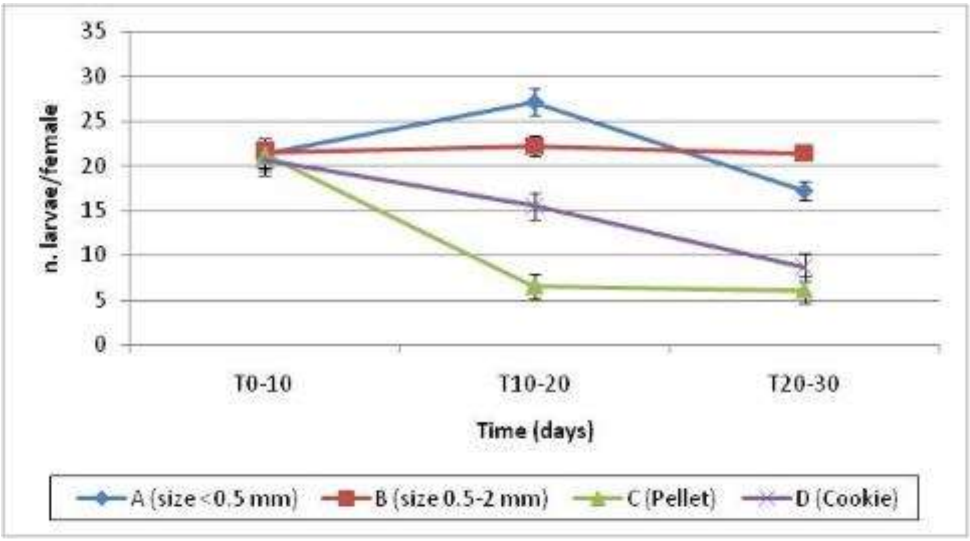

Figure 1. Production of larvae/female on different feed forms. Bran sizes <0.5 mm (A); bran sizes 0.5-2 mm (B); bran assembled in pellets (C); bran assembled in “cookies” (D). The mean values ± SE (n= 10) represent the number of larvae produced by female every 10 days.

The total productivity at the end of the month confirmed the differences between feed forms recorded during the oviposition period (F=15.51; df=3,36; p<0.001). Indeed, feed form A and B provided significantly more larvae (65.7 and 65.3 larvae, respectively) than assembled feed forms C and D (33.9 and 44.8 larvae, respectively) (Figure 2).

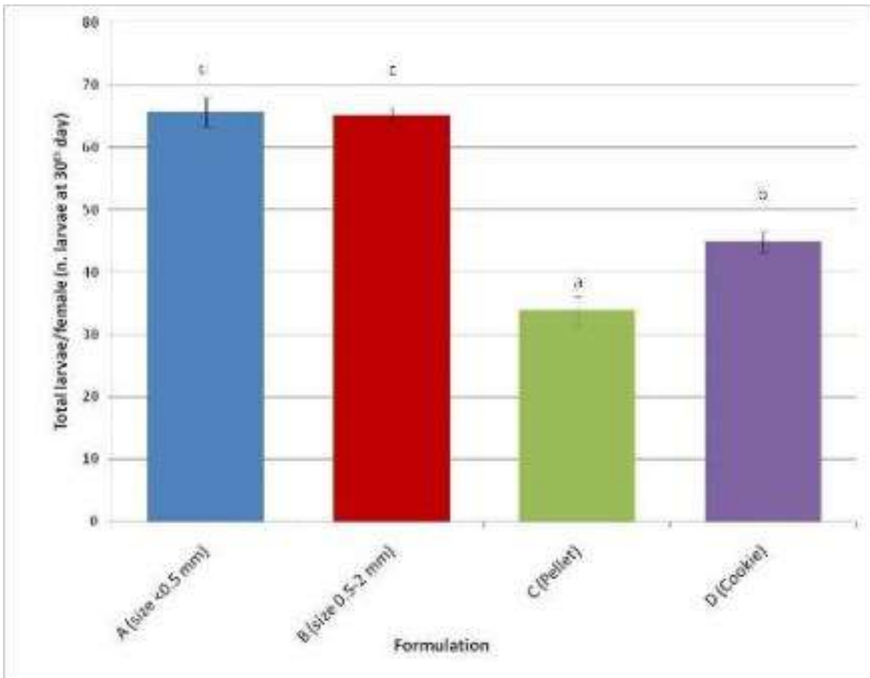

Figure 2. Total production of larvae/female on different feed forms. Bran sizes <0.5 mm (A); bran sizes 0.5-2 mm (B); bran assembled in pellets (C); bran assembled in “cookies” (D). The mean values ± SE (n= 10) represent the total number of larvae produced by female at 30th day.

However, the number of larvae produced was significantly greater on the cookie-assembled bran (D) compared to the pelletized form (C). Thus, in our study, the assembled diets negatively influenced the productivity of the adults, with 51.8% (C) and 68.4% (D) of larvae

produced compared to ground bran. Furthermore, the significant difference between pellet bran and cookie bran highlights the possible influence of other factors (hardness, shape, hygroscopicity, etc.).
Adult productivity estimated through the number of larvae in the diet (according to Berggreen *et al*., 2018) provides more realistic results for breeders of *Tenebrio molito*r. However, adults may cannibalise eggs (Deruytter*et al*., 2019; Frooninckx*et al*., 2022) and the feed form may also have affected egg survival. Newborn larvae may have had difficulty feeding, considering that the particle size of some feeds negatively affects larval growth (Naser El Deen *et al*., 2022). Recently, pelleted feed has been used to feed growing larvae with good results (Brai*et al*., 2023; Hwang *et al*, 2023), nevertheless, experiences on newborn larvae are lacking. All these aspects should be investigated for the best use of pelleted diets from the oviposition stage of *T. molitor*.

Conclusion

In this study, preliminary results showed that pelleted bran is less suitable than powdered bran (size <0.5 mm and 0.5-2 mm) and cookie-assembled bran for the oviposition of *Tenebrio molitor*. This limits the advantages of using pelleted feed (lower dustiness and lower transport costs) in a complete rearing cycle of yellow mealworm. However, further studies are needed to understand the effects on the development of newborn larvae to make the use of pelleted feed more efficient.

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NATURAL ENEMIES OF THE FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) IN JORDAN AND PALESTINE

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Abstract

The fall army worm (FAW) has recently invaded many countries in Africa and Asia causing serious damage to many crops, especially in corn fields. The search for natural enemies of this pest was conducted in infested corn fields in the Jordan Valley and eastern desert of Jordan, Gaza, and the West Bank. The egg-larval parasitoid, *Chelonus inanitus* (L., 1767) (Brachonidae: Hymenoptera) was found in three areas in Jordan. The general predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae) was collected from the different corn fields in Jordan and Palestine. The species *Lapidura riparia* (Pallas, 1773) (Dermaptera: Labiduridae) was found in Jordan. The FAW egg parasitoid, *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae), the coccinellid predator, *Hippodamia tredecimpunctata* (Linnaeus, 1758), and a predatory bug, *Anthocoris* sp., were found in Palestine. Other undetermined insect species associated with the fall army worm belonging to several families of Hymenoptera and Diptera were also collected. All specimens were deposited in the University of Jordan Insect Museum.

Key words: *Spodoptera frugiperda, Natural enemies, Jordan, Palestine.*

Introduction

The fall army worm (FAW) *Spodoptera frugiperda* has achieved the status of a cosmopolitan pest, also in many countries it is a quarantine pest. Therefore, the search for efficient biological-control agents for this pest would have a global impact, particularly because of its resistance to insecticides and Bt plants (Wengrat et al. (2021). A total of 353 FAW larval host plants belonging to 76 plant families, mainly Poaceae (106), Asteraceae (31) and Fabaceae (31) are known so far (Montezano, 2018). Many studies on its hosts and natural enemies in different parts of the world were conducted. Molina-Ochoa et al. (2003) conducted an inventory for the parasitoids and parasites of fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) in the native distributional areas (North, Central and South America and the Caribbean Basin). Tepa-Yotto, et al. (2021) reported that in the Americas, about 150 parasitoids were found to be associated with FAW. Computer modeling to predict the habitat suitability of fall armyworm FAW and its major parasitoids (*Chelonus insularis*, *Cotesia marginiventris*, *Eiphosoma laphygmae*, *Telenomus remus* and *Trichogramma pretiosum*) under current and future climate scenarios were utilized. This provided important data needed for decision making in worldwide biological control programs and long-term management of FAW (Tepa-Yotto et al., 2021).
Tendeng et al. (2019) recorded *Chelonus* sp. and *Campoletis* sp. (Hymenoptera) and Nematode *Hexamermis* sp. from Senegal. Abang et. al. 2021 conducted field surveys and laboratory studies on parasitoids associated with FAW and Southern army worm (SAW) (*Spodoptera eridania*) in Cameroon. Sisay *et al.*, (2019) surveyed the distribution of FAW and its natural enemies in Ethiopia, Kenya, and Tanzania in 2017 and 2018. Edmardash et al. (2011) listed 16 species of Cheloninae (Braconidae) from Egypt and provided a key for

species identification. *Orius albidipennis* and *Orius laevigatus* were collected from maize fields in Egypt (Karaman 2009). From India, Shylesha et al. (2018) recorded two egg parasitoids, three larval parasitoids, one predator and one entomopathogenic fungus.

Lists of parasitoids from Palestine were presented by Bodenheimer (1930 and 1937). Kugler (1966) listed 81 parasites from 56 Lepidoptera hosts. *Chelonus inanitus* (L.) was a common parasitoid of *Spodoptera littoralis* (Boisduval) in the Middle East, mostly in alfalfa fields (Gerling 1969). Hamdan and Abu-Awad (2007) studied the effect of host plants on the predatory bug, *O. laevigatus* in Palestine. Rechav and Orion (1975) studied the development of *Chelonus inanitus* immature stages. This species was released in a cotton field to study its ability to control *Spodoptera littoralis* (Boisduval) (Rechav, 1976) but it was found to be not effective. Rechav (1978) studied its oviposition, host preferences and sex ratio. In their study on the Egyptian Cheloninae Edmardash et al. (2011) listed *Chelonus inanitus* (L), 1767. *Chelonus (Microchelonus) basalis* Curtis, 1837 *Chelonus (Microchelonus) sulcatus* Jurine, 1807 *Phanerotoma (Phanerotoma) dentata* (Panzer, 1805) *Phanerotoma (Phanerotoma) leucobasis* Kriechbaumer, 1894 from Palestine. Najajrah, et al. (2019) listed 35 species of lady beetle family (Coleoptera: Coccinellidae) with data on their geographic distribution and ecological significance.

The FAW was first observed in August 2020 in Jordan (Katbeh-Bader et al., 2020) and because its introduction was recent no studies on its the natural enemies were conducted. However, studies on natural enemies on Lepidopteran pests or other pests can be found in earlier literature. Katbeh-Bader et al. (2000) listed the Heteroptera of Jordan based on the specimens preserved in the University of Jordan Insects Museum. Some potential natural enemies of the FAW may be found in this list such as *Orius niger* Wolff, 1811 (Anthocoridae), four species of the genus *Geocoris* (Lygaeidae) and 17 species of Reduviidae. Three hemipterans were collected from *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) heavily infested tomato plants in the Jordan Valley (*Orius albidipennis*, *Orius* sp. (Anthocoridae) and *Nesidiocoris tenuis* (Miridae). In addition, the parasitic wasp *Bracon* (*Habrobracon*) *concolorans*, (Hymenoptera: Brachonidae) was recorded for the first time from Jordan (Al-Jboory et al., 2012). A total of 66 parasitoid species in 11 families from the superfamily Chalcidoidea were recorded previously from Jordan (Noyes, J.S. 2003: Universal Chalcidoidea Database). Some potential natural enemies of the FAW may be found in these species.

The objectives of this paper are to record the natural enemies of the FAW found in our study in Jordan and Palestine (West Bank and Gaza).

Materials and Methods

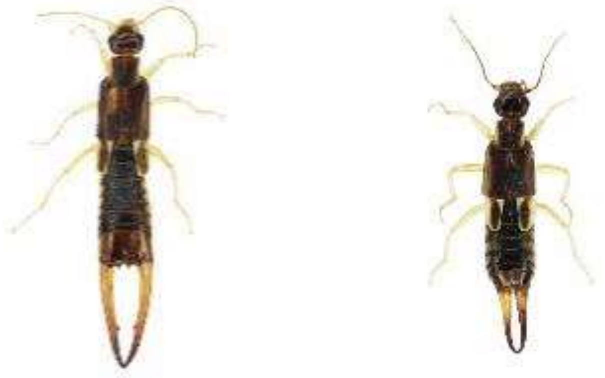
Several field trips were conducted to corn fields in the Jordan Valley, Eastern Desert of Jordan, Gaza and Jericho (West Bank) during September to December 2021 to inspect for the infestation with FAW and the presence of natural enemies of the pest. Egg masses, larvae, and pupae of the FAW were collected from infested corn plants and adults were collected from pheromone traps. Plant host, location (coordinates), date, collector name, and notes regarding any relevant ecological data were recorded. Male-genitalia were mounted on slides with glycerin for positive identification of the FAW, since the wing scales were usually removed from adult moths collected by pheromone traps. Collected larvae were reared on corn leaves to detect emergence of natural enemies. Edmardash et al. (2011) identification key was used to identify *Chelonus inanitus*. Collected insect samples were sorted into putative species and preserved in 75% ethanol. All specimens were kept in the University of Jordan Insects Museum.

Results and Discussion

A promising species, *Chelonus inanitus* (L., 1767) (Brachonidae: Hymenoptera) was found in three areas, one corn field in Dayr Alla in Jordan Valley and two corn fields in Al Hallabat in Eastern Desert (Figure 1A). *Chelonus inanitus* is a well-known natural enemy of the FAW. It was seen in large numbers in heavily infested, unsprayed corn field in the Jordan Valley. *Chrysoperla carnea* (Figure 1B) was found in the different corn fields. Its larvae are natural enemies of many insect pests like whiteflies, aphids, mealybugs, scale insects, psyllids, leafhopper eggs, thrips, beetle larvae, leaf miners and other caterpillars. *Lapidura riparia* (Pallas, 1773) (Dermaptera: Labiduridae) (Figure 1D) is another common natural enemy of many pests. It is a cosmopolitan species found mainly in tropical to subtropical regions.



A B C




D E

Figure 1. A. Chelonus inanitus. B. Chrysoperla carnea C. Telenomus remus D. Lapidura riparia E. Hippodamia tredecimpunctata.

Important general predators were recorded previously in Jordan (Katbeh-Bader et al. 2000) should be taken in consideration in conservation control since all of them are expected to be found in corn field in both Jordan and Palestine. *Geocoris* (s. str.) *megacephalus* (Rossi, 1790) (Hemiptera: Lygaeidae) has already been observed in Lebanon feeding on early instars of the FAW (Zinatt, personal communication). *Orius niger* Wolff, 1811 (Hemiptera: Anthocoridae), was also observed in corn fields in Jordan. *Geocoris* (S. str.) *ater* (Fabricius, 1780), *Geocoris* (s. str.) *phaeopterus* (Germar, 1837), *Geocoris* (*Eilatus*) *chloroticus* Puton, 1888 (Hemiptera: Lygaeidae) may eventually be collected in Jordan. The diverse parasitoid species from the superfamily Chalcidoidea (Noyes, J.S. 2003: Universal Chalcidoidea Database) should also be taken into consideration in conservation control programs.

The FAW egg parasitoid, *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae), was found (Figure 1C). And 3 species of general predators, *Chrysoperla carnea*, *Hippodamia tredecimpunctata* (Linnaeus, 1758) (Figure 1E), and *Anthocoris* sp. were found. The relatively small number of parasitoids found in this study in Palestine is mainly due to the use of insecticides in the surveyed fields Such fields were treated with insecticides such as Lufenuron, Emamectin benzoate, Chlorfluazuron, and Diesel.

The selection of a candidate parasitoid species for a biological control program should be based on pest risk clearance, its reproductive performance, its functional and numerical response profile, high behavioral host selection, resilience at low host population densities and its dispersal ability (Tepa-Yotto, et al., 2021).

Magaña et al. (2022) stated that *C. inanitus* may be an interesting parasitoid of *Spodoptera* species. They mass reared this parasitoid on *E. kuehniella* mature eggs which resulted in increased the female percentage in the offspring and shortened the parasitoid life cycle. In addition, a high number of females parasitizing simultaneously reduced the emergence of non-parasitized hosts. However, information on the performance of *C. inanitus* under field conditions is rare indicating that it could be highly sensitive to broad-spectrum pesticides (Rechav, 1976). Its release in a cotton field to control *S. littoralis* was not very successful because it was affected by the application of organophosphates used to control *S. littoralis.*

Chelonus inanitus has 3 larval instars. The parasitoid female lays its eggs in the host egg under the outer shell of the egg but outside the host egg cell. Later the 1st instar parasitoid penetrates to the yolk and enters the haemolymph of the embryo before the host hatches. The 3 instars of *C. inanitus* float on the host haemolymph between the Malpighian tubules.

Wengrat (2021) recorded *Telenomus remus* on *Spodoptera* spp. in Brazil for the first time and studied its phylogeography. They suggested that the *T. remus* strain distributed by anthropogenic action into the Americas, Africa, and Asia is able to adapt to different agricultural landscapes. Therefore, reintroductions of natural populations of *T. remus* from different geographical origins may be an efficient tactic for classical and augmentative biological control of *S. frugiperda* in different parts of the world. Accurate identification of *T. remus* and its genetic strains is essential for the success of biological control programs using this parasitoid. Liao et. al. (2019) reported *Telenomus remus* for the first time parasitizing *Spodoptera frugiperda* in southern China. Kenis et al., 2019 considered *Telenomus remus* as a candidate parasitoid for the biological control of FAW in Africa.

Conclusion

Two important natural enemies of the FAW are recorded in this study. The egg-larval parasitoid, *Chelonus inanitus*, is recorded from Jordan for the first time, and the egg parasitoid, *Telenomus remus* which was previously introduced into Palestine was also found in our study in Palestine. Other nonspecific natural enemies such as aphid lions, earwigs, lady beetles and predatory bugs were also found. This qualitative study should be considered as a preliminary one taking in consideration the time and season limitations. Therefore, additional natural enemy species are expected to be found if more extensive collecting is conducted during most seasons of the year in a wider geographical range in both Jordan and Palestine.

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EVALUATION OF *PSEUDOMONAS* FOR THE MANAGEMENT OF TOMATO DISEASE AND PESTS

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Abstract

The biopesticides sector is currently experiencing significant growth, with numerous discoveries being made in the development of new products based on microorganisms and plant extracts, which are fuelling a growing global market. Currently, around 75% of all biopesticides in use are *Bacillus*-based products and their toxins. The objective of this study was to elucidate the effects of a *Pseudomonas* isolate as a biopesticide against tomato crop diseases and pests, and the modes of action involved in antagonism. *Pseudomonas* isolates were isolated from rhizospheric tomato soils at Melk zhar (Agadir, Morocco). These isolates were tested against the fungus *Botrytis cinerea*, the mite *Tetranychus urticae* and the insect *Bemisia tabaci*. The results demonstrated that the isolates exhibited strong inhibitory effects against *B. cinerea*, with inhibition levels reaching up to 60%. The pest test results indicated that three isolates, Q110B, Q036B, and Q172B, exhibited significant mortality against *T. urticae*, and *B. tabaci*. Isolate Q036B exhibited a higher mortality rate than Q172B and Q110B. The results of this study indicate that selected Pseudomonas strains have good efficacy against tomato diseases and pests. They could potentially serve as an alternative biopesticide to chemicals for use in integrated pest management programs in agro-ecosystems.

Keywords: Tomato, *Pseudomoans, Botrytis cinerea, Tetranychus urticae, Bemisia tabaci*.

Introduction

Tomatoes are one of the most cultivated crops in Mediterranean greenhouses (Abou Hadid, 2013). In Morocco, greenhouse tomatoes cover approximately 16,000 hectares, producing around 1.5 million tons per year. They are considered an essential crop grown in the Souss Valley (Ait Hou et al., 2015). However, tomato productivity faces significant constraints and risks from increased pathogenic agents and pests, making it imperative for farmers to reduce the damage caused by these threats. Chemical pesticides have long been used to combat pests and diseases affecting tomato crops. However, these products can have negative impacts on the environment and human health. Additionally, the emergence of pathogen resistance is exacerbated by the use of chemical fungicides (Chung et al., 2009). For instance, Elad et al. (1992) reported that B. cinerea developed rapid resistance to specific fungicides, including benzimidazoles, dicarboximides, and sterol biosynthesis inhibitors. Similarly, Halime et al. (2019) reported a high level of resistance of B. cinerea to Fenhexamid in Moroccan tomato greenhouses. Moreover, the use of chemical products causes soil pollution and poses health risks to humans (Martínez-Romero et al., 2008). To find a safer fungicidal control, alternative strategies have been considered. Among these, biopesticides have emerged as a promising alternative. Biopesticides are biological control agents derived from natural organisms such as bacteria, fungi, or plants. Non-phytopathogenic bacteria from rhizospheric, endophytic, or halophilic environments have frequently been reported to protect plants against phytopathogens (Van Loon et al., 1998; Magnin-Robert et al., 2007; Compant et al., 2010; Verhagen et al., 2011). Bacterial genera such as Bacillus, Pseudomonas, and Enterobacter, along with fungi belonging to the Pythium and Trichoderma genera, and actinomycetes, have shown significant biocontrol potential (Lange et al., 1993; Chernin et al., 1995; Amkraz et al., 2010; Gao et al., 2018). Certain strains of Pseudomonas have demonstrated the ability to inhibit the growth of numerous plant pathogens while being relatively harmless to the environment (Qessaoui et al., 2020; 2021). Their modes of action vary, ranging from competition for nutrients to the production of insecticidal, antifungal, or antibacterial metabolites. Therefore, the objective of this study is to test the effect of Pseudomonas strains against diseases and pests of greenhouse tomatoes.

Materials and method

Pseudomonas isolates

The three Pseudomonas isolates (Q172B, Q110B, and Q036B) were obtained from an experimental farm of the INRA in Agadir, southwestern Morocco. These bacteria were characterized in a previous study as *Pseudomonas* sp. (Qessaoui et al., 2019).

In vitro selection of antagonistic Pseudomonas against B. cinerea

In vitro evaluation of *Pseudomonas* isolates was carried out using dual culture technique on PDA (Kaur, 2003). A heavy inoculum was applied as a band of 1.5 cm length equidistantly on three opposite edges of the agar medium in Petri plates using an inoculation loop. A mycelial disc of 5 mm diameter from a 7 day-old culture of B. cinerea was placed at the center of the Petri plate. Three replications were made for rhizobacteria. Plates containing the pathogen alone served as control. Plates were incubated for five days at 25°C (Kaur et al., 2007). After incubation, the mycelial growth inhibition percentage (IP) was calculated using the following expression.

$$IP= R1-R2/R1*100$$

Where R1 is radial growth of the fungus in the control and R2 is radial growth of the fungus in the treated plates . The bacterial isolates showing maximum zones of inhibition were selected for further studies.

Effects of *Pseudomonas* isolates on *B. tabaci adult*

Tomato leaves were collected, thoroughly washed with tap water, rewashed with sterile distilled water and then dipped for 1 min in each bacterial suspension (10^8 cfu/mL) for each isolate. Control tomato leaves were dipped in only distilled water for 1 min. The inoculated leaves were dried and introduced individually in sterile tubes. Fifteen adult whiteflies were then introduced in tubes containing the treated tomato leaves. The tubes were sealed by muslin tissue to allow ventilation. Three replicates were evaluated for each treatment, and the bioassay was replicated thrice. The tubes were incubated at T° = 25±2°C, RH = 70±5% and adult mortality was calculated 72h after treatment application.

Acaricidal activity

Three Pseudmonas bacterial strains Q110B, Q036B and Q172B were assessed for their ability to cause mortality to homogeneous age adults of *T. urticae* on tomato (Solanum lycopersicon) leaves. Fresh leaves of tomato were collected from unsprayed plants growing in a greenhouse (INRA experimental farm, Agadir, Morocco). The leaves were first washed with tap water and rewashed with sterile distilled water, and then dried under a laminar flow hood. The effect of bacterial isolates on *T. urticae* adults was studied under laboratory conditions using a leaf dip bioassay (Bouharroud et al. 2007). A leaf cage was prepared from Petri dishes (9 cm) containing Whatman paper soaked in sterile distilled water. A 1.5 cm diam. hole was made in the lid of Petri dishes and covered with muslin. Tomato leaflets were dipped in strain concentration (108CFU/ml) for 20 s. The treated leaflets were dried under a laminar hood then transferred to leaf cages. The control leaflets were dipped only in sterile distilled water. Fifteen T. urticae adults (male to female sex ratio: 1–1.1) were then transferred to treated leaflets. Four replicates for each leaf cage were used. The cages were incubated at 24±2°C with a photoperiod of 16 : 8 h (L : D), and T. urticae mortality rate was assessed 72 h after treatment. The bioassay was replicated three times. Two-spotted mite mortality rates were corrected using Abbott’s formula (Abbott 1925):

$$CrrM = \frac{DMN - DMNC}{MTN - DMNC} * 100$$

where: CrrM – corrected mortality, DMN – dead mite number, DMNC – number of dead mites in control, MTN – total mite number.

Statistical analysis

The mycelial growth inhibition percentage (IP) was calculated for each Pseudomonas isolate. To determine the efficacy of *Pseudomonas* strains on T. urticae, and *B tabaci* mortality data were analyzed using Abbott’s formula for acaricide activity (Abbott 1925). The data were subjected to Analysis of Variance test (ANOVA) using Statistical software, STATISTICA (Ver. 6). Any difference mentioned is significant at P< 0.01 using the Newman–Keuls multiple range test.

Results and discussion

In vitro selection of *B. cinerea* antagonistic *Pseudomonas*

The results demonstrated that the three Pseudomonas strains tested exhibited significant antagonistic potential (IP > 65%) against B. cinerea (Table 1) compared to the control. This inhibition was evidenced by a reduction in the colony diameter of the mycelium compared to the control (Figure 1).

Acaricidal activity

All three Pseudomonas strains caused significant mortality in T. urticae adults 72 hours after treatment (Table 1). The symptoms induced by the Pseudomonas isolates in T. urticae included reduced movement and the appearance of brown-black coloration (Aksoy et al., 2008). The highest mortality rate was achieved by strain Q036B, which resulted in approximately 97% mortality (Table 1).

The efficacy of *Pseudomonas* isolates against *B. tabaci* adults

All three Pseudomonas isolates (Q110, Q036B, and Q172B) had an effect on the mortality of B. tabaci adults (Table 1). The most significant effect of Pseudomonas was observed with Q036B.

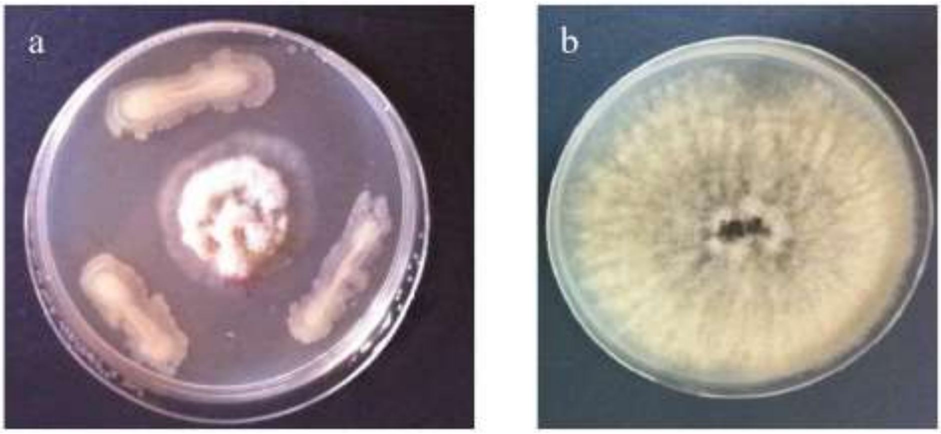

Figure 1. Inhibition of B. cinerea by Pseudomonas isolates in PDA medium. a: treated with Pseudomonas, b: control

Table 1. In vitro screening of bacterial isolates based on mycelial growth inhibition of B.cinerea.

Strain	IP % (Botrytis)	$CrrM$ % (T. urticea)	Mortality % (B. Tabaci)
Q036B	65,28±2,41[a]	97.6 ± 5.4 [a]	74% [a]
Q172B	66,86±5,64[a]	88.0 ± 14.4 [a]	63% [a]
Q110B	65,48±5,15[a]	84.4 ± 9.7 [a]	63%[a]

The principal findings of this research indicate that bio-inoculation with Pseudomonas isolated from rhizospheric soil effectively inhibits Botrytis cinerea development. These Pseudomonas strains show potential for plant protection against pests, causing significant mortality in B. tabaci under laboratory conditions and displaying acaricidal and repellent activities against Tetranychus urticae adults. Mechanistically, the isolates produce bioactive metabolites such as proteases, cellulases, chitinases, and siderophores (Qessaoui et al., 2021), which inhibit conidia germination, germ tube elongation, and mycelial growth of B. cinerea. Studies have demonstrated their secretion of antibiotics like lipopeptides, 2,4-diacetylphloroglucinol, and phenazine-1-carboxylic acid (PCA), reducing pathogenic infections directly (Lugtenberg and Kamilova, 2009; Mitter et al., 2013; Jaaffar et al., 2017; Paulin et al., 2017). Furthermore, siderophore production and volatile compounds (e.g., HCN) contribute to their biocontrol effectiveness against fungi (Ramette et al., 2003; Kumari and Khanna, 2014). These findings support the role of enzyme-producing bacteria in inhibiting phytopathogenic fungi (El-Tarabily, 2006), highlighting their potential as effective biofungicides and bioinsecticides. Additionally, Pseudomonas strains have been observed to induce mortality in Drosophila melanogaster due to their strong hemolytic activities, including lipases, chitinases, and hydrolases (Vodovar et al., 2006). This study represents a preliminary step towards future research supporting agroecological practices that balance industrial needs with ecological sustainability.

Conclusion

The findings of this study indicate the potential of Pseudomonas isolates as effective biopesticides for the management of tomato diseases and pests. The demonstrated inhibitory effects against B. cinerea and the significant mortality rates observed in T. urticae and B. tabaci underscore the promising role of these isolates in integrated pest management (IPM) programs. In particular, the higher efficacy of isolate Q036B suggests that it could be a valuable addition to the reduction of reliance on chemical pesticides. The results support further research and development of Pseudomonas-based biopesticides as sustainable alternatives for enhancing crop protection in agro-ecosystems.

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PHOSPHATE SOLUBILIZATION BY THE FLUORESCENTS *PSEUDOMONAS*

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Abstract

One of the most common strategies to increase agricultural production is to improve soil fertility. Phosphorus is the second macronutrient essential for the growth and development of plants. However, the solubility of this macronutrient is very low, and 75 to 90% of phosphate fertilizers are precipitated by iron. The use of rhizospheric microorganisms appears to be the most effective biological method for enhancing the solubilization of phosphate, as some bacteria are capable of degrading Ca3(PO4)2 to available phosphate (P_2O_5). Four isolates (R25, R36, R4, and R10) of fluorescents *Pseudomonas* were isolated from the rhizosphere soil of tomato plants in the Souss-Massa of Morocco. The strains were tested for their ability to solubilize phosphates on solid and liquid media of NBRIP. The phosphate solubilization was estimated by measuring the diameter of the clear halo on the solid media and by measuring the evolution of absorbance at 820 nm and pH during five days in the liquid media according to the Olsen method. The most significant clear halo was obtained by R25 (15.00 ± 5.94 mm). On liquid NBRIP media, R36 produced the greatest quantity of available phosphate (P_2O_5) 96 hours after inoculation (32.65 ± 0.01 µg/ml). This strain demonstrated the ability to acidify the media, resulting in a pH of 3.73 ± 0.16, in comparison to the control, which exhibited a pH of 6.18 ± 0.10. This phenomenon can be attributed to the production of organic acids by fluorescent Pseudomonas in the media. This finding has the potential to enhance the yield of tomato crops grown in soil with limited phosphate or available phosphate.

Keywords: *Pseudomonas, phosphate solubilization.*

Introduction

One of the most common strategies for increasing agricultural production is the improvement of soil fertility. Phosphorus is an essential macronutrient for the growth and development of plants, following nitrogen. However, it is considered to be one of the elements that limits plant growth. Chemical fertilizers represent the primary strategy for ensuring the availability of phosphorus in agricultural soils. However, it has been estimated that between 75 and 90% of phosphate fertilizers added are precipitated by iron, aluminum, and calcium complexes present in soils (Gyaneshwar et al., 2002; Turan et al., 2006). This accumulation of this form is a significant challenge in agriculture. The use of rhizospheric microorganisms represents the most effective biological approach to enhance the solubilization of phosphate in the soil and to provide sufficient quantities for plant nutrition (Pradhan and Sukla, 2005; Singh et al., 2011). The ability of certain microorganisms to convert the insoluble phosphate to an accessible form is an essential trait for plant growth-promoting rhizobacteria (PGPR) to enhance plant performance. Phosphate-solubilizing rhizospheric bacteria have been identified as a promising source for use as a biofertilizer in agriculture (Kucey et al., 1989). The population of microorganisms capable of solubilizing phosphates varies depending on the type of soil. These bacteria can be found in both fertile and deficient soils (Oehl et al., 2001). A significant proportion of them is located in the rhizosphere, which contains between five and twenty times more phosphate-solubilizing bacteria than non-rhizospheric soil (Bowen and Rovira, 1999). A multitude of microbial species are capable of solubilizing phosphates, with *Pseudomonas* and *Bacillus* exhibiting a notable proclivity for this element. These bacteria are predominantly found in soil and are adept at resisting adverse environmental conditions. The phosphate-solubilizing capacity of these bacteria is frequently evaluated on media containing calcium phosphate as the sole source of phosphates. A variety of media are recommended for this type of study, including Pikovskaya medium (Pikovskaya, 1948), the PVK bromophenol blue (BBP) and National Botanical Research Institute Phosphate (NBRIP) medium (Nautiyal, 1999). The mechanism of phosphate solubilization is initiated by the release of H+ ions, the production of organic acids and chelating substances. The production of organic acids represents the primary mechanism. Several studies have indicated a correlation between phosphate solubilization and a decrease in medium pH (Hinsinger, 2001). The purpose of this study was to assess the ability of four Pseudomonas isolates from tomato rhizospheric soil to solubilize phosphate.

Material and methods

Isolation of bacteria from tomato roots Isolation was performed as described by Qessaoui et al., (2019). Samples of rhizospheric soil were collected from tomato greenhouses of the experimental farm at the Regional Agricultural Research Center of Agadir in Morocco. Extract samples were diluted and dilutions were spread on King B medium and incubated at 26°C for 48h. Results were expressed as colony forming units per gram (CFU g-1) of rhizospheric soil Fluorescent colonies were purified by streaking and were stored at -80°C in 40% glycerol.

Phosphate solubilization

Phosphate solubilization by the isolated fluorescent *Pseudomonas* isolates was tested in solid media by the method described by Nautiyal1999. Ten microliters of the culture of each strain was spotted on the surface of the solid media in Petri dishes. The solubilization capacity was assessed by the transparent area formed around the colony. Ten days after incubation at 30 °C, the diameter of the solubilization halo (DSH) was determined by the following formula

DSH=THD−CD

where THD is the total halo diameter, and CD is the colony diameter.

Quantitative estimation of phosphate solubilization in broth was carried out in Erlenmeyer flasks (250 ml) containing 100 ml of NBRIP medium. The flasks were incubated at 28 °C for 5 days at 120 rpm. Then, 5 ml of each isolate culture was centrifuged at 3000 rpm for 20 min. Phosphate concentration in culture supernatant was estimated as described by Olsen and Sommers[71]. Three replicates were used, and a standard calibration curve was made with KH_2PO_4 solution (Sigma-Aldrich).

Results and discussion

Phosphate Solubilization on Solid Medium

The results showed that these four isolates formed a clear halo of solubilization on NBRIP medium. The most significant clear halo was observed for isolate R25 (15.00±5.94 mm), while the smallest halo was obtained with isolate R10 (7.75 mm) (Table 1). These results were compared with a bacterial control that showed no solubilization on solid medium. This halo of solubilization indicates the production of substances that solubilize phosphate; a larger halo suggests a greater production of these substances in the medium.

Table 1. Solubilisation halo diameter by P*seudomonas* isolate

	R10	R4	R36	RF25
DSH (mm)	7,75±2,06	10,50±0,58	12,00±3,16	15,00±5,94

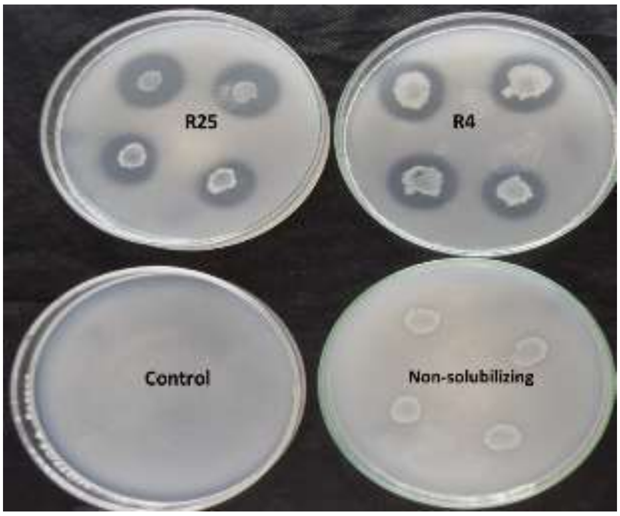

Figure1. Effect of *isolates* on the solubilization of phosphate (solid medium) after 8 days of incubation

Phosphate solubilization on liquid medium

To confirm the phosphate solubilization observed on solid medium, these isolates were tested for their ability to solubilize phosphate in liquid medium. The results showed that all four isolates effectively solubilized phosphate in liquid medium. The results showed that phosphate solubilization in liquid medium began 24 hours after inoculation, with the highest rate of solubilization observed 96 hours after treatment (Figure 2). This solubilization was indicated by a clarification of the medium's appearance compared to the control medium, which did not contain any bacteria (Figure 3). The analysis of variance showed significant efficacy for all four isolates in solubilizing phosphate. Isolate R36 produced the highest quantity of available phosphate (P_2O_5) 96 hours after inoculation (32.65±0.01 µg/ml). After 96 hours, a decrease in the concentration of soluble phosphate was observed for all isolates (Figure).

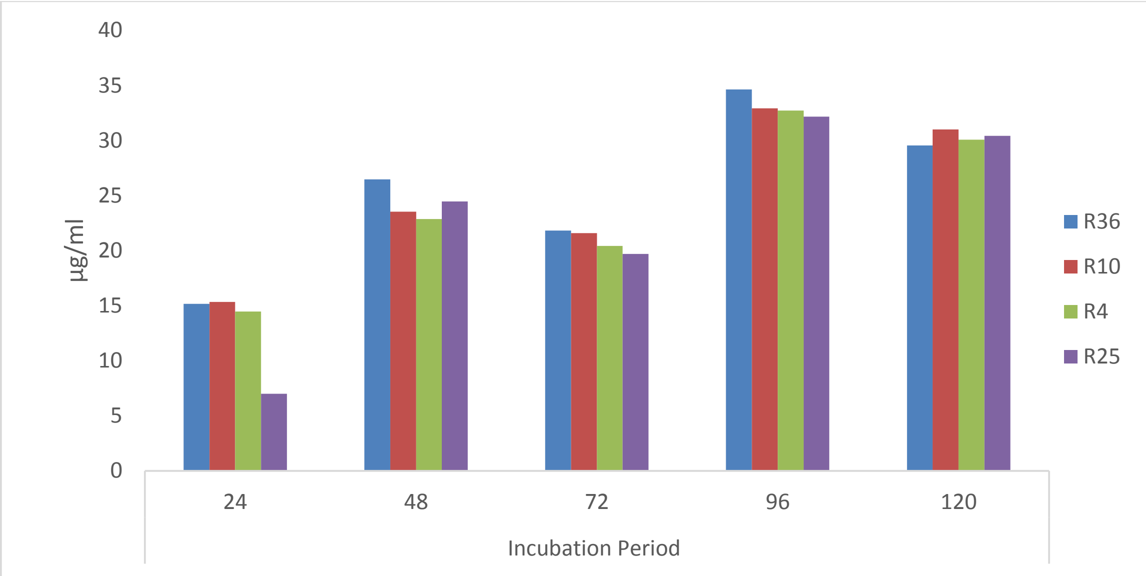

Figure 2. Evolution of Phosphate solubilization on liquid medium

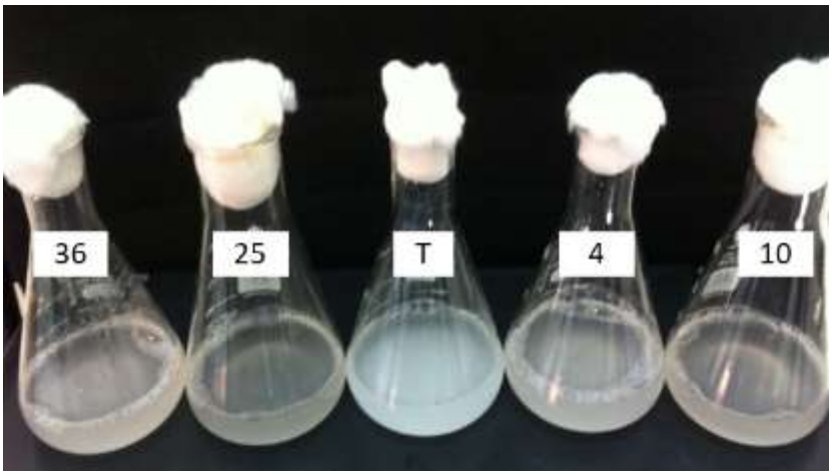

Figure 3. Phosphate solubilization on liquid medium manifested by a clarification of the medium's appearance compared to the control

Monitoring the pH of the Solubilization Medium:

During the incubation period of the solubilization medium, pH monitoring was conducted every 24 h. The results showed a significant decrease in pH for all isolate, compared to the control (Figure 5). The results showed that the pH of the medium decreased from pH 7 as early as 24 h after treatment, reaching stability around pH 4 by 72 h. These results of pH decrease indicate acidification of the medium, primarily attributed to the production of organic acids by the selected isolates.

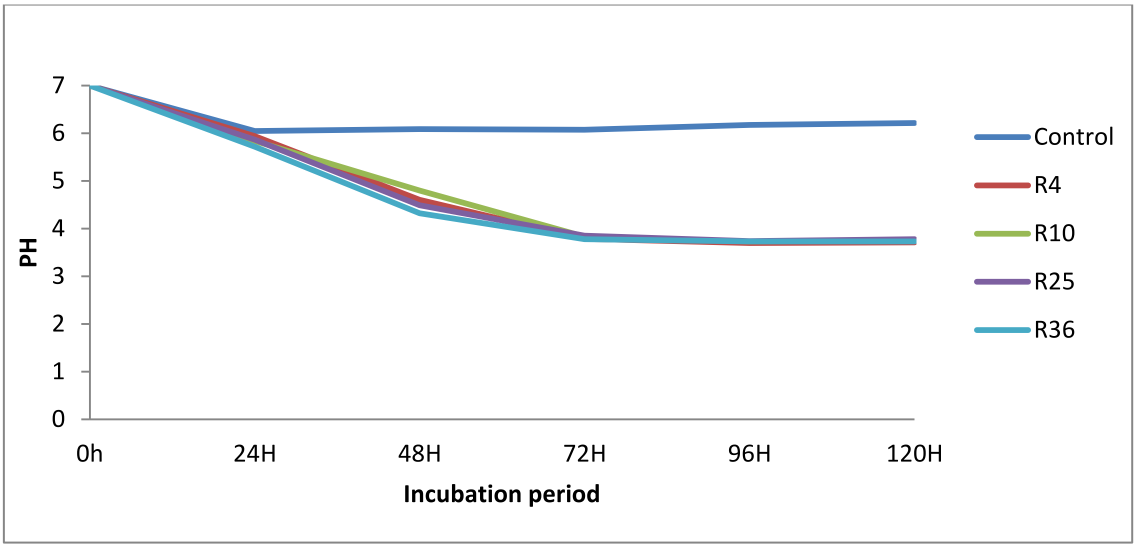

Figure 4. Monitoring the pH of the Solubilization Medium:

The results indicate that these four bacterial isolates formed a clear halos of phosphate solubilization on NBRIP medium, demonstrating their proficiency in phosphate solubilization. This characteristic is crucial for rhizospheric bacteria as it enhances phosphate absorption by plants, essential for their growth and development. Previous research highlighted the significant phosphate solubilization capabilities of *Pseudomonas* species (Joseph et al., 2005; Qessaoui et al., 2019). Many studies identified microbial genera such as *Pseudomonas*, Bacillus, *Rhizobium*, and *Enterobacter* among bacteria, and *Aspergillus* and *Penicillium* among fungi, as pivotal in phosphate solubilization (Xiao et al., 2011; Keditsu and Srivastava, 2014). The primary mechanism through which *Pseudomonas* solubilizes mineral phosphate involves the production of organic acids and acid phosphatase, crucial for the mineralization of organic phosphorus. Organic acid production induces acidification of the microbial cell and its surroundings, facilitating the release of phosphate from mineral phosphate through proton substitution for calcium (Goldstein, 1999). Qessaoui et al. (2019) demonstrated that isolates of *Pseudomonas* sp. (Q6B, Q14B, Q7B, Q1B, and Q13B) significantly enhanced seedling height, plant length, and collar diameter. These effects were attributed to their ability to solubilize phosphate, produce siderophores, ammonia, and indole-3-acetic acid, and colonize the roots of tomato plants. These findings underline the importance of selecting the most effective bacterial strains for agricultural applications, particularly in improving soil fertility and enhancing crop growth. Among these isolates, particularly isolate R36, with its healthy phosphate solubilization capacity, holds promise for future research aimed at developing effective and sustainable biofertilizers.

Conclusion

The study highlights the potential of rhizospheric *Pseudomonas* bacteria in enhancing phosphate solubilization. These strains could prove instrumental in improving soil fertility and agricultural productivity. The ability of these bacteria to convert insoluble phosphates into bioavailable forms suggests their practical application in sustainable agriculture, reducing the need for chemical fertilizers and promoting environmentally friendly farming practices.

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ANTIOXIDANT ACTIVITY OF EXTRACTS FROM DIFFERENT COMPONENTS OF THE INFRUCTESCENCES OF TWO CHESTNUT CULTIVARS

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Abstract

The chestnut has great economic importance in Portugal. Farmers and entrepreneurs of this crop have faced several problems that have led to a decrease in production due to the emergence of new pests and microbiological development during pre- and post-harvest stages. The main aim of this work was to investigate some chemical and biological parameters in different components of the chestnut infructescence – hedgehog, outer pellicle, integument+seed – at the harvest time in two varieties – Longal and Martaínha, to verify whether there are significant differences between the components and varieties after using an ecological extraction method. Three studies were carried out. In the first, the influence of the extraction method on the total reducing capacity, flavonoids, monomeric anthocyanins, hydrolyzable tannins, reducing power, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH) was analyzed using two methods. The methanol:water (1:1, v/v) proved to be suitable. The second study aimed to replace the mixture of methanol:water with ethanol:water (1:1, v/v) (an environmentally friendly solvent) and to use ultrasound to shorten the extraction time. When analyzing the effects of the extraction method and the solvent mixture on the total reducing capacity, reducing power, and DPPH radical scavenging capacity of chestnut’s hedgehog, the ethanol:water (1:1, v/v) method with ultrasound was the most effective in most situations. When comparing the two varieties and fruit components (third study), Longal had the highest extraction yield. The outer pellicle was the component of the infructescence with the highest values in both varieties, followed by the hedgehog and the integument+seed.

Keywords: *Chestnut, Solvents, Extraction method, Antioxidant activity.*

Introduction

The European chestnut (Castanea sativa Mill.) is an imposing tree that can reach a height of up to 30 meters and a diameter of 2 meters to one meter above the ground throughout its life (Baltazar, 2015; Dias, 2020). This species belongs to the group of dicotyledonous angiosperms, is monoecious, and belongs to the family Fagaceae within the genus Castanea. The chestnut tree has great economic importance, as it provides valuable nuts for human and

animal feed. Its wood is highly appreciated in construction and carpentry, while the leaves and bark contain tannins that are beneficial in the treatment of bleeding and diarrhea, the infusion of the leaves is also used against fever and whooping cough. In addition, the tree provides excellent quality firewood as fuel (Baltazar, 2015; Paiva, 2007).
Studies by Braga (2014), Kalinová et al. (2014), Fuente-Maqueda et al. (2020) and Cerulli et al. (2021) demonstrate that the chestnut fruit is rich in phenolic compounds, which are associated with health benefits, thanks to their antioxidant properties and anti-inflammatory effects. Such compounds are fundamental not only for human nutrition, but also have considerable potential for applications in the food and beverage industry, contributing to the competitiveness of companies that process nuts. An example of the use of these phenolic compounds in the various components of the chestnut tree is their use as natural preservatives, due to their ability to inhibit microbial growth and increase the shelf life of food products. One of the traditional methods for extracting phenolic compounds is solvent extraction, which is widely used in the industry. This process combines a solvent with the use of heat to facilitate the mass transfer of the compounds. However, this method has disadvantages, such as the degradation of heat-sensitive compounds and high energy consumption. Therefore, it is crucial to employ more efficient extraction methods for phenolic compounds (Barba et al. 2016; Carvalho et al. 2018; Larue et al. 2021). Furthermore, phenolic compounds can vary in the different components of a plant product and between different varieties. Therefore, the main objective of this work was to investigate some chemical and biological parameters in different components of the chestnut infructescence, namely: hedgehog, outer pellicle, and integument+seed, at the harvest time in two chestnut varieties – Longal and Martaínha, to verify the existence of significant differences between the components and both varieties after using an ecological extraction method.

Material and Methods

The present work involved three studies. The first was related to the study of the extraction method to be applied in the future to the chestnut samples, namely: hedgehog, outer pellicle, and integument + seed, in order to quantify the total reducing capacity, flavonoids, monomeric anthocyanins, hydrolyzable tannins, reducing power, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH), based on the works of Lameirão et al. (2020) and Fuente-Maqueda et al. (2020). The first work mentioned involves an aqueous extraction, while in the second methanol:water (1:1) was used. As the methanol:water (1:1) mixture proved to be adequate to determine the parameters proposed in the present work, the second study focused on the effect of replacing the mixture of methanol:water (1:1) with ethanol:water (1:1) because ethanol is a green solvent, just like water. It was also desirable to change the extraction method involving agitation for 24 hours at room temperature as used by Fuente-Maqueda et al. (2020) by ultrasound, in line with what was reported by Lameirão et al. (2020). These authors used ultrasound at 70 °C (50% amplitude) for 40 minutes. In the present work, the extraction was carried out without temperature control, since the equipment used does not have any thermostatic bath attached and because the operation of the ultrasounds causes heating of the sample, making it impossible to establish and maintain a constant temperature throughout the extraction. In the first and second studies, the following conditions were used: (A) Room temperature - Approximately 1.5 g of lyophilized sample was weighed into a 250 mL goblet, and 150 mL of water or methanol:water (1:1) or ethanol:water (1:1) was added. In both cases, the mixtures were left at room temperature for 24 h in agitation at 300 rpm; (B) Ultrasound - Approximately 1.5 g of lyophilized sample was weighed into a 250 mL goblet and 150 mL of water or methanol:water (1:1) or ethanol:water (1:1) solution was added. Ultrasound was applied for 5 minutes and 70% amplitude (Qsonica, Q500 Sonicator, 20 kHz). The total reducing capacity was determined by the Folin-Ciocalteu method described by Falcão et al. (2007), the reducing power and DPPH radical scavenging capacity by Delgado et al. (2010), the monomeric anthocyanins by Bchir et al. (2012) and Rajasekar et al. (2012), the tannins by Elfalleh et al. (2012), and the flavonoids by Viuda-Martos et al. (2011). In the third study, the best method was applied to the hedgehog, outer pellicle, and integument+seed of the Longal and Martaínha varieties at the harvest time for both varieties.

Results and Discussion

In this section, the results of the three studies carried out as part of this work are presented. In the first study, the use of water and methanol:water (1:1) was studied (Table 1). For these experiments, the matrix chosen for the extraction was the outer pellicle of the fruit.

Table 1. Parameters evaluated in the extracts prepared with methanol:water (1:1) (stirring and ultrasound) and water.

Extraction type	Extraction yield (%)	Total reducing capacity (mg Galic acid/g d.w.)	Flavonoids (mg Quercetin /g d.w.)	Anthocyanins (mg Cyanidin-3-glucoside /g d.w.)	Tannins (mg Tanic acid/g d.w.)	Reducing power[2]	DPPH (mg Ascorbic acid/ g d.w.)
MeOH:Water 24h	9.8 ± 0.6^{a}	54.7 ± 3.1^{a}	179 ± 15^{a}	0.008 ± 0.004^{b}	4.83 ± 0.08^{b}	0.135 ± 0.006^{a}	34.7 ± 2.4^{a}
MeOH:Water US	11.1 ± 0.9^{a}	55.9 ± 2.5^{a}	179 ± 21^{a}	0.020 ± 0.002^{a}	6.22 ± 0.14^{a}	0.130 ± 0.004^{a}	37.0 ± 4.0^{a}
H_2O US	5.6 ± 0.6^{b}	24.0 ± 2.2^{b}	81 ± 1^{b}	[1]ND	2.27 ± 0.27^{c}	0.094 ± 0.006^{b}	14.3 ± 2.0^{b}

[1]ND = Below the detection limit; [2]Absorbance at 700 nm of an extract solution with a concentration of 0.125 mg extract/mL Different lowercase letters per column indicate significant differences (*p<0.05*) between the different types of extraction.

The results obtained in the first study showed that in the extraction where the combination of the solvent methanol:water (1:1, v/v) was used, either with ultrasound or stirring for 24 hours, better results were obtained than with water, proving to be a more efficient solvent. When comparing the extraction methods, in most situations, no significant differences were found between the use of 24-hour stirring and ultrasound, except for anthocyanins and tannins. For both types of compounds, higher concentrations were obtained with the application of ultrasound. Considering these results and the fact that the ultrasound method is faster, it was decided to use methanol:water (1:1)+ultrasound in the following tests.
In the second study, it was decided to test the use of ethanol instead of methanol because it is an environmentally friendly solvent. Table 2 shows the results of the test comparing ethanol:water (1:1) with methanol:water (1:1). In this study, the hedgehog was used.

Table 2. Extraction yield and antioxidant activity of chestnut hedgehog when extracted with methanol:water and ethanol:water (1:1) under stirring for 24 hours or ultrasound (US).

Extraction type	Extraction yield (%)	Total reducing capacity (mg Galic acid /g d.w.)	Reducing power (mg Ascorbic acid / g d.w.)	DPPH (mg Ascorbic acid / g d.w.)
MeOH:Water 24h	14.7 ± 0.6^{b}	23.5 ± 2.0^{b}	60 ± 7^{c}	142 ± 4^{a}
EtOH:Water 24h	15.0 ± 0.8^{b}	39.7 ± 7.6^{a}	85 ± 4^{b}	94 ± 3^{b}
MeOH:Water US	14.5 ± 0.1^{b}	24.2 ± 4.4^{b}	71 ± 7^{c}	142 ± 5^{a}
EtOH: Water US	16.8 ± 0.3^{a}	36.6 ± 3.0^{a}	121 ± 13^{a}	106 ± 12^{b}

Note: Different lowercase letters per column indicate significant differences (*p<0.05*) between the different types of extraction.

Significant differences were found between the extraction methods. The ultrasound extraction showed the best results. In a study carried out by Vázquez et al. (2008) on the antioxidant activity and total phenolic compounds of chestnut and eucalyptus outer pellicle extracts, in which extraction solutions based on ethanol:water and methanol:water were used in different concentrations, the authors observed that when they used the solution of ethanol:water at 50:50 obtained an extraction yield of 8.28%, lower than that determined in the present work. This fact may be due to the application of different extraction methods since, in the aforementioned study, stirring was applied for 2 hours at the boiling temperature of the solvent. In general, in the present work, the ethanol:water (1:1) + ultrasound stood out in relation to the different parameters analyzed, except for the DPPH test, where the best results were obtained with methanol:water (1:1) + stirring or ultrasound. It was decided to carry out the subsequent extractions with ethanol:water (1:1) and ultrasound since ethanol is considered an environmentally friendly solvent that is more sustainable and has less environmental impact.

In the third study, the hedgehog, the outer pellicle, and the integument + seed of two chestnut varieties were analyzed. The results are presented in Table 3. The outer pellicle was the component of the infructescence with the highest values in both varieties, followed by the hedgehog and the integument+seed. The integument+seed of Longal variety presented higher total reducing capacity, reducing power, and DPPH radical scavenging capacity than Martaínha.

Table 3. Total reducing capacity, reducing power, and DPPH radical scavenging capacity of the different components of the chestnut infructescence when extracted with ethanol:water (1:1) for 5 minutes in ultrasound.

Parameters	**Part of infructescence**	**Longal**	**Martaínha**
Total reducing capacity (mg Galic acid /g d.w.)	Hedgehog	33.3±1.5^{b}	27.7±2.5^{b}
	Outer pellicle	75.1±4.0^{a}	91.1±11.8^{a}
	Integument+seed	15.1±6.7^{c}	4.9±0.7^{c}
Reducing power (mg Ascorbic acid / g d.w.)	Hedgehog	0.070±0.009^{b}	0.068±0.005^{b}
	Outer pellicle	0.160±0.013^{a}	0.178±0.006^{a}
	Integument+seed	0.059±0.047^{b}	0.025±0.002^{c}
DPPH (mg Ascorbic acid / g d.w.)	Hedgehog	0.092±0.010^{b}	0.094±0.011^{b}
	Outer pellicle	0.228±0.019^{a}	0.277±0.020^{a}
	Integument+seed	0.013±0.001^{c}	0.006±0.001^{c}

Note: Lowercase letters compare the samples to each other for a given variety. Different letters indicate significant differences ($p<0.05$).

In the study carried out by Vázquez et al. (2008), described previously, an average total reducing capacity of 45.6 mg gallic acid/g sample d.w. for the outer pellicle was obtained, lower than that determined in the present work. Again, the differences found between the two studies may be due to the extraction method. In another study carried out by Barreira et al. (2008) on the antioxidant activity of extracts from flowers, leaves, outer pellicle, integument, and chestnut seed, it was observed that the highest values of total reducing capacity expressed in mg of gallic acid/g extract were determined in the outer pellicle in relation to the integument and seed. The average values obtained for the total reducing capacity for the outer pellicle, integument, and seed were equal to 25.4, 103, and 0.73 mg of gallic acid/g sample d.w., considering the extraction yield. In the present work, the integument was found attached to the seed, explaining the higher values obtained. Fuente-Maqueda et al. (2020) obtained total reducing capacity values of 93.3, 56.0, and 1.8 mg gallic acid/g sample d.w. for the hedgehog, outer pellicle, and seed, respectively, slightly different from our values probably due to the different extraction methods applied.

Conclusions

In the first study, it was found that methanol:water (1:1) + ultrasound gave the best results when compared to the extraction with water + ultrasound and methanol:water (1:1) by stirring for 24 hours. In the second study, the extractions made with ethanol:water (1:1) with ultrasound provided good results. Regarding the components of chestnut, the outer pellicle was the component of the infructescence with the highest values in both varieties, followed by the hedgehog and the integument+seed. Some differences between varieties were also found.

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FARMERS' WILLINGNESS TO REDUCE THE USE OF HERBICIDES IN MANAGING WEEDS IN RICE CROPS

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Abstract

Weeds management are the main crop protection issue in rice cultivation, responsible by about 30% of production losses in the field. Rice cultivation provides stability revenues to rural populations, in swamp ecosystems, wetlands, and lowlands, even in soils with high salinity, where other crops are not economic viable. To reduce yield losses, rice farmers rely on chemical control which generated intense selection pressure for the evolution of herbicide-resistant weed populations. This study focused on rice farmers' perceptions and practices for weed management. The applied methodology was a questionnaire, carried out personally with 82 rice producers, in three Portuguese rice-growing regions, complemented by focus groups' meetings. The results reveal that *Echinochloa* spp. is the weed of greatest concern, followed by *Oryza sativa* var. *sylvatica*. Most growers are not, or average satisfied with the efficacy of pre-emergence herbicides for *Echinochloa* spp. control, and they are even less satisfied with the efficacy of post-emergence herbicides. Almost a quarter of producers (26.8%) changed previous year's herbicide application scheme, but only 12 producers (14.6%) changed any cultural practice related to plant protection in the last five years. The study results confirmed the low adoption of alternative physical and cultural measures, due technical, economic and climate constrains, namely the adoption of rotation (due to learning process and economic viability), and stale seedbed (available water restrictions and the delay on sowing time). To tackle the reduction herbicide resistance risk, it is essential to act at the level of the agriculture innovation system.

Keywords: *Weeds, rice, non-chemical control, European Ecological Pact.*

Introduction

Rice crop is one of the most important crops in the World and European Union, due the importance of rice in human nutrition but also because allows to obtain revenues from marginal lands for agriculture. This crop grows in swamp ecosystems, wetlands, lowlands, and very saline soils, generating revenues in rural areas where other crops could not thrive. It has environmental benefits, contributing to the diversity of fauna and flora, and playing a fundamental role in the integrated management of these particularly sensitive ecosystems.

The Portuguese rice area represents around 6% of the total rice area in Europe (EC, 2022), distributed over three regions: *Tejo* and *Sorraia* valleys (*Ribatejo* region), *Sado* river valley (*Alentejo* region) and *Mondego* valley (*Beira Litoral* region) (Clara, 2022; INE, 2023). The current average productivity (in the triennium 2020-2022) is 5.62 kg/ha, in 27,518 ha with a production of 155,573 tons. (INE, 2023). The degree of self-provisioning for rice was 60% in 2020/2022 (GPP, 2023), with a *per capita* consumption of 14.2 kg per year (2022/2021) (idem). The annual *per capita* consumption worldwide is 54 kg, while in the EU the figure is only 6 kg (EC, 2020a). The Portuguese rice consumption is the highest in Europe.

Weed resistance affects the main rice-producing countries of the European Union (UE) and is the key crop protection problem in rice, with losses close to 30% of production (Oliveira *et al*., 2021). The cost of herbicides is the main cost of the crop, 18% of the total, and weed management is the main concern of rice farmers in Portugal (idem).
The herbicide-resistant weed populations are evolving rapidly (Espig *et al*, 2022; Heap, 2024; Norsworthy *et al*., 2012), namely in rice crops (Karim, *et al*. 2004; Calha *et al*., 2023). The impact of pesticides on the environment constitutes a high risk, requiring urgent mitigation measures, in line with the goals established by Farm to Fork strategy (EC, 2020b; 2020c). However, the withdrawal of many herbicides and less diversity of modes of action increased the risk of herbicide resistance. To tackle these problems, it is necessary to find alternative ways to control weeds and better use of pesticides in rice crops (Karim *et al*., 2004; Paiman *et al*, 2020).
There are few studies on farmers' adoption of herbicide alternatives, despite numerous studies on sustainable pesticide use. In contrast to the many studies of herbicide resistance's biophysical aspects, its sociocultural dimensions have historically been overlooked until recently, resulting in few targeted practice change initiatives (Espig *et al*., 2022). To further the reduction use of pesticides, it is necessary to empirically focus on how the actors and processes that compose pesticide use are assembled, held in place, and work in different ways to open or close possibilities of pesticide use reduction (Argüelles and March 2023). Ferrero *et al*. (2021) analyzed the relationship between agronomic practices and weedy rice (*Oryza sativa sylvatica*), but not the producer’s willingness to adopt alternative methods of weed control. In this paper, we present the results of the research on rice farmers' perceptions and attitudes towards weeds and cultural practices related to spontaneous vegetation management, based on a survey complemented by a focus group meeting. Our questions are “Are rice producers willing to adopt alternative methods to herbicide use? What are the main constraints to those changes?”

Materials and Methods

The inquiry methodology by questionnaire was carried out in person, in the main three rice-producing regions (in 2018 and 2019), complemented by three focus group meetings (in 2019 and 2020). The data were analyzed by the IBM® SPSS® Statistics software version 25.0, using non-parametric methods for the analysis of the normative and ordinal variables. The chi-square test was applied to identify differences among the rice crop regions.
In complementarity, three meetings using focus group methodology were done in order to discuss the results and collect contributions, namely, to understand better the different perceptions and cultural practices applied for weed management in each region. It allowed going deeper in the alternative cultural practice’s issues, such as the possibilities, constraints, and particularities of the non-chemical alternatives to control rice weeds.
It was interviewed 82 rice growers distributed relatively equitably among the three rice-growing regions, after the rice cropping season, in the months of lower agricultural activity, from September to February. Technicians from producer associations and technicians from regional agricultural services carried out the survey.
The survey was structured in three sections: 1) Background of the cultural system; agricultural holding area; agricultural plot; ownership and cultivation system, crop rotation, and rice varieties. 2) Field operations: indication of the entire technical itinerary, from soil preparation for sowing to harvesting, with emphasis on main weeds, herbicide application in the last years, the farmer's perception of the most challenging weeds, and the efficacy of the herbicides. 3) Technology and organization: associations and technical advice, training, agri-environmental measures, and technology innovation.

The focus group meetings were held one year after the inquiries. One meeting in each rice-growing region, with around 20 people per group, involving the various stakeholders of the rice sector (producers, irrigation associations, researchers, technicians, producer organizations, companies input suppliers). The discussion focused on weed resistance to herbicides. The main questions were: the perception of weed resistance to herbicides in the region; which strategies were being implemented to tackle the weed resistance, and what their results were; which crops are best suited for crop rotation; how to implement stale seedbed.

Results and discussion

Most of the interviewees follow Integrated Crop Production and have been rice producers for more than 25 years. There are only two producers of organic rice. There are differences in the land tenure structure in the three regions. The mean rice areas cultivated are: 11.5 ha in *Mondego*; 16.7 ha in *Sado*; and 35.8 ha in *Sorraia* (in this region, there is a greater specialization in rice farming).

Main weeds and weeds resistance

The main weeds that most concern farmers were *Echinochloa* spp., followed by *Oryza sativa* var. *sylvatica, Alisma* spp., and *Heterantera* spp. It should be noted that emerging weeds such as *L. fascicularis* appear with greater importance in rice paddies in *Sorraia* region. In *Mondego* region, *O. sylvatica* is the biggest concern for farmers, after *Echinochloa* spp. *Cyperaceae* began to gain importance in the southern region of the *Sado* river valley.
Rice growers were also asked to rate the occurrence of new weeds in the last three years. More than a fifth of respondents mentioned the appearance of *L. fascicularis* in the last three years. One or two references were made to other weeds, such as *Heterantera*, *O. sylvatica*, *C(Cyperus?). difformis,* E. *phyllopogon* and *Leersia oryzoide* and *Leptochloa fusca* subsp. *fascicularis* that recently appeared in Portugal (Santos, 2022). Concerning the occurrence of new weeds, there are no significant differences among rice production regions.
When assessing awareness of herbicide resistance, around 90% of rice farmers stated that existing herbicides no longer control *Echinochloa* spp. as it controlled in the past, and they are most concerned with this weed.

The herbicides in weed-controlling

The study revealed that in a universe of 82 farmers, 71 used pre-emergent herbicides, of which 71 used oxadiazon and only four farmers used clomazone. Clomazone was only used in the *Sado* valley, associated with dry sowing in the line with buried seed. Most growers are 'Not satisfied' or 'Average satisfied' with the efficacy of pre-emergence herbicides for *Echinochloa* spp. control. There are no significant differences among the three rice-producing regions.
Post-emergence herbicides are farmers' main tool for weed control. Growers are even less satisfied with the efficacy of post-emergence herbicides in controlling *Echinochloa* spp. than with pre-emergent herbicides. More than half are "Not satisfied or dissatisfied" with post-emergent herbicides efficacy.

Production modes as control measures

Most farmers (90%) have been producing rice in monoculture for at least five years. Stubble and straw management significantly diverge among regions. In the *Mondego* valley there is a preference for burning (56%), while in the *Sado* valley almost only straw and stubble are incorporated without burning (96.2%).

In *Mondego* the incorporation of straw and stubble with a disc harrow on dry soil "lowering" operation, is an old practice that has fallen out of use since the employment of leveling the beds with laser technology. More than 96% of producers use this laser leveling. Tillage with a rotary ley allows for reducing the seed bank that develops after sowing. This technique is more used in *Mondego* valley than in the others two regions.
Regarding the irrigation and drainage structure, we verify that the inlet and outlet of water is independent for each bed. In 57% of the cases, the inputs and outputs of water are independent for each paddy field. In 42% of the cases where the water transits between beds, and a mix situation.
Considering a risk assessment, it is meaningful to associate the trends expressed by the rice growers surveyed within the field assessment, in which the following dissemination of resistance to penoxsulam in several *Echinochloa* spp. populations: 90% in *Mondego* 50% in *Sado* valley rice fields and 25% in *Tejo* and *Sorraia* valleys (Calha, 2016).
These results clarify the different use of penoxsulam in the three regions: larger use in *Mondego*, followed by the *Sado*, and finally the *Sorraia*. Once resistance builds up, resistant *Echinochloa* weeds are managed primarily by switching to herbicides with different mode of action (MoA) and, in some cases, by applying different agronomic practices (Harker et al, 2013).

Innovation in weeds management
Rice farmers were asked about changes to the usual herbicide application schedule, and whether they had changed any cultural practices related to plant protection in the last five years. Almost a quarter of producers (26.8%) changed previous year's herbicide application scheme. The main changes were in the herbicides used and date of application. Only 12 producers (14.6%) changed any cultural practice related to plant protection in the last five years, and of these, only one introduced crop rotation. Most of the cultural changes occurred in terms of sowing form and density. The results show a high dynamic in the change in the use of herbicides (26.8% of producers, from one year to the next), while changes in cultural practices are slow (14.6% over a period of five years). These results are more significant if we considering that 92.7% of producers receive technical support, and 90.2% are certified in integrated crop production.

Focus groups
The focus groups clarify some of the survey results. Weed resistance and herbicide efficacy decrease were mentioned in all regions, and *Echinochloa* spp. was identified in all regions as a top concern.
Regarding the strategies to be applied, the groups proposed different strategies, without consensus among the members of the focus groups. However, the need for better knowledge of herbicide use was highlighted. Crop rotation was considered by all focus groups as a means of weed control. However, at the same time, it was considered a difficult or impossible technique to apply, both from an economic and technical point of view, due to the characteristics of the soil in which rice is cultivated. The following were identified as possible crops for rotation: maize, sorghum, and peas. A stable seedbed is recognized as a good technical option for reducing problematic weeds such as *O. sylvatica* and *Echinochloa* spp., but it presents implementation difficulties due to the scarcity of water in some regions and the delay on sowing time that this technique implies in rice cultivation (Table 1).

Table 1. Summary of focus group

Questions	*Mondego* region	*Sado* region	*Sorraia* region
Q1. What is the perception regarding weed resistance in the region?	Increased level of resistance to herbicides. Emergence of new weeds	Water grass (*Echinochloa spp.)* are the most problematic. Doubts about whether we are dealing with herbicide resistance or lack of effectiveness in herbicide application.	Herbicide resistance for some weeds. Lack of efficacy is not always associated with herbicide resistance.
Q2. What strategies are being implemented in that direction, are there results?	Negative to dry soil preparation. Positive: rotation and other cultural practices; change in sowing date, rotation of herbicides and height of water blades after harvest. New solutions were not very feasible: cleaning of marrows, manual weeding and thermal weeding.	Several solutions were tested: dry seeding with buried seedlings, tillage, herbicide mix, and more effective water management. No agreement on the most effective solution. Consensus on the option of manual weeding, but with the problem of a lack of workforce.	Alternation of herbicides with different modes of action (most common) and localized application of herbicides. Crop practices such as tillage, change in soil preparation mode, improved irrigation management, dry sowing with buried seed, rapid soil covering.
Q3. Which crops are most adapted for a rotation, and what to consider in a three-year economic assessment?	Corn and pea. Positive: increases production and decreases the cost of the crop. However, there is the problem of uneven land and runoff from alternative crops.	Leguminous crops, especially chickpeas (low profitability and lack of organization). Difficulty in implementation: saline and heavy soils (hinders cropping alternatives); restrictions in terms of available water (the alternative is autumn/winter crops)	Corn and sorghum. Possibility of producing forage legumes or producing legumes for agro-industry. Implementation difficulties: saline soils and heavy soils (hinder cultural alternatives); problem of unevenness of the land; technical and economic difficulties for the implementation of alternative crops. Herbicide efficacy (with increased cost) preferred to the use of crop rotation.

Questions	*Mondego* region	*Sado* region	*Sorraia* region
Q4. False seeding, with or without flooding? How to be implemented?	Positive, but the three-week delay in sowing causes crop losses.	Water for irrigation is only available from late April or early May.	Late sowing problems (lack of early, productive varieties) and possibility of available water restrictions. The alternative is post-harvest tillage, taking advantage of the still favorable temperature.
Q5. What results would you like to see from this project?	Technical manual for crop practices. Mapping of weeds and herbicide resistance. Presentation of best practice cases in other countries, with an exchange of experiences.	Decision support tools for weed control. Raising public awareness about efforts in rice production and technological advances. Mediation between the project team and official entities to improve the search for solutions and their dissemination.	Technical solutions to improve the effectiveness of herbicides and the production of new herbicides; agricultural tools that allow for other forms of soil preparation and the improvement of the "tillage" technique. Dissemination of project results. Financial compensatory measures for the implementation of crop rotation and false sowing.

Source : Calha *et al.* (2023, pag. 16)

Conclusions

The survey results confirmed the low adoption of alternative physical and cultural measures, with the main reasons identified in focus groups, particularly for rotation and stale seedbed. These constraints are primarily due to climate and soil conditions: saline and heavy soils limit the cultivation of other crops, and the climatic conditions for sowing and harvesting make the stale seedbed option difficult. Crop rotation faces additional challenges, such as the learning curve for cultivating new crops, access to production factors, and knowledge of production practices. Furthermore, the viability of new crops and difficulties in commercialization, such as lack of commercial contacts, low market power, and low productivity, are significant barriers.

Weed control predominantly relies on chemical means, despite the high costs and dissatisfaction with herbicide efficacy. Rice farmers acknowledge the growing issue of herbicide resistance, exacerbated by incorrect application, poor choice of active substances, and timing errors. To adapt, farmers are adopting new products and mixing herbicides, with over a quarter of respondents changing their application schemes from the previous year, using nearly three different Modes of Action (MoA) over five years. On average, they apply fewer than two different herbicides *per* year, with almost all using ALS inhibitor herbicides.

Cultural practices are recognized for their importance in mitigating reduced herbicide efficacy.
The specificities of local ecosystems, farmers' knowledge gaps, and the complexity of learning new crops or techniques make non-chemical alternatives very risky. Despite the costs and herbicide resistance risks, farmers continue to rely on pesticides. Addressing this requires enhancing the innovation ecosystem by developing applicable scientific and technical knowledge, promoting social capital to build skills and relationships among economic agents, and implementing political measures to support sustainable practices. The problem of herbicide resistance is worsening, especially with the reduction in available active substances and Modes of Action. Adopting non-chemical weed control practices is crucial to reducing herbicide costs and meeting the European Green Deal's requirements for herbicide use reduction.
The application of these alternative control methods cannot be widespread but must be studied on a case basis, and require technical follow-up, adapted to the region and the plot. The procedure of learning new crops or practices makes non-chemical alternatives very risky for farmers. To reduce resistance risk and maintain the sustainability of the rice cropping system it is essential to act at the level of the innovation ecosystem: from the emergence of scientific and technical knowledge applicable to new solutions, to the evolution of social capital, promoting capacities and relationships between economic agents, and political measures supporting and stimulating innovation and implementation of greener and more sustainable practices.

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EXPLORING INSECTS DETECTION PERFORMANCE IN CORN PEST CONTROL WITH MOBILENET-SSD NEURAL NETWORK AND HIGHER-RESOLUTION INPUT

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Abstract

Building upon previous research in the domain of corn pest detection from some species of insects (Coleoptera Order) using Convolutional Neural Networks (CNNs), this study investigates the impact of a higher input resolution on the performance of the MobileNet-SSD neural network architecture. Our initial study focused on optimizing a deep-learning model for low-power mobile devices to detect various corn pests while preserving beneficial species. In order to further improve the detection capabilities of MobileNet-SSD, the present research explores the effect of a higher resolution of 512×512 pixels, as input for the MobileNet-SSD-v1 and v2-Lite models. These adjustments aimed to provide a more detailed input representation to facilitate enhanced detection performance. The evaluation metrics include average accuracy per class and mean average precision (mAP), as used in the previous study, to ensure consistency in performance assessment. Our findings indicate that training the networks on the augmented dataset at a higher resolution resulted in improved detection performance, with both models demonstrating enhanced mAP scores (MobileNet SSD v1: mAP = 0.912; MobileNet SSD v2 Lite: mAP = 0.919). Additionally, MobileNet SSD v2 Lite consistently outperformed MobileNet SSD v1 in terms of mAP across both training phases. This research contributes to a broader understanding of the relationship between input resolution and detection performance in CNNs, providing valuable insights on refining the approach to further enhance the precision and efficiency of modern pest detection systems in agricultural contexts.

Keywords: *Pest control, insects, Coleoptera, smart agriculture, neural network.*

Introduction

The demand for effective pest management solutions in agriculture is an important objective, alongside the need for sustainable and eco-friendly practices. Traditional methods relying heavily on chemical pesticides face environmental concerns and diminishing effectiveness over time. As a response, there has been a shift towards integrating artificial intelligence (AI) technologies into agricultural practices, offering innovative solutions for pest detection and management.

Previous research has demonstrated the potential of AI-driven techniques in various agricultural domains, including disease management in sunflower crops (Gulzar *et al.*, 2023), fruit classification in horticulture (Gulzar, 2023), and citrus fruit disease detection (Dhiman *et al.*, 2023). These studies showed the effectiveness of advanced algorithms, particularly neural networks, in addressing diverse agricultural challenges.

Insect pest detection and identification have been particularly emphasized in recent research, with studies employing a range of neural network models and methodologies. The most popular object detection algorithm was YOLO, followed by Faster R-CNN and VGG16 (Maican *et al.*, 2023). Noteworthy results in terms of mean average precision (mAP) are summarized in Table 1.

Table 1. Ranking of some insect identification neural networks by maximum mAP

Model	Dataset	Maximum mAP
YOLOv5m	Dataset from Maryland Biodiversity Project (Sava *et al.*, 2022)	99.2%
YOLOv5-X	7046 images, 23 pest classes (Ahmad *et al.*, 2022)	98.3% (mAP@0.5)
YOLOv7	4865 images of coccinellids, seven classes (Wang *et al.*, 2023)	Up to 97.31% (AP@0.50)
Faster R-CNN	36,000 images of harmful and non-harmful beetles (Butera *et al.*, 2022)	92.66%
YOLOv5	100,000 annotated images of small insects (Kranthi *et al.*, 2002)	92.4% (mAP@0.50)
Improved Faster R-CNN	AgriPest21 dataset: 21 pest classes, 25,000 images (Wang *et al.*, 2021)	78.7%
AgriPest-YOLO	Pest24 dataset: 25,000 images of small pests (Zhang *et al.*, 2022)	71.3% (mAP@0.50)

However, most existing research faces challenges when applied to low-power agricultural mobile systems, which require lightweight neural networks both to conserve battery life and to accommodate their limited processing capabilities. In this context, MobileNet networks have received limited attention despite their potential advantages in resource-constrained environments.

In our previous research (Maican *et al.*, 2023), we focused on developing optimized MobileNet SSD networks, for crop pest detection. Utilizing a two-step transfer learning approach, we aimed to enhance the accuracy of these networks, particularly MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite. Despite using a reduced training set (2648 images), both models demonstrated similar and good overall accuracies (MobileNet-SSD-v2-Lite: mAP = 0.8923; MobileNet-SSD-v1: mAP = 0.908), highlighting their potential for real-time pest control.

Building upon these findings, the main objective of our current study is to investigate the impact of input image resolution on the performance of MobileNet-SSD networks for crop pest detection. Specifically by increasing the input resolution from 300 to 512 pixels, we aim to assess whether higher-resolution images yield improved accuracy in pest identification. This adjustment aimed to provide a more detailed input representation to facilitate enhanced detection performance.

Materials and methods

In this study, we modified the MobileNet SSD v1 and MobileNet SSD v2 Lite networks to process images with a resolution of 512×512 pixels. This adaptation aimed to enhance the networks' ability to detect and classify objects with finer details, ultimately improving their overall performance. The training process was conducted on the Nvidia Jetson AGX Orin platform using PyTorch 2.0.0 for JetPack 5.1.1. Additionally, we utilized Nvidia TensorRT for real-time inference optimization on Nvidia GPUs.

The training dataset consisted of 2648 images after augmentation, sourced from the iNaturalist web portal (iNaturalist, 2023). These images represented four beetle species know as pests that cause substantial damage to crops such as corn, wheat, sunflower, and beans (*Anoxia villosa*, *Diabrotica virgifera virgifera*, *Opatrum sabulosum*, and *Zabrus tenebrioides*). Additionally, the dataset includes images of the beneficial *Coccinella* sp., an

aphid predator in agricultural ecosystems. This inclusion aimed to evaluate the network's ability to distinguish between pest species and beneficial organisms.
The training process involved both a common transfer learning procedure, as well as a novel two-step transfer learning procedure, which consisted of the following steps:

- Initial Transfer Learning: in the first step, we performed transfer learning using the Open Images dataset to recalibrate the pre-trained MobileNet-SSD models for insect detection task. This involved retraining the models on an Open Images subset of 2605 images, containing only two classes: Beetle and Ladybug.
- Refinement Transfer Learning: in the second step, we further refined the pre-trained models using our custom dataset, organized according to the Pascal VOC format. This dataset included images of the five insect species mentioned earlier, allowing the models to learn specific features relevant to our pest detection task.

The training and evaluation procedures were conducted using two PyTorch scripts: 'train_ssd.py' and 'eval_ssd.py', respectively. These scripts were used for model training, validation, and performance evaluation, including metrics such as mean average precision (mAP). Further optimization for real-time inference was achieved using Nvidia TensorRT and detectNet – an object detection framework that performs optimized real-time inferencing on Nvidia Jetson GPUs. Detailed information on the training and evaluation procedures can be found in our previously published work (Maican *et al.*, 2023).
To summarize, in order to ensure consistency in performance assessment between the results obtained by increasing the image resolution and those from our previous research, experiments were conducted under identical conditions:

- The same two MobileNet-SSD models (MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite) were trained but modified to process images with resolutions of 512×512 pixels;
- Both models were trained on the same dataset, with the same images in the training, validation, and test sets;
- Identical values were employed for hyperparameters such as batch size, number of workers, and epochs;
- The same evaluation metrics were employed (average accuracy per class, and mAP@.50).

Results and discussion

In our previous work, we demonstrated that training the MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite networks on the unaugmented dataset yielded inferior results compared to training on the augmented dataset. Therefore, in this research, all training was conducted on the augmented dataset. For experimentation, we downloaded pre-trained versions of the two neural networks: MobileNet-SSD-v1 pre-trained on the PASCAL-VOC dataset, and MobileNet-SSD-v2-Lite pre-trained on the COCO dataset. We conducted the following experiments (Figure 1):

3. Single transfer learning training:
    3.1. Training the downloaded MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite directly on the augmented dataset (5 classes, 2648 images, input resolution: 512×512 pixels);
    3.2. Comparing the results from step 1.1 with the results obtained through the same procedure in the previous research, but with an input resolution of 300×300 pixels;
4. Two successive transfer learning trainings, as described in the Materials and Methods section (Two-step Transfer Learning):
    4.1. Training the downloaded MobileNet-SSD-v1 and MobileNet-SSD-v2-Lite on the Open Images subset of 2605 images (2 classes: Beetle and Ladybug, input resolution: 512×512 pixels);

4.2. Training the MobileNet-SSD-v1 and SSD-v2-Lite networks pretrained at 2.1. on the augmented dataset (5 classes, 2648 images, input resolution: 512×512 pixels);
4.3. Comparing the results from 2.2. with the results obtained through the same procedure in the previous research, where the input resolution was 300×300 pixels.

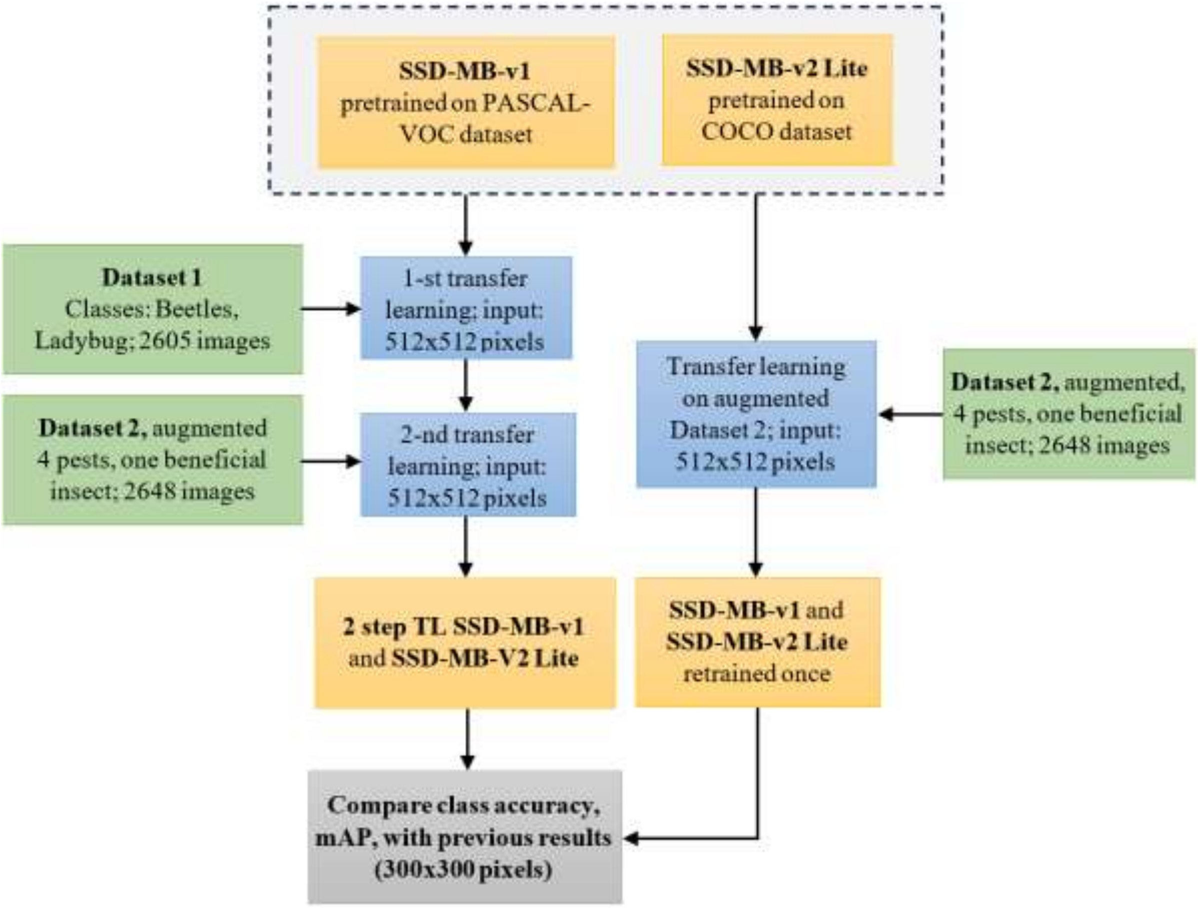

Figure 1. Schematic representation of the training and performance evaluation procedures; 2 step TL: two-step transfer learning; color scheme: orange - neural network models; blue - training procedures; green - datasets; grey - evaluation (adapted from Maican *et al.*, 2023)

To compare the results obtained from the experiments conducted in the current work, using a resolution of 512×512 pixels, with those from the previous work, where a resolution of 300×300 pixels was employed, we analysed the mean average precision (mAP), and the accuracy per class, for both MobileNet SSD v1 and MobileNet SSD v2 Lite.
The mAP metric provides an overall measure of the performance of the object detection models across all classes, capturing their ability to detect objects accurately and localize them within the image.
Conversely, the accuracy per class is a measure of the performance of the object detection neural networks for each individual class, showing their ability to differentiate between various pest species. This allows for a nuanced evaluation of their capabilities across different categories.
In the case of the Single Transfer Learning training at the resolution of 512×512 pixels, both MobileNet SSD v1 and MobileNet SSD v2 Lite achieved higher mAP values compared to the previous work at 300×300 pixels (Table 2). Specifically, for MobileNet SSD v1, the mAP increased from 0.887 in the previous work to 0.912 in the current work. Similarly, for

MobileNet SSD v2 Lite, the mAP increased from 0.884 to 0.891. This indicates that training the networks on the augmented dataset at a higher resolution improved detection performance.

Table 2. Average accuracy per class and mAP for single transfer learning training

Resolution: 512 × 512 (current work)				**Resolution 300 × 300 (previous work)**			
Mobilenet SSD v1		**Mobilenet SSD v2 Lite**		**Mobilenet SSD v1**		**Mobilenet SSD v2 Lite**	
Anoxia	0.998	Anoxia	0.977	Anoxia	0.987	Anoxia	0.991
Diabrotica	0.768	Diabrotica	0.766	Diabrotica	0.726	Diabrotica	0.727
Ladybug	0.977	Ladybug	0.909	Ladybug	0.874	Ladybug	0.891
Opatrum	0.909	Opatrum	0.909	Opatrum	0.924	Opatrum	0.909
Zabrus	0.909	Zabrus	0.895	Zabrus	0.947	Zabrus	0.903
mAP	0.912	mAP	0.891	mAP	0.887	mAP	0.884

Examining the results from the Two-step Transfer Learning training pipeline, at the resolution of 512×512 pixels, both networks showed better performance compared to the previous work (Table 3). The mAP values increased from 0.908 to 0.915 for MobileNet SSD v1 and from 0.892 to 0.919 for MobileNet SSD v2 Lite. This further confirms the advantage of using the augmented dataset at a higher resolution for training.

Table 3. Average accuracy per class and mAP for two-step transfer learning training

Resolution: 512 × 512 (current work)				**Resolution 300 × 300 (previous work)**			
Mobilenet SSD v1		**Mobilenet SSD v2 Lite**		**Mobilenet SSD v1**		**Mobilenet SSD v2 Lite**	
Anoxia	0.989	Anoxia	0.986	Anoxia	0.907	Anoxia	0.985
Diabrotica	0.787	Diabrotica	0.795	Diabrotica	0.777	Diabrotica	0.807
Ladybug	0.988	Ladybug	0.998	Ladybug	0.944	Ladybug	0.894
Opatrum	0.909	Opatrum	0.906	Opatrum	0.906	Opatrum	0.951
Zabrus	0.902	Zabrus	0.907	Zabrus	0.903	Zabrus	0.905
mAP	0.915	mAP	0.919	mAP	0.908	mAP	0.892

Comparing the performance of the two networks at the resolution of 512×512 pixels, one can notice that MobileNet SSD v2 Lite consistently outperformed MobileNet SSD v1 in terms of mAP. This suggests that MobileNet SSD v2 Lite is more effective in handling object detection tasks at the higher resolution. The accuracy per class analysis reveals that while higher input resolutions lead to improved detection performance in most cases, there are specific instances where lower resolutions yield better results. For example, in Table 2, using MobileNet SSD v1, higher accuracies at the 300×300 pixel resolution were observed for the classes *Zabrus* (0.947 at 300×300 pixels vs. 0.909 at 512×512 pixels) and *Opatrum* (0.924 at 300×300 pixels vs. 0.909 at 512×512 pixels). Similarly, for MobileNet SSD v2 Lite, the classes *Zabrus* (0.903 at 300×300 pixels vs. 0.895 at 512×512 pixels) and *Anoxia* (0.991 at 300×300 pixels vs. 0.977 at 512×512 pixels) also showed higher accuracies at the lower resolution.

In Table 3, the trend persists. For MobileNet SSD v1, the class *Opatrum* achieves higher accuracy at 300×300 pixels (0.906 at 300×300 pixels vs. 0.909 at 512×512 pixels), and *Zabrus* (0.903 at 300×300 pixels vs. 0.902 at 512×512 pixels). For MobileNet SSD v2 Lite, the classes *Diabrotica* (0.807 at 300×300 pixels vs. 0.795 at 512×512 pixels) and *Opatrum* (0.951 at 300×300 pixels vs. 0.906 at 512×512 pixels) also exhibit slightly better performance at the lower resolution.

These variations suggest that while higher resolutions are generally advantageous, the optimal resolution can vary depending on the specific class. This nuanced behavior highlights the complexity of object detection tasks, particularly in agricultural environments, and the importance of selecting the appropriate resolution for each specific use case.

Conclusions

This study investigated the impact of input resolution on the performance of two MobileNet-SSD neural network architectures for crop pest detection, focusing on the Coleoptera order. By increasing the input resolution from 300×300 to 512×512 pixels, we aimed to assess whether higher-resolution images would yield improved accuracy in pest identification. Our findings suggest several key conclusions:

5. Both MobileNet SSD v1 and MobileNet SSD v2 Lite demonstrated enhanced mAP scores when trained on datasets with a resolution of 512×512 pixels compared to 300×300 pixels. This improvement indicates that higher-resolution images provide more detailed input representations, facilitating better detection performance.
6. The use of a two-step transfer learning approach resulted in improved detection performance for both MobileNet SSD v1 and SSD v2 Lite at the higher resolution.
7. Most of the time, MobileNet SSD v2 Lite slightly outperformed MobileNet SSD v1 in terms of mAP across both training phases at the resolution of 512×512 pixels.
8. The findings of this study have practical implications for pest control in agriculture. By using higher-resolution images on neural networks for limited-power devices such as MobileNet SSD, farmers and agricultural professionals can benefit from more accurate and efficient pest detection systems, leading to improved crop management practices and reduced environmental impact.

Overall, this research contributes to a deeper understanding of the relationship between input resolution and detection performance in convolutional neural networks, offering valuable insights for optimizing pest detection systems in agricultural contexts. Future studies may explore additional factors such as dataset size, augmentation techniques, and model architecture modifications to further enhance the precision and efficiency of crop pest detection systems.

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IMPROVING ENERGY EFFICIENCY IN ROMANIAN AGRICULTURE: MECHANICAL ENGINEERING'S CHALLENGES AND CONTRIBUTIONS

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Abstract

Romania's agricultural sector serves as a vital pillar of the nation's economy, ensuring food security and economic stability for its population. This study investigates the complex array of issues surrounding energy utilization in Romanian agriculture, with a specific focus on the significant role played by mechanical engineering in addressing these challenges. Traditional farming methods, such as irrigation, plowing, and harvesting, often rely on outdated machinery and inefficient practices, resulting in excessive energy waste. By leveraging advanced technologies like smart sensors, automation, and data analytics, mechanical engineering solutions can optimize crop production, minimize energy usage, and mitigate environmental harm. However, the adoption of these innovations encounters formidable obstacles within the Romanian context, including financial constraints and limited access to modern equipment and technology.The integration of energy-efficient practices through mechanical engineering interventions is pivotal for promoting sustainable and eco-friendly farming techniques in Romania. These innovations have the potential to significantly reduce energy consumption, resulting in cost savings for farmers and a reduction in greenhouse gas emissions. Nonetheless, the widespread implementation of such advancements faces resistance due to initial financial burdens and deficiencies in infrastructure and knowledge.This paper aims to delve into the challenges associated with energy consumption and efficiency in Romanian agriculture, highlighting the vital contributions of mechanical engineering in addressing these issues. By raising awareness about the inefficiencies inherent in conventional farming practices and their associated costs and environmental repercussions, the study seeks to advocate for the adoption of sustainable and energy-efficient methodologies in Romanian agriculture.

Keywords: *agriculture, energy efficiency, electric tractors.*

Introduction

The agricultural industry plays a crucial role in ensuring the food supply for humanity. To perform a wide array of activities within the agricultural and food production chain, spanning from fundamental tasks like soil cultivation, planting, pest control, harvesting, irrigation, and drying, to intermediate processes like storage and packaging, as well as more advanced functions such as sourcing essential supplies and constructing farm infrastructure, a dependable energy source is essential (Becerril et al., 2016; Gorjian et al., 2021). In order to conduct operations in the field and facilitate large-scale production, the utilization of agricultural machinery becomes imperative. This necessitates the consumption of resources like fossil fuels and the procurement of various materials. While the mechanization of agriculture has undoubtedly improved profitability, it has also led to increased energy requirements, heightened water usage, and the elevation of greenhouse gas emissions, posing significant sustainability challenges (Balafoutis et al., 2017).

Agricultural equipment, such as tractors, combines, wagons, loaders, pickups, and trucks, holds a vital role within the agricultural sector, as they are utilized for a wide range of farm activities across small to large-scale farms worldwide (Malik et al., 2020). Enhancing agricultural processes through optimization, automation, mechanization, and efficient resource management has the potential to promote sustainability in the agricultural sector, leading to increased production and income. Nonetheless, this approach is accompanied by elevated energy requirements for agricultural operations. Moreover, to meet the growing global food demand, the expansion of agricultural land and the workforce are essential. Within this framework, precision agriculture (PA) emerges as a more beneficial solution, offering food security with reduced labor demands, enhanced environmental stewardship, and decreased energy consumption (Piechocki et al., 2018, Saiful et al 2020).

Material and methods

PV technology in agriculture. Photovoltaic (PV) technology in agriculture refers to the use of solar panels or solar cells to capture sunlight and convert it into electricity for various applications in farming and agriculture. This technology offers several benefits, including sustainability, cost savings, and increased energy independence. Agrivoltaics, or the concept of agrophotovoltaics (APV), involves the concurrent utilization of agricultural fields for both farming and photovoltaic power generation. This innovative approach addresses the issues of limited available land while expanding photovoltaic capacity and preserving valuable arable land for a wide range of crops (Dupraz C., et. al., 2011).

Electric vehicle technologies. Electric vehicles (EVs) typically refer to automobiles that employ one or more electric motors for propulsion. These EVs are further categorized into two main types: Battery Electric Vehicles (BEV) and Hybrid Electric Vehicles (HEV), as depicted in Figure 1.

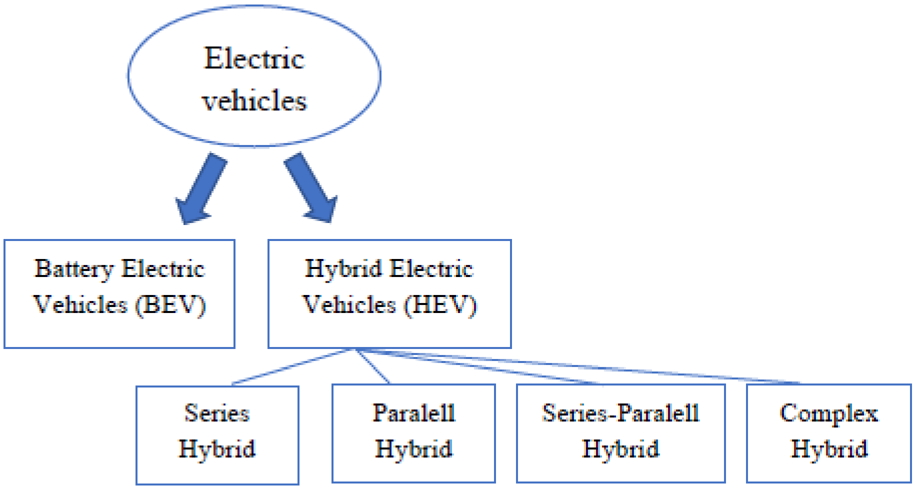

Figure 1. Electric Vehicles classification

While the agricultural and forestry sectors have historically been major contributors to global emissions due to their reliance on diesel-powered machinery, there is a notable shift towards achieving environmental sustainability in these industries. The adoption of electric vehicles (EVs) for agricultural purposes is now a pivotal step in ensuring sustainable food production and reducing greenhouse gas emissions (Magalhaes et al., 2017). Multiple agricultural researches indicates that designing and developing an effective tillage system can result in achieving a better desired soil behavior while using less draft force and energy. It is widely recognized that the oscillation of tillage equipment can effectively lower the draft and drawbar power needs, offering a potential solution to this issue (Shagoli et al 2010). In the context of the transition to a low-carbon economy, the production and use of energy from renewable sources is one of the ways to reduce GHG emissions. Romania has a wide range of renewable energy resources that can be used in the actions proposed in the rural development projects. There is also a wide range of potential renewable energy production resources from agriculture (crop residues resulting from agricultural harvesting or animal waste). These resources, together with other renewable resources (solar, wind, geothermal, etc.) can be used to obtain renewable energy to be used in the production process of farms or processing units, thus helping to reduce energy costs (electricity and heat) and contributing to efforts for developing a low-carbon economy (Romania: new financing for renewables in agriculture, 2021). Romanian agricultural operations face limited digital integration primarily because of the substantial expenses associated with digital infrastructure and the scarcity of financial resources. Nevertheless, Romanian farmers exhibit a readiness and awareness to enhance farm management by embracing digital technologies (Rodino et al., 2023). In Romania, reduced soil tillage methods commonly employ equipment like the chisel plow, which minimizes soil disturbance while promoting aeration and water infiltration. Strip-till machines, another popular choice, till only narrow strips where seeds will be planted, preserving soil structure and reducing erosion. Disk harrows, used for shallow tillage, effectively manage crop residues and prepare seedbeds with minimal soil turnover. No-till drills directly plant seeds into the residue-covered soil, maintaining soil health and moisture. These machines collectively contribute to sustainable farming practices, enhancing soil conservation and crop yields.

Results and discussions

Romania's strength regarding renewable energy is the wide range of available renewable energy resources and weakness the low level of production and use of renewable energy resources in the agricultural and forestry sector. Opportunities in the programme area are connected to widespread access to renewable energy technologies, while increase in energy prices is seen as a threat to the area. The initial expense for any electric vehicle, including electric tractors (Figure 2), is typically two to three times greater than that of a similarly capable internal combustion engine (IC engine) vehicle. The cost of the battery constitutes a significant portion, ranging from 30% to 50% of the total vehicle cost.

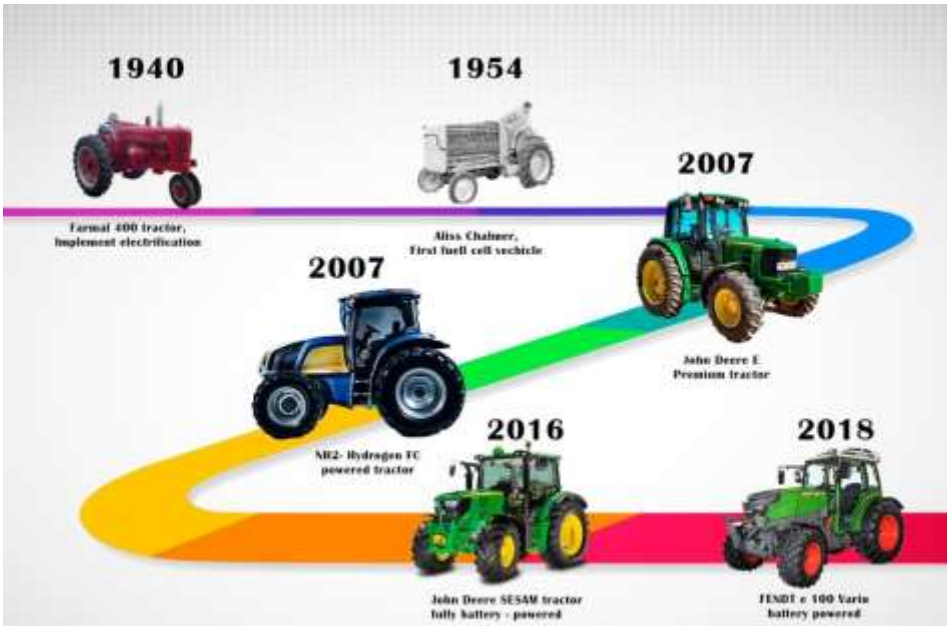

Figure 2. Historical development of hybrid tractors, adapted from (Ghobadpour et al., 2019).

Romania possesses the requisite resources for implementing the technology of cultivating straw cereal crops using a reduced tillage system, thanks to the research conducted by INMA Bucharest. This research has resulted in the development of specialized equipment (as depicted in Figure 3) capable of simultaneously performing tillage and sowing of straw cereal crops, even on partially prepared or unprepared fields. This innovative approach significantly mitigates issues related to soil compaction, energy usage, and labor costs (Marin et al., 2011).

Figure 3. Technical equipment endowed with working parts for preparing the soil and sowing straw cereals

Table 1 offers an insightful comparison of electric tractors gaining popularity in Romania, showcasing models from Fendt, John Deere, Farmtrac, Solectrac, and Rigitrac. Each tractor is evaluated based on its battery capacity, power output, operating time, charging time, and key features, providing a clear understanding of their performance and suitability for different farming needs. For example, the Fendt e100 Vario, with a 100 kWh battery and 67 hp, operates for up to 5 hours and charges quickly in 5 hours. It's noted for its versatility and zero emissions, making it ideal for diverse farm tasks. The John Deere SESAM, featuring a robust 130 kWh battery and 400 hp, offers up to 4 hours of operation and a rapid 3-hour fast charge, emphasizing advanced technology for sustainable energy use.The Farmtrac FT25G is compact and affordable, with a 21 kWh battery and 25 hp, providing up to 6 hours of operation, suitable for small-scale farms. Solectrac's eUtility model, equipped with a 40 kWh battery and 70 hp, ensures up to 8 hours of efficient and eco-friendly performance, ideal for medium-sized farms. The Rigitrac SKE 50, with an 80 kWh battery and 50 hp, offers up to 6 hours of robust operation, handling various terrains effectively. This detailed comparison underscores the growing adoption of electric tractors in Romania, highlighting their environmental benefits and potential to enhance sustainable farming practices.

Table 1. Comparison of Leading Electric Tractors in Romania

Brand & Model	Battery Capacity	Power Output	Operating Time	Charging Time	Key Features
Fendt e100 Vario	100 kWh	67 hp	Up to 5 hours	5 hours (fast charge)	Versatile, zero emissions, suitable for various farm tasks
John Deere SESAM	130 kWh	400 hp	Up to 4 hours	3 hours (fast charge)	High power, sustainable energy supply, advanced tech
Farmtrac FT25G	21 kWh	25 hp	Up to 6 hours	6 hours	Compact, affordable, practical for small-scale farms
Solectrac eUtility	40 kWh	70 hp	Up to 8 hours	4-6 hours	Eco-friendly, efficient, good for medium-sized operations
Rigitrac SKE 50	80 kWh	50 hp	Up to 6 hours	6 hours	Robust design, all-electric, suitable for various terrains

Oscillating mode tools have several advantages over non oscillating one. Oscillating tools requires less draft as compared to non-oscillating one. Therefore, oscillatory tillage tool can resolve the problems related to tillage applications.

Figure 4. Elastic supports for the working bodies of cultivators

Many studies have been conducted regarding the influence of the angle of attack of vibratory working tools (Figure 4), with the main objectives of the research being be to find the most efficient oscillation angle for draft reduction and low power requirement. At the end of January 2021, the National Rural Development Programme of Romania was approved. The SWOT analysis carried out to elaborate the document revealed the current situation of renewable energy consumption in agriculture, noting that the production and use of energy from renewable sources in agriculture (RES) are low. Only 2.5% of the total production in Romania came from agriculture compared to 9.8% in the EU27.

Through measure 4.1 the type of support is in the form of grants with reimbursement of eligible costs actually incurred and payed in advance. Eligible beneficiaries may be farmers (except for unauthorized individuals), cooperatives (agricultural cooperatives and agricultural cooperative societies), and producer groups (set up on the basis of the national legislation in force, serving the interests of the members; and have eligible costs for investments in installations whose main purpose is the production of electricity). Measure 4.2 "Support for investments in the processing / marketing of agricultural products" - grants will be awarded for eligible activities such as the production and use of energy from renewable sources (solar, wind, geothermal), by heat pumps, exclusively for own consumption and also grants for investments to improve energy efficiency. The launch of financing measures is eagerly awaited - there is major interest from rural communities to submit and implement projects that generate energy produced sustainably, thus succeeding to reduce greenhouse gases generated by agricultural activities (Romania: new financing for renewables in agriculture, 2021).

Conclusions

The introduction of energy-efficient machinery and electric vehicles in Romanian agriculture is becoming increasingly popular due to their ability to lower operational expenses and mitigate environmental impact. These vehicles provide a greener alternative to conventional fossil fuel-powered equipment. Despite this, obstacles such as significant initial investment and insufficient charging infrastructure must be overcome to achieve broader adoption. Incorporating electric vehicles into Romanian agriculture offers potential for greater efficiency and reduced emissions, supporting the country's environmental sustainability goals. Ongoing research and investment are essential to fully realize the benefits of electric vehicles in Romania's agricultural industry.

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PNEUMATIC CONVEYING SYSTEMS IN THE AGRI-FOOD INDUSTRY – STATE OF THE ART

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Abstract

Pneumatic conveying systems for agri-food materials have become a crucial part of controlling the flow of raw materials along with finished goods in an era defined by the fast pace of technical development and higher productivity demand within the agri-food industry. The agri-food sector must contend with several obstacles, such as strict regulations governing food safety, elevated product quality standards, and high operational efficiency requirements. The selection of pneumatic conveying systems, whether low, medium, or high pressure, is subject to various criteria, including the distance to be transported, the necessary throughput rates, the properties of the material, and budget constraints. Pneumatic conveying systems offer several benefits to the agri-food industry, such as effective handling, space optimization, cost savings on material shipping and storing, and the supply of an inert transport environment that prevents contamination or deterioration of agri-food products. This paper presents a multi-point perspective overview of today’s status of pneumatic conveying systems research and development, aspects such material handling efficiency, pressure control and monitoring, integration with Industry 4.0 technologies, environmental sustainability, and safety features. Taking all into account, the state of the art in pneumatic conveying systems is a result of a blend between industry-specific adaptation, technological innovation, and an increasing focus on efficiency and sustainability.

Keywords: *pneumatic systems, material handling, conveyors, agri-food.*

Introduction

Conventional conveyors are predisposed to dust and foreign matter entering the systems, generating material quality degradation and consequent losses from material that is no longer appropriate to be used. Pneumatic conveyors are progressively replacing traditional ones. One of the reasons for this migration is product contamination, still a key worry for end-users.

Pneumatic conveying, an essential component in a wide range of sectors, from food processing to pharmaceuticals, mining to chemical engineering, has withstood the test of time due to its effectiveness in transferring bulk materials over long distances. The handling of granular materials, ranging from micrometers to millimeters in size, poses significant challenges in the packaging industry. The flowability of these materials is crucial for the efficient operation of bulk solids handling equipment. Apart from cohesive effects, the flow regimes are primarily influenced by particle collisions and friction. Flowability is also affected by particle characteristics such as size, shape, density, and surface properties, as well as environmental conditions like humidity and temperature. However, the impact of complex grain-scale interactions on bulk behavior is not yet fully understood (Jones, 2021).

The main market participants are investing in the introduction of new technologies. Industry forecasts suggest that the global market for conveying systems is expected to see significant growth within the next three to five years (Klinzing, 2018).

Recent advancements in pneumatic conveying technology hold substantial potential to revolutionize material transport across various sectors by offering more energy-efficient and environmentally friendly solutions. These improvements could significantly lower costs, enhance productivity, and reduce environmental impacts, benefiting industries like pharmaceuticals, food, cement, minerals, and power (Abe, 2023).
This paper provides a comprehensive overview of current research and development in pneumatic conveying systems, focusing on aspects such as material handling efficiency, pressure control and monitoring, integration with Industry 4.0 technologies, environmental sustainability, and safety features. It suggests that the state-of-the-art ~~p~~pneumatic conveying systems emerge from a combination of industry-specific adaptations, technological advances, and a growing emphasis on efficiency and sustainability.

Material and methods

The forms of pneumatic conveying are the dilute phase and the dense phase. Each type moves materials using air pressure and an enclosed line or tube. The difference between them is their method of creating air pressure and how the material travels through the system.
There are two methods for moving materials using dilute, suspension, or lean phase pneumatic conveying: positive and negative. In a positive or blowing phase system, a fan or blower generates pressure in the line to suspend the material. At the end of the line, a separator or filter removes the material (Javaid, 2023).

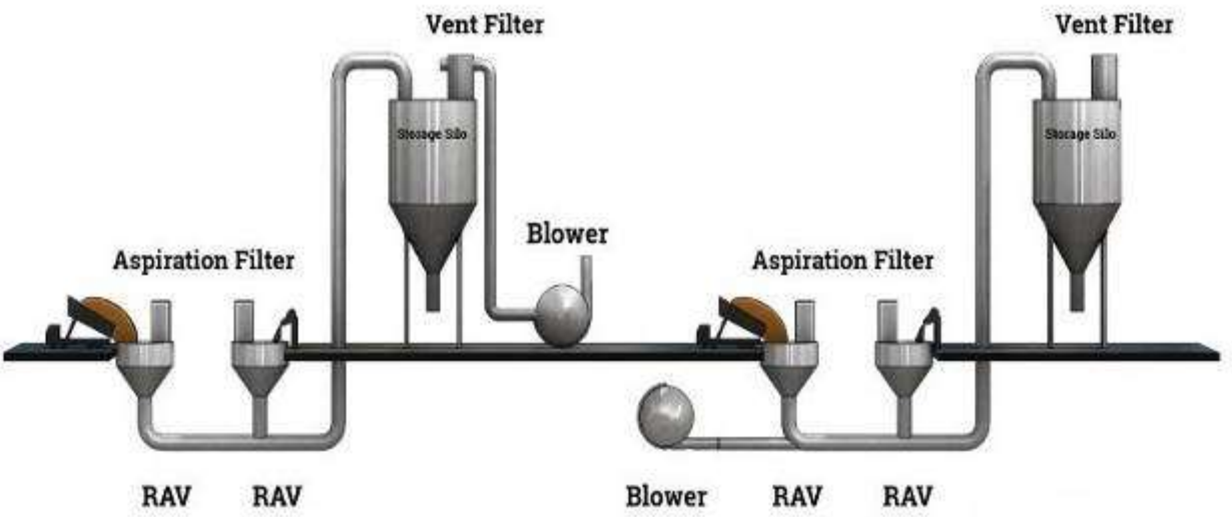

Fig.1 Dilute phase pneumatic conveyor (Javaid, 2023)

Dense-phase pneumatic conveying uses a small amount of air to move a large amount of material in slugs, much like extruding. A dense phase system pushes a denser concentration of bulk solids at low velocities, as can be seen in Figure 3 below.

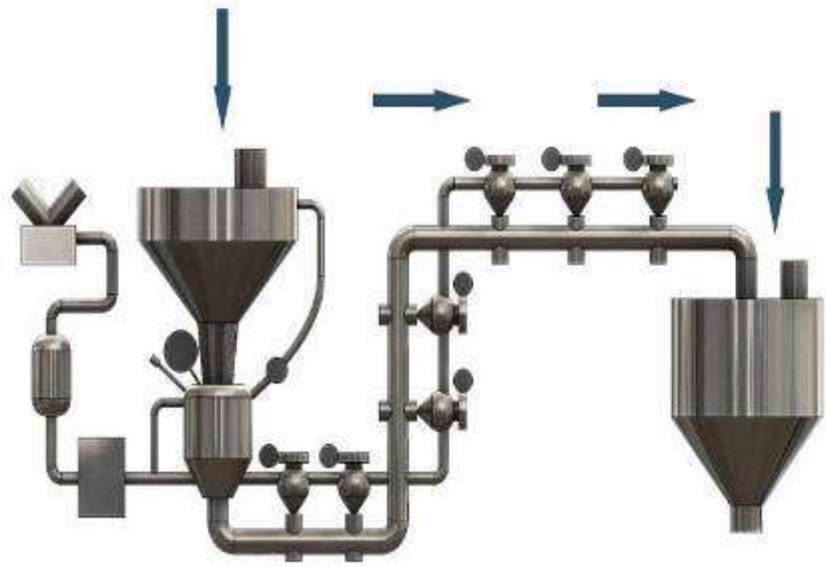

Fig.2 Dense phase pneumatic conveyor (Javaid,2023)

The dilute phase moves materials at high velocities under pressure and has some breakage during transport. The dense phase is used for fragile materials since it operates at low pressure. The dilute phase has a high air-to-product ratio, while the dense phase has a low ratio. Figure 3 below shows the difference between the dilute phase on the left and the dense phase on the right (Javaid, 2023).

Fig.3. Dilute and dense phase pneumatic conveying (Javaid, 2023)

Results and discussion

With the advent of Industry 4.0 and the increasing integration of industrial IoT technologies, cloud systems, and advanced analytics, the field of traditional pneumatic conveying is set for significant changes. At the heart of this evolution is the concept of 'digital twinning'. Digital twinning goes beyond a mere technological trend; it represents a transformative method that unites the physical and digital realms. Essentially, it involves creating a dynamic, virtual representation of a physical system or entity. This digital twin, continuously updated with real-time data from sensors and other sources, reflects the condition and performance of its physical counterpart (Salihler, 2023).
This advanced technology enhances operational efficiency, reduces power consumption and component wear, and offers real-time, comprehensive insights into the conveying system. Its predictive capabilities allow for proactive measures against potential blockages, ensuring smoother operations. Furthermore, digital twins enable better planning and optimization of

the process, contributing to improved workflow coordination and overall operations management (Premarathna, 2023).

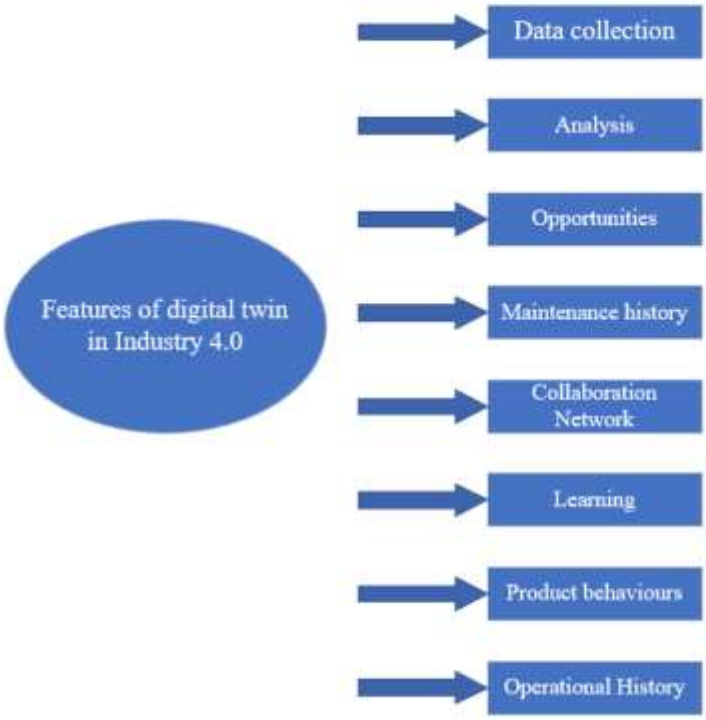

Fig.4. Features of Digital Twin in Industry 4.0 (Javaid,2023)

A fundamental framework for digital twin implementation is demonstrated in Figure 5, illustrating the division between the physical space, the digital space, and an optional web space that facilitates remote monitoring and control via 3D graphical representation (Premarathna, 2023).

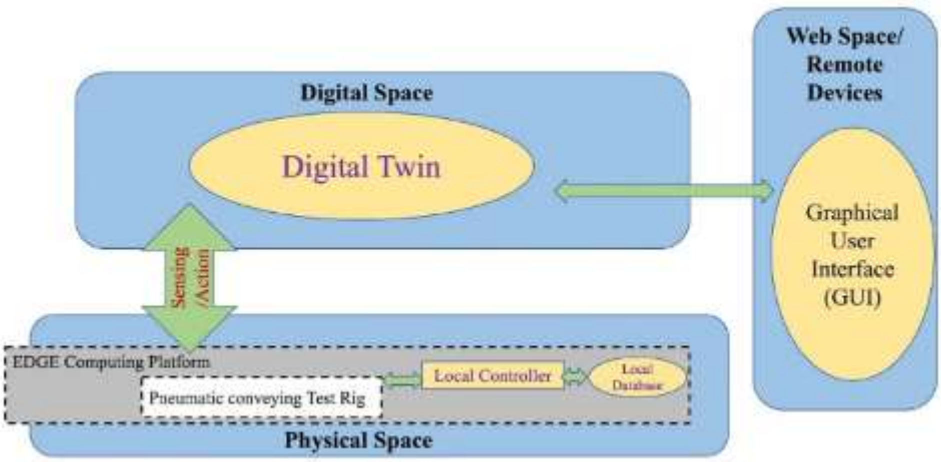

Fig. 5 Fundamental Framework for a Digital Twin (Premarathna, 2023)

One of the most advanced pneumatic conveyors is the Gericke Pulse-Flow PTA Dense Phase presented in Figure 6, which utilizes pulse-flow technology to achieve efficient dense phase conveying. It minimizes air consumption while ensuring gentle handling of materials, making it suitable for fragile products (Gericke, 2023).

Fig.6. Gericke Pulse-Flow PTA Dense Phase conveyor (Gericke, 2023)

One of its main advantages is that has enhanced material flow control, reduced energy consumption, and minimized wear on system components. Gericke says the system can be Custom-engineered for each application, matching the product design to the desired throughput rates, conveying distances, and other requirements in either batch or continuous operation (Plastics technology, 2019).

Aerocon has conducted research and tests on VAC-U-MAX Aero-Mechanical Conveyors which enable the user to move certain products at critical speeds, approaching the equivalents of dense or dilute phases in the system. High speed, lean (or dilute) phase conveying fluidizes the material and handles particles more gently than pneumatic conveying. The dynamics of fluidization within the cable-disc configuration, around the corners, causes minimal impact between particles and against the housing. The result is less particle degradation, less conveyor wear, than with dilute pneumatic conveying (Anonymous, 2024).

Fig.7. VAC-U-MAX Aero-Mechanical Conveyor (Anonymous, 2024)

The E-Finity system presented in Figure 8 is a patented continuous dense phase system from the Schenck Process, which provides gentle and energy-efficient conveying of granular and pelleted products. Traditional continuous dense phase systems use blowers and rotary valves, which can cause unstable air leakage due to the increased pressure in the pipeline. To overcome this problem, E-Finity uses precise pressure monitoring to control the valve and manage the airflow, automatically compensating for any leakage or changes in temperature. With the E-Finity conveying system, Schenck Process can carry out comparative monitoring of conveying air, pressure, feed rate, leakage, and power consumption.

Fig.8 Advances in pneumatic conveying technology showcased at UK Test & Innovation Centre

The news is that Schenck Process FPM is becoming Coperion - Effective August 1, 2024, the company will officially transition its name under Coperion. Coperion designed the High-pressure ZVH rotary valve presented in Figure 9, used for continuous gentle conveying of coarse granular or pellet size, friable or Temperature-sensitive materials such as plastic pellets, coffee beans, grains, etc.
A Dense-phase rotary valve system is ideal for achieving high conveying capacities, even at long distances. These parameters are possible due to the rotary valve’s ability to withstand 3.5 bar(g) while minimizing leakage and through use of the Coperion LMR-X air control unit.

Fig.9 High Pressure Rotary Valve

Another research for the food and beverage sector was conducted by Bürkert, designing FLOWave, a sensor that is easy to integrate into an existing installation and its control infrastructure. The ability to offer improved control systems that reduce waste, save time, and maintain rigorous hygiene standards are distinct advantages for the industry. Figure 10 shows the sensor installed on a food sector pneumatic conveyor (Bürkert, 2020).

Fig.10 FLOWave sensor mounted on the pneumatic conveyor (Bürkert, 2020)

Conclusions

In the agri-food sector, pneumatic conveying systems are essential for effective and controlled movement of products. Proper handling, space efficiency, cost savings, and contamination protection are just a few advantages they provide. Technological advancements, particularly digital twins, enhance operational efficiency, provide real-time insights, and improve planning and monitoring.

Innovative solutions for material flow control and hygiene standards are demonstrated by case studies such as the Bürkert FLOWave sensor and the Gericke Pulse-Flow PTA Dense Phase conveyor. The emphasis on energy conservation and environmental friendliness is growing, lowering costs, and reducing environmental impacts. Numerous industries, including pharmaceuticals, food, cement, minerals, and power, benefit from these improvements. Integration of digital twinning and IoT technologies is set to transform pneumatic conveying systems, emphasizing efficiency and sustainability.

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PLECTOSPHAERELLA CUCUMERINA − NEW AND EMERGING PATHOGEN OF LETTUCE IN BALKAN REGION

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Abstract

In March 2021, unusual plant stunting, collar and root rot of lettuce during rosette stage were observed in two commercial fields in Serbia. Initial above-ground symptoms were yellowing and wilting of leaves, while below-ground symptoms were collar and root rot. Eventually, whole plants wilted, collapsed and died. In total, twenty *Plectosphaerella* spp. isolates obtained from 35 symptomatic lettuce plants were identified on the basis of morphology and evaluated for their pathogenicity. To confirm the species identity, internal transcribed spacer (ITS) region and the D1/D2 region, of a selected representative isolate 13-3-c, were amplified and sequenced by using primer pairs ITS1/ITS4 and N1/N2, respectively. Sequence analysis of both regions revealed 100% nucleotide identity with *Plectosphaerella cucumerina* isolates from different countries deposited in the NCBI GenBank. Neighbour-joining analysis was conducted based on combined ITS and D1/D2 regions, and the tree was constructed with the substitution models. Combined phylogeny confirmed that the sequences shared a common clade with *P. cucumerina*. To our knowledge, this is the first molecular identification of *P. cucumerina* on lettuce or any other crops in Serbia and Balkan region. *P. cucumerina* has already been known as a pathogen of lettuce and other hosts grown in many countries worldwide, as well as in some European countries. This emerging pathogen may cause significant economic losses in lettuce production in Serbia and in the other Balkan countries. Our results may help to develop efficient monitoring and detection of *P. cucumerina* and effectively manage the spread of the disease.

Keywords: *Lactuca sativa, soilborne pathogen, detection.*

Introduction

Leafy vegetables, especially lettuce (*Lactuca sativa* L.), are economically important crops with a relevant role in the diet all over the world, and are grown worldwide under intensive cultivation systems (Das and Bhattacharjee, 2020). Fungal pathogens are major threat for producers and can cause significant losses under favourable conditions. Recently, a new fungal disease of lettuce caused by *Plectosphaerella cucumerina* in Serbia and the Balkan area was detected (Mihajlović et al., 2024). *Plectosphaerella* species are causal agents of root and collar rot, vascular and leaf symptoms in different hosts (Raimondo and Carlucci, 2018). This filamentous ascomycete is cosmopolitan in distribution, and acts as both and airborne pathogen being able to infect aboveground and belowground plant parts (Palm et al., 1995; Carlucci et al., 2012; Gao et al., 2016). *P. cucumerina* causes brown-black lesions, which, on older leaves, are surrounded by a yellow halo, causing the progressive defoliation of the plants and, on rare occasions, plant death. Its ability to survive as a saprotroph in soil, its polyphagous nature and the ability to contaminate seeds, make its management difficult (Domsch and Gams, 1972). *P. cucumerina* has already been known as a pathogen of lettuce and other hosts grown in many countries worldwide, as well as in some European countries such as Belgium, England, Italy, Netherland and Switzerland (Zhang et al., 2019). In Serbia, it was discovered recently (Mihajlović et al., 2024). However, there is no any additional information about the pathogen features, or the damage it causes in lettuce plants. Therefore, the objectives of this study were: 1) to estimate the disease incidence in inspected fields and 2) to characterize the causal agent of collar and root rot of lettuce caused by *P. cucumerina* using morphological and molecular methods.

Materials and methods

In March 2021, unusual plant stunting, collar and root rot of lettuce during rosette stage were observed in two commercial fields in Banat region of Serbia. To identify the causal agent, a total of 35 symptomatic plants were collected from the fields. Diseased tissue from the collar of all collected plants were cut into small pieces, surface sterilized with 70% ethanol for 1 min, rinsed three times in sterile distilled water and plated on potato dextrose agar (PDA) for 10 days at 25°C. From the developed colonies mono-hyphal-tip isolates were derived and used for pathogenicity tests. Cultural characteristics and morphological features of the isolates were determined on PDA (Palm et al., 1995; Carlucci et al., 2012). To confirm the species identity, internal transcribed spacer (ITS) region and the D1/D2 region, of a selected representative isolate 13-3-c were amplified and sequenced by using primer pairs ITS1/ITS4 (White et al., 1990) and N1/N2 (O’Donnell and Gray, 1995), respectively. Neighbour-Joining method phylogenetic tree was constructed from ITS and D1/D2 regions of ribosomal RNA gene sequences of 17 selected *Plectosphaerella* sp. isolates. The phylogram was generated with MEGA 11 using the p-distance model. Bootstrap analysis was performed with 1,000 replicates. In pathogenicity assay 10 isolates were tested. Five 30-day-old lettuce plants (cv. Majska kraljica) per isolate were root-dipped in the conidial suspensions (1×10^5 conidia/ml) prepared from 10-day-old colonies grown on PDA, transplanted into 1 L pots containing sterile substrate (Floragard, Germany) and maintained in a greenhouse at 25-28°C under a 12-h photoperiod (Cai et al., 2021). Five plants treated with sterile distilled water were used as controls. Inoculated plants were inspected daily for the occurrence of symptoms. After the appearance of disease symptoms, the pathogen was re-isolated on PDA. Then, the morphological features of the re-isolated fungi were compared and matched with the original ones used for inoculation.

Results and discussion

Unusual symptoms on lettuce plants were noticed in two commercially cultivated fields in the Banat region of Serbia, (44°58'N, 20°32'E; 44°45'N, 20°43'E) during March 2021. Disease incidence in the fields (≈ 0.9 ha each) was approximately 15 and 20%. Initial aboveground symptoms were yellowing and wilting of leaves, while belowground symptoms were progressive collar and root rot.

Figure 1. Symptoms of collar root rot on lettuce plant collected from the field

Eventually, whole plants wilted, collapsed and died (Figure 1). Isolation resulted in 20 morphologically uniform monoconidial isolates, derived from 35 diseased lettuce plants. The isolates formed white to creamy colonies, gradually becoming salmon pink in colour, slimy, or moist in appearance, with a sparse aerial mycelium after 10 days on PDA at 25°C. Numerous hyphal coils with conidiophores and conidia were formed. Conidia were hyaline, smooth-surfaced, ellipsoid to ovoid, septate or aseptate, 4.5-10.07×1.2-3.7 μm in size (n=100). Chlamydospores were absent (Figure 2). Morphological characteristics of the isolates were similar to the description of *Plectosphaerella cucumerina* (Lindf.) (anamorph: *Plectosporium tabacinum*) (Palm et al., 1995; Carlucci et al., 2012).

Figure 2. Left: Culture characteristics of a Plectosphaerella cucumerina isolate 13-3-c, grown on potato dextrose agar for 10 days. Right: Conidia of a Plectosphaerella cucumerina isolate 13-3-c (X40)

The ITS sequence analysis revealed 100% nucleotide identity between representative isolate 13-3-c isolate (OR880564) and *P. cucumerina* isolates from different countries deposited in the NCBI GenBank, including isolate MH860704 (Vu et al., 2019), while the D1/D2 region showed 100% nucleotide identity between the 13-3-c isolate (OR880567) and *P. cucumerina* isolate KY662256 (Su et al., 2017). Sequence was deposited in GenBank (ITS - OR880564 and D1/D2 - OR880567). Combined phylogeny confirmed that the sequences shared a common clade with *P. cucumerina* (Figure 3).

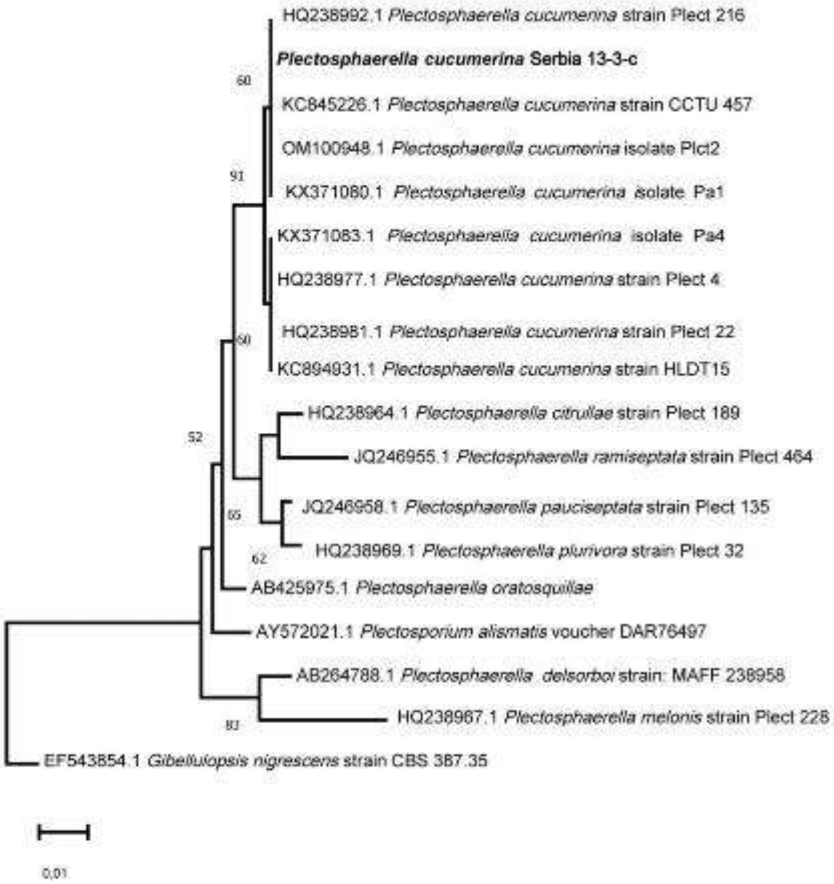

Figure 3. Neighbour-Joining method phylogenetic tree inferred from ITS and D1/D2 regions, region of ribosomal RNA gene sequences of 17 selected *Plectosphaerella* sp. isolates. The phylogram was generated with MEGA 11 using the p-distance model. Bootstrap analysis was performed with 1,000 replicates, and bootstrap values (>50%) are shown next to the branches. There were a total of 377 positions in the final dataset. *Gibellulopsis nigrescens* isolate CBS 387.35 is included as an outgroup. *Plectosphaerella cucumerina* isolate obtained in this study from Serbia is indicated in bold.

The results of the pathogenicity test revealed that *P. cucumerina* is the causal agent of lettuce collar and root rot. Four weeks after inoculation, stunting, chlorosis and wilting of plants were observed, while collars and roots of inoculated plants exhibited typical decaying symptoms. No symptoms were observed on the control plants. The pathogen was re-isolated from symptomatic tissue as previously described, while isolation from control plants was unsuccessful. Koch's postulates were completed by confirming the identity of re-isolates based on morphological features.

P. cucumerina has been detected on numerous crops including tomato, fennel, soybean, muskmelon, potato, pepper, and other important commercial crops (Abad et al., 2000; Cai et al., 2021). In addition, it has already been known as a pathogen of lettuce and other hosts grown in many countries worldwide, including some European countries such as Belgium, England, Italy, Netherland and Switzerland (Zhang et al., 2019). In Serbia, it was discovered recently, but there is no information on the damage and distribution of diseases caused by *Plectosphaerella* species. Additionally, *P. cucumerina* on lettuce or any other crops is still not reported in other Balkan countries. In recent years, *P. cucumerina* has become more visible worldwide as a new or emerging pathogen on plants (Abad et al., 2000; Cai et al., 2021). The wide distribution and broad host range of *P. cucumerina* can present it as a severe threat to Balkan lettuce production region.

Conclusion

More research is needed to better understand biology of *P. cucumerina*, and minimize the spread of the pathogen to the other lettuce-producing areas. This emerging pathogen may cause significant economic losses in lettuce production in Serbia and in the other Balkan countries. Due to the economic importance of lettuce, the management of the disease should be considered in further studies.

Acknowledgment

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EFFICACY OF PYRAFLUFEN-ETHYL IN THE CONTROL OF SUCKERS IN RASPBERRY, APPLE AND PLUM ORCHARDS IN SERBIA

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Abstract

Suckers can have adverse effects in fruit orchards for various reasons. In addition to their competition with the plant, they also provide hiding places for insect pests, promote favorable environmental conditions for disease development, serve as entry points for plant diseases, and interfere with in-row weed management. The efficacy of a herbicide based on pyraflufen-ethyl (26,5 g a.s./l) in the control of suckers was studied in raspberry, apple and plum orchards across four field sites in Serbia (Kušići, Zminjak, Padinska Skela and Morović). The trials were set up following the standardized EPPO protocol, and herbicide application was done in spring, when the longest suckers were 10-15 cm long. In the split application treatments, the second treatment was done when the new suckers had reached a length of 10-15 cm. The efficacy of the tested product was assessed in comparison to the untreated control. Pyraflufen-ethyl was shown to be highly effective in controlling the number of suckers (>90%), both in single and split application in all three orchards. The highest level of efficacy in raspberry and plum orchards was observed in the split application treatment, when the tested product was applied at a dosage of 0,8 l/ha + 0,8 l/ha. When applied in combination with the adjuvant Alteox T prima, the highest efficacy levels were also observed in the split application treatments. In contrast, the efficacy of the inhibition of subsequent regrowth was comparatively lower, with efficacy percentages between 69 and 84% in raspberry, 50 and 64% in plum and between 51 and 65% in apple orchards.

Key words:*, herbicide efficacy, sucker control, pyraflufen-ethyl, orchards.*

Introduction

Plant suckers represent natural, but unwanted, vegetative growth of many deciduous fruit trees (Smith and Gutierrez, 2014). Suckers can occur either in the form of basal shoots, growing at the base of a tree (Lippi et al. 2023), or as crown suckers, when buds located at the point of branch curvature produce suckers, as a result of apical dominance (Meyer et al., 2016). In raspberry plantations, suckers develop from root buds, occupying the inter-row and must be removed to avoid competition with the crop (Graham et al., 2007).
In general, plant suckers have many potentially adverse effects in fruit plantations. In addition to their competition with the plant for light, water, and nutrients, potentially resulting in yield losses (Graham et al., 2007; Pacchiarelli et al., 2022), they also provide hiding places for insect pests (e.g. wooly apple aphid; Johnson et al., 2020), promote favorable environmental conditions for disease development and/or serve as entry points for plant diseases (e.g. fire blight in apples; Aćimović et al. 2023), and disrupt weed management within rows (Johnson et al., 2020).

Chemical control of plant suckers usually requires several treatments during the growing season, due to their constant regrowth. These treatments must be performed when the suckers

are herbaceous and reach a height of 15–20 cm. Alternatively, their management can also be conducted using physical techniques later in the season, when they have lignified (Pacchiarelli et al., 2022). However, manual removal of suckers is very expensive, and consequently only possible in cases when their presence is limited (Smith and Gutierrez, 2014). Chemical control of plant suckers can be performed using herbicides, plant growth regulators, or desiccants (Johnson et al. 2020). In conventional agriculture, the application of herbicides remains the most widely used control method for the management of plant suckers (Smith and Gutierrez, 2014), as it offers two main advantages - quick implementation and lower cost, when compared with other management techniques (Serdar and Akyuz, 2017).
Pyraflufen-ethyl is a fast-acting and effective contact herbicide, primarily used for POST-EM control of broadleaf weeds. It is also used to control suckers in fruit trees (Johnson et al., 2020). The efficacy of a herbicide based on pyraflufen-ethyl (26,5 g a.i./l) in controlling suckers was studied in raspberry, apple and plum orchards in Serbia.

Materials and methods

The efficacy of a herbicide based on pyraflufen-ethyl (26,5 g a.i./l; commercial product Kabuki 2.5 EC) in controlling suckers was studied in raspberry, apple and plum orchards at four locations in Serbia: Kušići (+43.493026, +20.075460), Zminjak (+44.745574, +19.463703), Padinska Skela (+44.957823, +20.433335) and Morović (+45.013222, +19.253138). The trials were set up following the standardized EPPO protocols: PP1/304(1) for apple and plum orchards (EPPO, 2017) and PP1/154(3) for raspberry plantations (EPPO, 2009).
In apple and plum orchards each plot contained three trees (EPPO, 2017), while in the case of raspberry plantations the plot size was 10 m long, and comprised two raspberry rows (EPPO, 2009). Herbicide application was done in spring, when the longest suckers were 10-15 cm long. In the split application treatments, the second treatment was done when the new suckers had reached a length of 10-15 cm.
In apple and plum orchards, the number of suckers and their length per tree were recorded prior to application (EPPO, 2017). On the other hand, in raspberry plantations, prior to the application, the number and mean height of suckers were recorded in several fixed squares in each experimental plot (EPPO, 2009). The assessments included counting the total number of newly regrown suckers per plot and the degree of regrowth of partly injured suckers (in cm). The assessments were done 7 and 14 days after application. In the split application treatments, assessments were done after the second application. In all assessments, the efficacy of the tested product was assessed in comparison to the untreated control.

Results and Discussion

Field trials conducted at all four locations have shown that pyraflufen-ethyl is highly efficient in controlling the number of suckers (>90%), both in single and split application in all three types of fruit orchards (Figures 1-3). Our results are in line with previous findings of Querzola et al. (2010) who have also shown pyraflufen-ethyl to be highly efficient in the control of suckers in pome and stone fruit orchards, both regarding its speed of action and its persistence. Similarly, Allegri and Manucci (2012) have documented high efficacy of sucker control in apricot and plum orchards, higher than that of the previously used gluphosinate ammonium. Lower efficacy of chemical sucker control in hazelnut orchards when using other herbicides, such as 2,4-D, glufosinate and paraquat was also documented by De Souza and Moretti (2020).

An overview of the documented levels of efficacy in different fruit orchards, both regards its efficacy in controlling the number of suckers and the average length of suckers is given in Figures 1-6.
The highest level of efficacy in raspberry plantations (location Zminjak; Figure 1) and plum orchards (location Zminjak; Figure 2) was observed in the split application treatment, when the tested product was applied at a dosage of 0,8 l/ha + 0,8 l/ha. The addition of the adjuvant did not drastically increase the efficacy of the tested product (approx. 2% higher efficacy in some locations) (Figures 1-3). Johnson et al. (2020) state that suckers in cherry orchards are more susceptible to pyraflufen-ethyl compared to those in apple orchards. Nevertheless, our results show high efficacy of in controlling the number of suckers in apple orchards in both locations and all four treatments (≥89%; Figure 3).

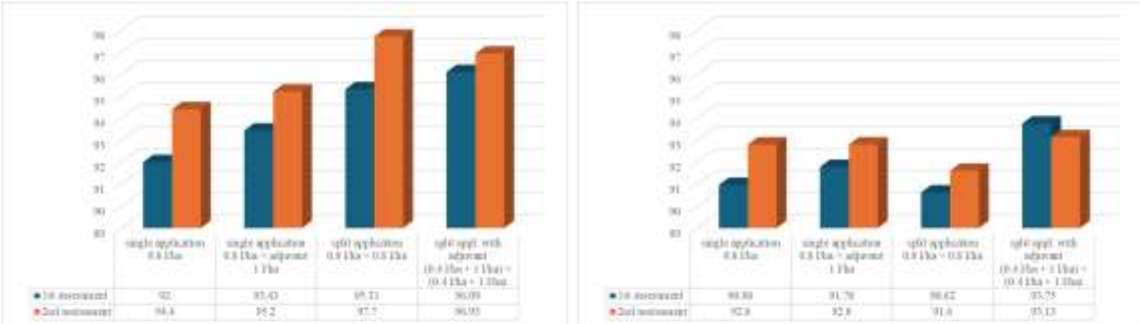

Figure 1. Efficacy of pyraflufen-ethyl in controlling the number of suckers in raspberry plantations in Serbia (a –location Zminjak; b –location Kušići).

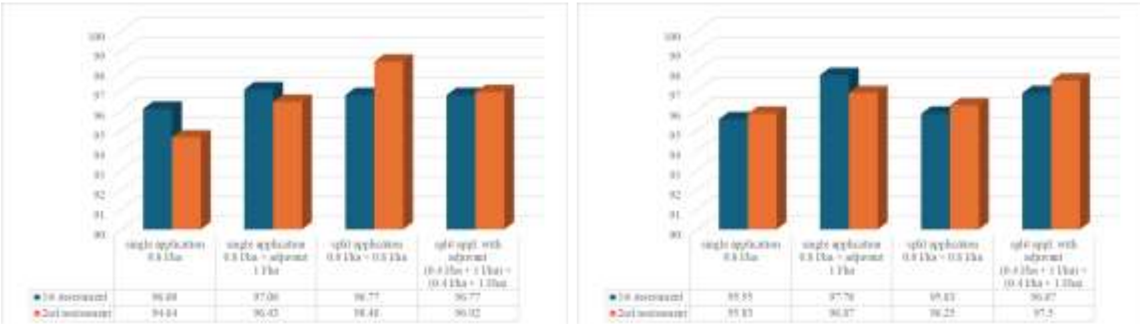

Figure 2. Efficacy of pyraflufen-ethyl in controlling the number of suckers in plum orchards in Serbia (a –Zminjak; b –location Padinska Skela).

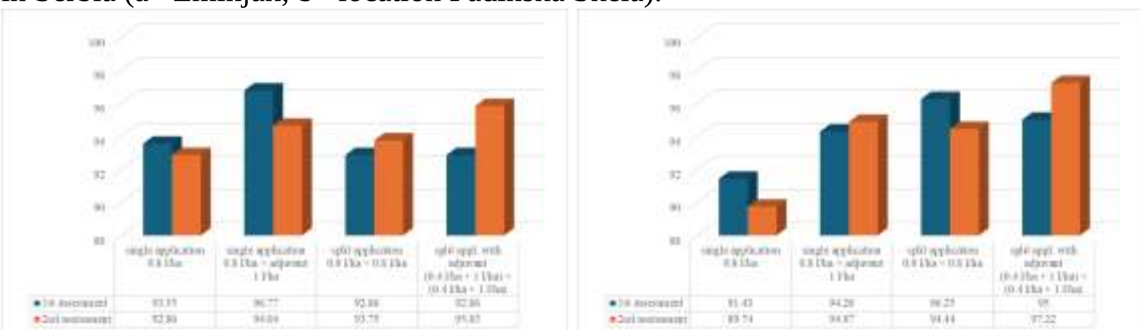

Figure 3. Efficacy of pyraflufen-ethyl in controlling the number of suckers in apple orchards in Serbia (a –location Morović; b –location Padinska Skela).

Meanwhile, the efficacy of the tested product in inhibiting subsequent regrowth (Figures 4-6) was comparatively lower, with the percentage of efficacy (observed in the second assessment) ranging between 69 and 84% in raspberry, 50 and 64% in plum and between 51 and 65% in apple orchards. It was slightly higher in split-application treatments, with the addition of the adjuvant Aleox T prima.

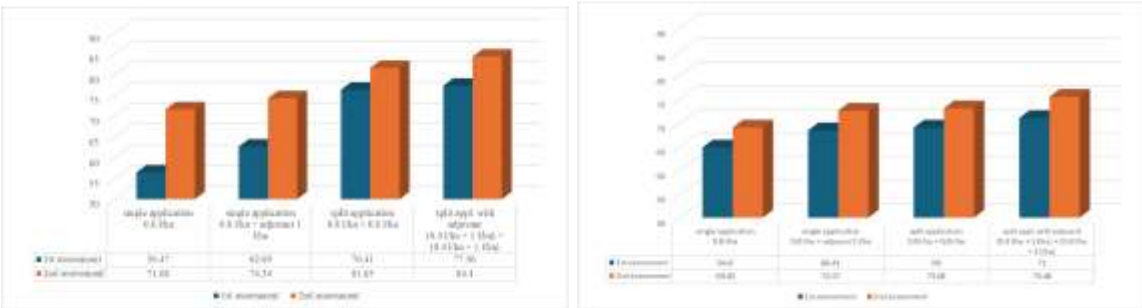

Figure 4. Efficacy of pyraflufen-ethyl in controlling the average length of suckers in raspberry plantations in Serbia (a –location Zminjak; b –location Kušići).

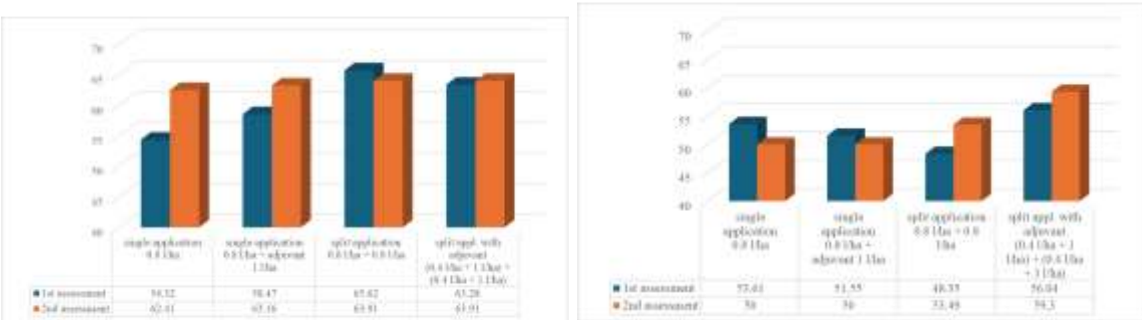

Figure 5. Efficacy of pyraflufen-ethyl in controlling the average length of suckers in plum orchards in Serbia (a – location Zminjak; b –location Padinska Skela).

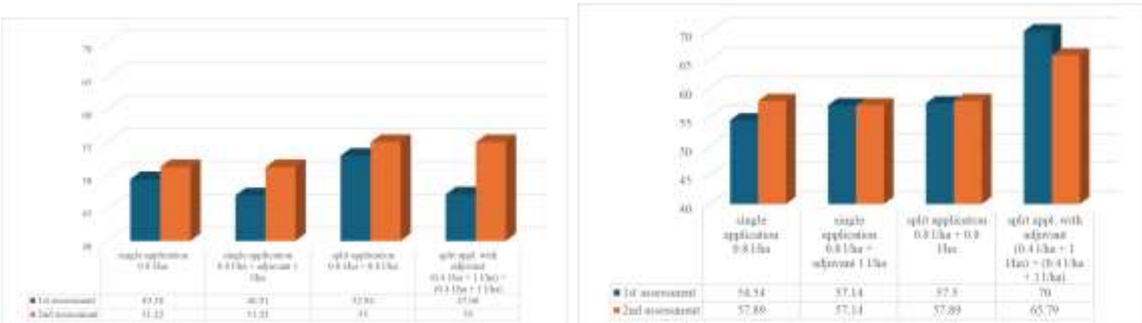

Figure 6. Efficacy of pyraflufen-ethyl in controlling the average length of suckers in apple orchards in Serbia (a –location Morović; b –location Padinska Skela).

Conclusion

In conclusion, it can be said that herbicides based on a.i. pyraflufen-ethyl are highly efficient in controlling plant suckers in raspberry, apple and plum orchards. When considering their effectiveness in controlling the number of suckers, split application treatments show the highest level of efficacy. In contrast, the efficacy of the tested product in inhibiting subsequent regrowth of new suckers is slightly lower, showing highest levels in the split application treatments with the addition of the adjuvant.

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EFFECTS OF THE INSECTICIDE LAMBDA-CYHALOTHRIN IN CONTROL OF *APHIS POMI* DE GEER IN APPLE ORCHARDS

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Abstract

Apple production has become highly demanding and requires great commitment. During the year, a large number of plant protection products (PPPs) for different harmful species, have been applied. The green apple aphid *Aphis pomi* (Hemiptera: Aphididae) represents one of the most economically important apple pests. It feed in dense colonies onon undersides of leaves and young shoots. If adequate control measures for this pest are not implemented, it could cause great damage, especially for young apple plants. In 2023, the trials were carried out according to the standard EPPO methods to establish the level of apple protection in the control of *A. pomi*. Two products based on lambda-cyhalothrin, with different amounts of active ingredients (50 g a.i./L, CS) and (100 g a.i./L, CS), were applied. In apple orchards variety Idared, Red Delicious and Granny Smith, at localities Kać, Mala Remeta, Rumenka and Budisava (Serbia), the PPPs were foliar applied by backpack sprayer at an amount/concentration of 0.2 L/ha and 0.01%, respectively. The experiment was set up in four replications in randomized block design. By visual examination, when the apple was in the BBCH 75 stage, the presence of *A. pomi* adults was registered. The obtained results were analyzed using one-way ANOVA and LSD test, while the efficacy was calculated according to Henderson-Tilton. Three days after the application the efficacy was between 82.3% and 99.4%, while after seven days it was 79.5-95.6%, depending on the applied product and locality. Fourteen days after the treatment efficacy was high and it ranged from 81.9 to 97.5%. In all assessments, the number of green apple aphid in the variants with insecticide was significantly lower comparing to the control. The populations of *A. pomi* in the mentioned localities showed a satisfactory sensitivity to lambda-cyhalothrin, which allows the use of this insecticide according to the recommendations of the anti-resistance strategy.

Key words: *Aphis pomi***,** *apple, lambda-cyhalotrin, efficacy.*

Introduction

Nowadays, the apple is among the most widespread types of fruit worldwide. Agroecological conditions in Serbia are suitable for its cultivation, yet they also favor the development of numerous harmful insect species, as well as disease-causing agents. In addition to aphids (Aphididae) and mites (*Tetranychus urticae*, *Panonychus ulmi*), economically significant pests in apple orchards are the codling moth (*Cydia pomonella*), fall webworm (*Hypanthria cunea*), apple blossom weevil (*Anthonomus pomorum*) and apple ermine moth (*Hyponomeuta malinellus*). *Aphis pomi*, known as the green apple aphid, is European in orgin, and widespread in Asia, North Africa, and North America. Consequently, its presence is also significant in Serbia. Throughout its development, it remains on a single host, which can be an apple, pear, quince, or whitebeam. The aphid damages leaves and green shoot tips by sucking juices from them. As a result, leaves may curl or dry under heavy infestation, while the shoots exhibit delayed growth and deform. This is more significant in nurseries and newly planted seedlings. On older trees and shrubs, the occurrence can remain below the damage threshold for many years, due to the action of natural enemies (Kereši et al., 2019). This paper aims to evaluate the efficacy of the insecticide lambda-cyhalothrin since it has been used for many years, in controlling the green apple aphid in apple orchards at four locations in the territory of Vojvodina.

Material and Methods

The control of the green apple aphid was carried out in apple orchards, with the Idared, Red Delicious, and Granny Smith varieties, located in Kać, Mala Remeta, Rumenka, and Budisava in Serbia. The experiments were set up according to standard OEPP methods: OEPP/EPPO, PP1/152 (4) (2012) for experiment design and data analysis; OEPP/EPPO, PP 1/258 (2) (2022) for the efficacy of insecticides in controlling *A. pomi* and OEPP/EPPO PP 1/135 (4) (2014) for phytotoxicity. The experiment was designed using a randomized block system with four repetitions, with each basic plot consisting of four trees. The treatment was applied foliar, using a backpack sprayer with 1000 liters of water per hectare. At the time of treatment (June 26 and 27, 2023), the apple was in the BBCH 75 phenophase. Visual assessments of the orchards registered the presence of the green apple aphid. Four assessments were made: right before treatment, and then three, seven, and 14 days after treatment. The efficacy of the products was assessed by counting the number of live aphids on 10 marked apple shoots per repetition. Insecticides based on lambda-cyhalothrin were applied in the recommended quantity/concentration of 0.2 l/ha and 0.01%. The efficacy was determined using the Henderson Tilton method (Wentzel, 1963), as well as the least significant difference (ANOVA).

Results and Discussion

Results on the control of *A. pomi* in M. Remeta, Kać, Rumenka, and Budisava are shown in Tables 1-4. The initial number of *A. pomi* before the application of insecticides ranged from 39.0 to 356.2, varying across the locations.

Table 1. Average number of green apple aphids (*A. pomi*) before treatment

Insecticide	Mala Remeta	Kać	Rumenka	Budisava
	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd	$\bar{x}$ ±Sd
lambda-cyhalothrin CS (50 g a.i./l; 0.2 l/ha)	43.2±3.8 c	48.2±5.3 b	356.2±46.8 a	39.0±9.5 ab
lambda-cyhalothrin CS (100 g a.i./l; 0.01%)	76.0±5.7 a	88.7±12.4 b	308.0±42.6 a	57.5±8.5 a
Control	58.5±7.3 b	184.0±88.9 a	335.2±54.0 a	51.0±17.6 a
F	32.04	7.20	1.01	2.23
p	<0.01	<0.01	<0.01	0.12
LSD 5%	6.92	69.43	214.33	25.93

$\bar{x}$ –average number; ±Sd- standard deviation

At all localities, the number of aphids three days after the application of lambda-cyhalothrin (50 g a.i./l CS and 100 g a.i./l CS) was significantly lower compared to the control. The efficacy of the insecticide ranged from 74.7 to 99.4%, depending on the product and localities (Table 2). Additionally, seventh day after pesticide application, the number of aphids was noticeably reduced compared to the control. The efficacy of the insecticides ranged from 72.1 to 95.6% compared to the control (Table 3). 14 days after the application, the efficacy varied between 77.9 and 97.5%, depending on the applied products and the examined localities (Table 4).

Table 2. Average number of green apple aphid (*A. pomi*) and efficacy of insecticides three days after treatment

Insecticide (L/ha, %)	Mala Remeta		Kać		Rumenka		Budisava	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
lambda-cyhalothrin CS (50 g a.i./l; 0.2 L/ha)	2.0±0.8 b	**97.1**	27.5±6.0 b	**78.0**	2.5±1.3 b	**99.4**	10.7±4.2 b	**74.7**
lambda-cyhalothrin CS (100 g a.i./l; 0.01%)	3.25±1.7 b	**97.3**	10.5±2.6 b	**93.3**	3.0±0.8 b	**99.1**	9.00±1.4 b	**83.1**
Control	93.2±13.7 a	/	324.2±49.8a	/	380±11.0 a	/	47.25±10.4 a	/
F	172.47		147.87		275.26		44.01	
p	<0.01		<0.01		<0.01		<0.01	
LSD 5%	9.30		34.06		7.49		7.73	

$\bar{x}$ -average number; ±Sd-standard deviation; E%-efficacy

Table 3. Average number of green apple aphid (*A. pomi*) and efficacy of insecticides seven days after treatment

Insecticide (L/ha, %)	Mala Remeta		Kać		Rumenka		Budisava	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
lambda-cyhalothrin CS (50 g a.i./l; 0.2 L/ha)	28.7±1.7 a	**72.1**	23.0±2.0 b	**81.6**	22.5±9.6 b	**95.6**	2.0±1.8 b	**95.3**
lambda-cyhalothrin CS (100 g a.i./l; 0.01%)	23.5±5.9 a	**80.6**	16.7±8.9 b	**89.3**	26.0±7.5 b	**94.1**	3.5±1.0 b	**93.4**
Control	27.2±15.5 a		438.5±35.5a	/	479.2±90.1 a	/	65.0±13.7 a	/
F	1.05		520.39		102.45		68.94	
p	0.32		<0.01		<0.01		<0.01	
LSD 5%	30.90		24.72		61.03		9.99	

$\bar{x}$ -average number; ±Sd-standard deviation; E%-efficacy

Table 4. Average number of green apple aphid (*A. pomi*) and efficacy of insecticides 14 days after treatment

Insecticide (%, L/ha)	Mala Remeta		Kać		Rumenka		Budisava	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
lambda-cyhalothrin CS (50 g a.i./l; 0.2 L/ha)	1.7±0.5 b	**97.5**	27.7±3.3 b	**77.9**	25.0±12.3 b	**95.3**	2.7±1.7 b	**92.5**
lambda-cyhalothrin CS (100 g a.i./l; 0.01%)	3.0±0.8 b	**97.7**	28.2±15.6 b	**81.9**	38.0±10.9 b	**91.8**	3.2±0.5 b	**93.9**
Control	70.7±22.2a	/	500±55.8a	/	503±91.7a	/	68.5±19.8a	/
F	37.57		265.13		102.53		43.31	
p	<0.01		<0.01		<0.01		<0.01	
LSD 5%	15.39		39.09		63.13		13.65	

$\bar{x}$ -average number; ±Sd-standard deviation; E%-efficacy

Over the years, resistance to insecticides has become a growing concern in aphid control. The decreased susceptibility of aphids to insecticides belonging to the organophosphate and carbamate groups has been determined with a lesser extent of resistance observed in pyrethroids. Among the spectrum of aphid resistance mechanisms, the most commonly detected ones include increased capacity for insecticide deactivation due to excessive production of esterase enzymes, and changes in sensitivity of the target site of action (Elezović et al., 2006). Our professional community often presents data regarding the insufficient efficacy of applied insecticides, particularly those belonging to the organophosphate and pyrethroid groups, in managing plant aphids. However, there is no official data concerning plant aphids that have developed resistance to insecticides or any organized monitoring of this undesirable phenomenon. Some researchers have found that lambda-cyhalothrin can be recommended for effective control of insects from the order Hemiptera: Aphididae in apple orchards and other crops (Seni and Naik, 2017; Saabome, 2013; Pezzini and Koch, 2015). Moreover, products based on lambda-cyhalothrin (0.03%) have exhibited high efficacy (81-90%) in controlling aphids 24 hours after treatment (Ganchev and Atanasova, 2018).

Conclusions

Based on the conducted experiments and the results obtained regarding the efficacy of products based on lambda-cyhalothrin 50 g a.i./L CS and 100 g a.i./L CS in controlling A. pomi in apple orchards located in Mala Remeta, Kać, Budisava and Rumenka, the following conclusions can be drawn:

• At all examined localities, the number of A. pomi, three, seven, and 14 days after treatment, significantly decreased compared to the control. The efficacy of the insecticides ranged from 72.1 to 99.4%, depending on the applied products and the localities.

• Based on the achieved results, it can be concluded that the A. pomi populations at the mentioned localities have shown satisfactory sensitivity to lambda-cyhalothrin, which allows the continued use of this insecticide while following all recommendations of the anti-resistance strategy.

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PARAQUAT AND OTHER DESSICANTS – INFLUENCE ON PLANTS AND OTHER ORGANISMS

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Abstract

The paper provides an overview of various aspects of paraquat and other desiccants and bleaching herbicides, from various chemical groups, which have in common the drying and bleaching of leaf mass. In weed plants, they can act as total or selective herbicides, and applied in the mature stages of the crop, by drying the leaf mass, they promote the maturation of some important crops (sunflower, soybeans, potatoes). More or less, all these various herbicides are highly or significantly toxic, which greatly complicates their application. In the EU, there was an initiative to ban some of them (eg Paraquat). However, we are of the opinion that despite their significant toxicity, they should be considered as an alternative to some other herbicides.

Key words: *dessicant herbicides, bipyridyl herbicides, paraquat, weeds, crop plants, toxicity.*

Introduction

Weeds have always been encountered by humans since the beginning of the development of agriculture, simply because the selection of cultivated plants is most often carried out by cross-breeding. (Quarrie, 1997), which has a positive effect on quantitative properties, such as on crop yield (economically the most important property of cultivated plants), but significantly reduces the competitive ability of cultivated plants, i.e. native species that grow around them (which by definition are weeds). Man has always solved that problem manually, by weeding and other ways of physically removing weeds, so that the rapid development of plant physiology and organic chemistry in the first half of the 20th century created the preconditions for a completely different way of solving that important problem. Namely, that knowledge led to the fact that in the years before the WW2, weeds could be selectively removed chemically, without major negative consequences of those compounds on the health and yield of cultivated plants. After the WW2, these promising findings led to systematic research into the synthesis of various organic compounds and their testing in terms of weed removal efficiency and selectivity, i.e. the absence of significant negative effects of those phytotoxic compounds on crop plants (Ashton and Crafts, 1981; Corbet et al., 1984; Percival and Baker, 1991). Such an approach soon proved to be promising because, while routine application of herbicides (as these phytotoxic compounds are called) leads to a reduction in crop yield due to weed competition of 8-15%, without chemical weed removal with herbicides, this loss of total yield can and 50% of the potential total crop yield (Percival and Baker, 1991). The introduction into practice of a new agrotechnical measure, chemical crop protection, in addition to its benefits, also led to new unknowns and problems, f.e. the resistance of weeds to previously phytotoxic doses of herbicides. This becomes a serious problem, because investments in the development of new, phytotoxic formulations and herbicidal compounds are very expensive and demanding, and the profit can be small, because the question arises of the economic expediency of using these means, if there is a rapid development of weed resistance to some classes of herbicides (Percival and Baker, 1991; Travlos et al., 2020). This then entailed the need for a more careful study of the effects of herbicides, both on plants and on other living nature and ecosystems, due to health and ecological problems.

Effects of a Paraquat and other dessicants on weeds

Paraquat and its related diquat, as bipyridyl molecules (Averina et al., 1991), lead to desiccation of weeds and partially controlled desiccation of crop leaf mass (for faster ripening), inhibiting electron transport in PS_1 (Ashton and Crafts, 1981; Corbet et al., 1984), whereby those electrons, instead of the usual photosynthetic acceptors (Figure 1), transferred to oxygen and through the so-called Mehler's reactions or water-water cycle (Kleczkowski, 1993; Janjić et al., 1994; Asada, 2000) they produce reactive oxygen species, disrupting cellular metabolism, which leads to drying and death of plant foliage.

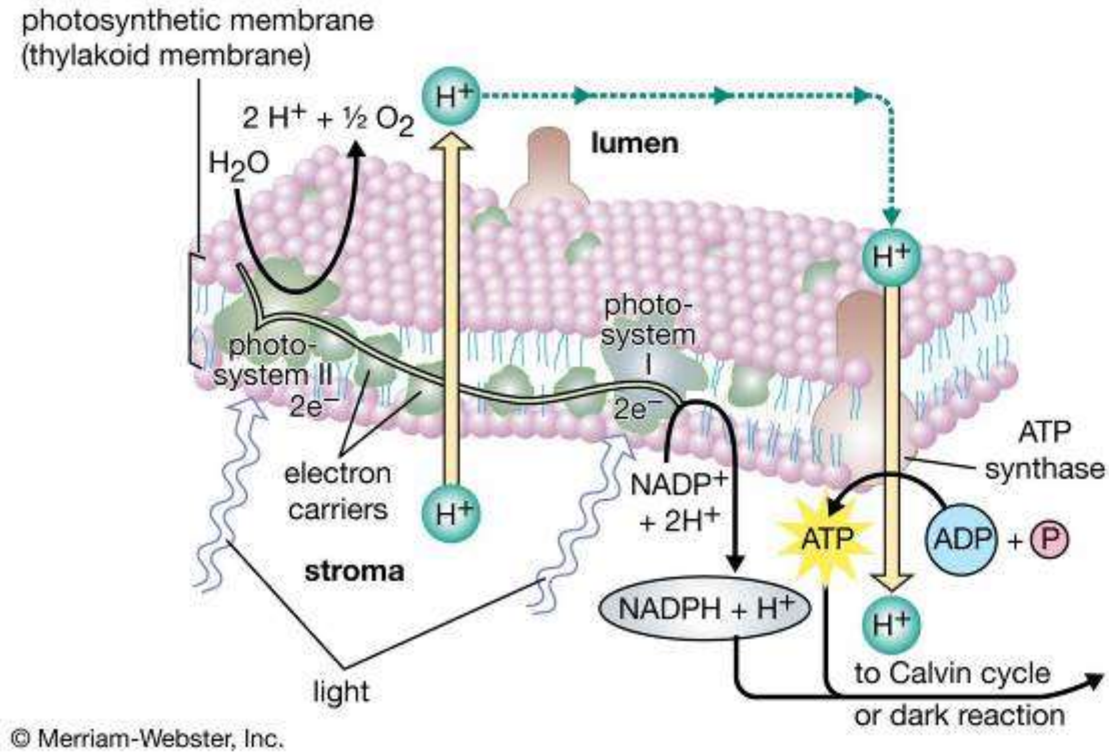

Figure 1. Transport of electrons in light phase of photosynthesis.

In addition to the well-known bipyridyl herbicides with desiccation properties, and due to the development of resistance to bipyridyls (Amsellem et al., 1993; Streller et al., 1994; Weaver et al., 2004; Qin et al., 2004; Yu et al., 2004; Asaduzzaman et al., 2022; Farago et al., 2022), naturally occurring or due to genetic modification, mainly due to the increased content of antioxidant small compounds (e.g. polyamines) or antioxidant enzymes (which inactivate reactive oxygen species, thereby preventing phytotoxic processes associated with bipyridyls), a new type of desiccant that induces the Mehler reaction (s.c. water-water cycle) has recently been developed (Gerwiuck et al., 1997). In addition to bipyridyl's, as PS_1 inhibitors, which induce Mehler's reaction, there is another type of desiccant herbicidess, which at the same time lead to leaf bleaching (indicates an increased production of reactive oxygen species), whose mechanism of action is mainly based on the inhibition of one of the pathways in the biosynthesis of chlorophyll or carotenoids (Sandman et al., 1990; Sherman et al., 1991; Jacobs et al., 1991; Averina et al., 1991). These are diethyl ether, pyridazinone and furanone types herbicides, as well as amitrole (Ashton and Crafts, 1981; Corbet et al., 1984; Percival and Baker, 1991). Such an approach, due to the appearance of weeds resistant to bipyridyl, influenced to the emergence of new desiccant herbicides (Ison et al., 2022), such as Voraxor®!? However, it should be mentioned that paraquat, although generally understood as a contact herbicide, has been observed to be taken up and transported through plants by the same pathways as polyamines. (Fujita and Shinozaki, 2021), which indicates possible new types of weed resistance to this and other desiccant herbicides (Thies et al., 1996; Farago et al., 2022; Lyu et al., 2022). In addition to other treatment practicies, the effect of paraquat, as well as other desiccants, can be enhanced by encapsulation processes (e.g. with chitosan), which prolongs the otherwise short-term contact effect of this herbicide (Kurniadie et al., 2022).

Effects of a Paraquat and other dessicants on crops

Although the application of herbicides is directed primarily against the harmful effects of weeds on crops, their influence on growth and development, as well as the quality and quantity of yields, it is also important to study the effects of herbicides on cultivated plants. That is why it is important to study the effect of desiccant herbicides, either because they belong to the group of total herbicides (bipyridyls, amitrol), or because of their persistence, both in the products of cultivated plants and food, and in the soil where the mentioned herbicides are applied. All in all, it is necessary to distinguish the phytotoxic from the health aspects of the application of herbicide-desiccants when they are used in cultivated plants. And the mode of action of desiccant herbicides on cultivated plants is very diverse. Bipyridyl herbicides (paraquat, diquat and some related compounds) haver many different effects on crop plants. In addition to inducing the production of reactive oxygen species (Asada, 2000), which disrupt cellular metabolism and lead to the decline of crop leaf mass (which is useful in the case of ripening of some crops, such as sunflower, soybean, potato), this group of herbicides leads to pheophytinization, which is another factor in the inhibition of photosynthesis and other metabolic processes (Averina et al., 1991).

This effect was studied in a series of papers by Nikolić and colleagues and it was found that, in addition to the confirmation of pheophytinization of chlorophyll under the effect of diquat (Nikolić and Janjić, 1995; Nikolić et al., 1996a, 1996b; Nikolić, 1997; Pavlović et al., 2014), importante changes in the photosynthetic membrane proteins (Milivojević and Nikolić, 1995, 1996, 1998, 1999; Milivojević et al. 1997) under the effect of diquat, among other things mediated by far-red light (Milivojević and Nikolić, 1998; Table 1 and 2), which is primarily adopted through PS_1, the main site of action of bipyridyl herbicides (Ashton and Crafts, 1981).

As indicated in the previous chapter, plant resistance to bipyridyl herbicides is possible either through enzymatic antioxidant systems or through small molecules with antioxidant properties. This topic was investigated in detail on cultivated plants, and it was found that resistance to bipyridyl herbicides in cultivated plants is increased by the action of small molecules (Hart et al., 1992, 1993; Zer et al., 1994; Gosset et al., 1996; Donahue et al., 1997; Fujita and Shinozaki, 2021), which probably exert complexation of ions of transitional metal elements (Fe, Cu, Zn, etc.), as well-known promoters of the Mehler reaction (Chang and Kao, 1997).

Table 1. Effect of diquat in the dark or under "white light" (WL) or far-red radiation (FR) during different time intervals (0 - 48 h) on ratios of chlorophylls (Chl) and carotenoids (Car) in primary leaves of soybean and maize. n.m.: not measured. According: Milivoijević and Nikolić (1998).

culture	Soybean						Maize									
parameter	Chl *alb*			Chl a/Car			Chl *alb*					Chl a/Car				
treatments	Sh	24 h	48 h	0 h	24 h	48 h	5 h	12 h	18 h	36 h	48 h	5 h	12 h	18 h	36 h	48 h
WL	2.1	2.3	2.4	5.3	5.6	4.6	2.6	2.5	2.3	2.5	n.m.	4.9	4.8	4.9	5.5	15.3
WL+ diquat	2.5	2.0	1.5	4.4	6.6	8.9	2.6	2.6	2.3	1.5	0.8	4.7	5.4	6.1	9.8	35.8
FR	2.2	2.1	1.7	3.4	3.4	3.9	2.6	2.4	2.4	2.1	2.1	4.9	4.6	4.9	4.6	4.9
FR+ diquat	2.1	2.1	1.5	3.5	4.4	6.9	2.6	2.4	1.5	1.9	0.9	5.2	4.6	15.8	6.8	11.6
Dark	2.1	2.3	2.6	5.3	4.1	4.2	2.8	2.7	2.5	1.6	1.2	5.0	4.7	5.2	5.7	6.0
Dark + diquat	2.3	2.4	2.1	4.1	4.1	5.1	2.7	2.6	2.4	1.4	1.0	4.9	3.6	5.7	13.8	10.7
LSD o.os	0.07			0.09			0.24					2.13				

Table 2. Effect of diquat in the dark or under "white light" (WL) or far-red radiation (FR) during different time intervals (0 - 24 h) on polypeptides of photosynthetic reaction centres (RC) and light-harvesting complexes (LHC) of photosystem $(PS)_1$ and PS_2 of soybean and maize chloroplast thylakoids. n.m.: not measured. According: Milivoijević and Nikolić (1998).

culture	Soybean										Maize									
parameter	PS_1		PS_2		LHC		PS_2/PS_1		LHC/PS_2		PS_1		PS_2		LHC		PS_2/PS_1		LHC/PS_2	
treatments	Sh→	24 h	5 h→	24 h	5 h→	24 h	5 h→	24 h	5 h→	24 h	Sh→	24 h	Sh→	24 h	5 h→	24 h	5 h→	24 h	5 h→	24 h
WL	7.1	3.0	23.2	38.6	35.4	34.3	3.3	1.8	1.7	1.0	6.9	5.2	22.2	33.8	36.7	29.8	3.2	6.5	1.7	0.8
WL+ diquat	4.0	2.6	19.1	25.4	40.7	43.7	4.8	9.8	2.1	1.7	1.9	4.0	24.7	33.6	42.6	25.6	13.0	8.4	1.7	0. 8
FR	5.3	2.6	24.6	23.8	23.9	43.7	4.6	9.2	1.0	1.8	2.9	5.8	28.4	25.7	37.1	27.9	9.8	4.4	1.3	1.1
FR+ diquat	3.5	2.0	12.9	15.0	50.7	34.3	3.7	7.5	3.9	1.4	1.2	3.6	21.2	23.4	45.9	23.4	17.7	6.5	2.2	1.0
Dark	3.5	3.1	19.9	22.0	39.0	46.3	5.5	8.7	2.0	1.7	1.5	4.0	25.0	26.3	35.8	30.9	16.7	10.0	1.4	1.2
Dark + diquat	4.9	1.8	34.9	30.0	34.9	37.0	4.7	16.7	1.5	1.2	n.m.	2.6	45.0	32.0	36.3	25.1	45.0	12.3	0.8	0.8
LSD o.os	0.8		10.5		9.3		1.5		1.2		1.1		5.1		8.5		7.6		0.4	

In addition to the effect on transition metals as mediators of the Mehler reaction, increased resistance to bipyridyl herbicides in cultivated plants is also possible through antioxidant enzyme systems (Peleg et al., 1992; Aono et al., 1995; Kuk et al., 2006; Wang et al., 2021). Since the induction of the production of reactive oxygen species on a small scale has a favorable effect on plant metabolism, it was considered, as in the case of some other types of herbicides (Ashton and Crafts, 1981), to treat crop plants with bipyridyls in small doses maybe induced the effect of the so-called. hormesis, i.e. increasing plant metabolism (which would theoretically have a favorable effect on crop yield), but such attempts did not give sufficiently promising results (Ferrari et al., 2021), although equivalent trials have given favorable results in the model plant *Arabidopsis thaliana* (Farago et al., 2022). In case of effect of some other types of desiccant herbicides, which at the same time lead to leaf bleaching (which indicates an increased production of reactive oxygen species, with mechanism of action based on the inhibition of one of the pathways in the biosynthesis of chlorophyll or carotenoids), we observe the binding of these herbicides to enzymes such as phytoene desaturase and protoporphyrinogen oxidase (Sato et al., 1991; Lee et al., 1993; Dalla Vekia et al., 2001; Di Baccio et al., 2001), which leads to their inhibition, and thus to increased production of reactive oxygen species. But a physiological difference was found in the degree of binding of those herbicides to the mentioned enzymes, which can be the basis for the possible resistance of cultivated plants to their effect (Sherman et al., 1991).

Effects of a Paraquat and other dessicants on health of human and animals

Paraquat, partly diquat, as well as other desiccants are poisonous substances. Paraquat is one of the most poisonous and according to the nomenclature applied in Serbia, it belongs to the most poisonous, the 1st group of poisons. This is partly due to the fact that paraquat induces the production of reactive oxygen species, which disrupt metabolism (Brown and Sejther, 1990) and damage human and animal cells, as well as plant cells, partly due to the high volatility of paraquat, which can damage the eyes, while diquat penetrates the skin. So that bipyridyl compounds easily penetrate into the body of humans and animals, even without inhalation or via food or liquid. Herbicides-desiccants from other chemical groups mostly cause poisoning by ingestion via contaminated food or liquid or when they are inadequately applied in the field, but unlike bipyridyl, which are highly toxic, but have a short-term effect due to rapid inactivation, desiccants from other chemical groups are persistent, so their prolonged action in the environment can lead to their accumulation and prolonged toxicity in human and animal organisms. Detoxification and relief of bipyridyl poisoning symptoms are performed in different ways, either by removing or inactivating transition metal ions (Krall et al. 1991; Sato, 1991), as cofactors with bipyridyl in the production of reactive oxygen species, or by using natural ones (Palipoch et al., 2022; Chen et al., 2023) or synthetic (Li et al., 2021) drugs. The consequences of intoxication with bipyridyl herbicides are very serious (damage to the eyes, lungs, liver, kidneys), but also lead to longer-term symptoms, such as causing Alzheimer's disease (Marshall and Prior, 2022). That is why, at the first instance of the Court of the European Union, Sweden, with the support of Denmark, Finland and Austria, and against the European Commission launched an initiative to cancel the license for the use of paraquat as a plant protection agent, due to health risks (Anonymus, 2007). At that decision-making level in EU, the court of EU gave support to that initiative.

Other aspects relating to a Paraquat and other dessicants

Because of these health risks, very sophisticated analytical techniques have been developed for the detection of bipyridyl herbicide residues in various human, animal, and plant tissues (Hao et al., 2013; Tsao et al., 2016; Guo et al., 2023). In addition, various techniques are being considered for the fastest possible inactivation of these highly toxic substances, should soil or water be polluted by them (Inthama et al., 2021; Hammami et al., 2022)

Conclusions and future perspectives about Paraquat and other dessicants

Bipyridyl herbicides, as well as other desiccants, showed high efficiency, either for the complete removal of weed vegetation (total herbicides: paraquat, diquat, amitrole), or for its selective removal (e.g. diethyl ether, pyridazinone and furanone types herbicides), however their high toxicity (paraquat) or persistence in the environment more and more raise the issue of restrictions and even bans on their use. But certainly, despite these restrictions, their high efficiency in removing weeds cannot be ignored. So developed countries, similar to in the case of some organochlorine insecticides (e.g. DDT), which are known to have high persistence and a proven harmful effect on the environment, they still have their stocks, simply because in palliative situations it is absolutely necessary to have such substances as the possibility of choosing a solution suddenly We are of the opinion that, taking into account all the limitations in the application of these types of pesticides, our country must also develop a strategy for responding to possible palliative situations, even with these highly toxic herbicides.

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THE ROLE OF VOLATILE ORGANIC COMPOUNDS IN PLANT-SOIL COMMUNICATION

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Abstract

Plants can communicate with each other and with the surrounding environment through volatile organic compounds (VOCs). VOCs will give plants a signal to communicate about different stressors and dangers to survival and well-being. When a plant is under stress or when it communicates with other plants or the soil, it releases VOCs. Sometimes, to protect themselves from herbivores and other predators, plants will release a VOC signal. Soil microorganisms and plant roots are the main sources of soil volatile organic compounds emissions. We have kept an observation on these VOCs which were released into the soil during the experiment. We have selected two distinct genotypes of basil-*Ocimum basilicum purpurescens* and *Ocimum basilicum* L.-were planted for observation. Headspace gas-mass chromatography (GCMS) was utilized to detect VOCs. GCMS headspace analysis was performed on the native samples. Examining both genotype types indicated the presence of monoterpenes (alpha-Pinene and (+)-3-Carene), which primarily reflect abiotic stress. Plant-to-plant communication was facilitated by the monoterpene cymene, which was detected. Alcohol was also shown to be among the other category of chemicals. While nonanal and decanal signals are indicative of insect pheromones, octanal signals *(Ocimum basilicum purpurescens* and *Ocimum basilicum* L), which is implication of inhibition for plant growth. Additionally, hexanal and benzaldehyde were also detected as indicators of damage, which can trigger defense responses in plants. The collected data show that volatile organic components should be closely monitored as signal substances and future observation will include other genotypic types. Whereas prompt identification of signal volatile organic compounds in soil and plants may improve plant's protection and yield.

Key words: *plant communication, volatile organic compound, terpenes, chromatography.*

Introduction

In the plants, there are numerous defense reactions and mechanisms that will enable survival in unfavorable conditions. For centuries, plants have adapted to natural climate and established a relationship with the environment in which they are established. In principle, a stressful situation causes a series of physiological changes in the plant that neutralize and preserve the basic vital functions in the organism. However, plants cannot move and change the site environment from which they show a negative response and less stable reaction. As they suffer pressures reflected through negative response and low yield quantity and quality. It is difficult to find an adequate explanation and interpret the signals that the plant release in response.

Therefore, detecting the answers that the plant sends, plant language and understanding plants could be the solution for real time decisions. Recent studies suggest that plants sense and respond to many changes in their environment, both above as plant–plant interactions aboveground and below ground, as root–root interactions.

The agronomic potential of identification and detection of VOCs as signal which can be a natural and environmentally friendly for protecting plants from various stresses and increase crop yield is invaluable. VOCs can ensure prevention of many diseases, as well as providing protection from environmental stresses when detected at the right moments. VOCs also, stimulate plant defense mechanisms for enhanced resistance/tolerance to the increasing stress, reduce reactive oxygen species (ROS), possess potent antimicrobial and allelopathic effects, and that may be important in regulating plant growth, development, and senescence through interactions with plant hormones.
Plants produce an amazing variety of metabolites, a few of these are involved in "primary" metabolic pathways, as well as secondary metabolites. These secondary metabolites are the result of different plants' responses, through the course of evolution, to specific needs. Among such metabolites, VOCs may play a dominant role (Pichersky et al., 2000). Volatile organic compounds are important mediators of mutualistic interactions between plants and their physical and biological surroundings. These volatiles rapidly indicate competition or potential threat before these can take place, and they regulate and coordinate adaptation responses in neighbouring plants, fine-tuning them to match the exact stress encountered (Ninković et al., 2020). Being released by almost any kind of tissues and type of vegetation as green leaf volatiles, nitrogen-containing compounds and aromatic compounds, plants VOCs can be emitted constitutively or in response to a variety of stimulus. They are involved in a wide class of ecological functions, as a consequence of the interactions of plants with biotic and abiotic factors (Spinelli et al., 2011). Several trials have shown the capacity of various VOCs produced by leaves to inhibit germination and growth of plant pathogens, yet the mechanisms of action remain unknown. Plants use VOCs to indicate a variety of responses, such as: indirect plant defense against insects (Mumm et al., 2017); plant-pathogen interactions (Anjali et al., 2023), pollinator’s attraction (Dudareva and Pichersky, 2000); plant-to-plant communication (Baldwin et al., 2016; Heil and Karban, 2010), thermo-tolerance and environmental stress adaptation (Holopainen and Gershenzon, 2010); defence from predators (War et al., Vivaldo et al., 2017). The types of interactions that occur between host plants and pathogens involve varied and complex mechanisms, whereas the first is recognition. There may be molecules produced in both host and parasite that can interact. Second, there must be metabolic changes in the host or pathogen or both that are triggered by the initial interaction step. Genetic mutations in either host or pathogen can change the specificity of such molecular interactions or their ability to trigger metabolic change (National Research Council US Committee on Biosciences, 1985).
The aim of this work was detecting volatile organic compounds (VOCs), as signals, in soil where two different genotypes of basil, *Ocimum basilicum purpurescens and Ocimum basilicum L*, were grown for observation.

Material and methods

For analysis was used a GC 8890 GC equipped with a split/splitless inlet. The Pal Sistem, PAL PSI 85, sampler was used to extract and transfer the VOCs in the sample to the GC. The sample was then analyzed using a 5977B GC/MSD configured with an inert extraction ion source. Agilent MassHunter Acquisition software version 10.0 was used for analytical data collection.
In this study 3g of soil was used for analysis and transferred in vials. In each vial, added water to total volume of 5ml. Non target analysis included scan mode of analysis which lasted 30 min.
The scanning mass was in the range of 50 to 500 AMI.

Results and Discussion

Several types of analytes, including terpenes, aldehydes, alcohols, and others, were identified in our investigation employing headspace technique in a soil sample. Table 1. Table 1 displays the results.

Table 1. List of detected VOCs in soil

Aldehydes	Terpenes	Alcohols/Phenols	Other	Kethon
Hexanal	(+)-3-Carene-	Phenol	Cyclotrisiloxane, hexamethyl	5-Hepten-2-one, 6-methyl-
Butanal, 3-methyl-	alpha-Pinene-	-	m-Menthane	
Benzaldehyde	Camphene	-	Cyclotetrasiloxane, octamethyl-	
Octanal	Cymene			
Nonanal	Eucalyptol			
Decanal				

As expected, the aldehydes and terpenes were the dominant group of analytes, and they are the main group of compounds relevant to soil and plant communication. Terpenoids are naturally occurring chemicals and are commonly found in the whole living world. Investigations have shown that all organisms can and often do emit VOCs. In this study we will focus on emission of VOCs in the soil. It is well known that plant VOCs mediate plant interactions with the surrounding environment (Erb and Kliebensteinb, 2020). Emission of VOCs by plants is ubiquitous and can take place from their above-ground (Mofikoya et al., 2019) and below-ground parts (Massalha et al., 2017). Many other VOCs are instead induced by abiotic (Loreto et al., 2006; Loreto and Schnitzler 2010) or biotic stresses (Dicke and Loreto, 2010). In this study, we identified terpenes (+)-3-Carene-, alpha-Pinene-, Camphene, Cymene, and Eucalyptol) that are directly communicators of abiotic stresses.

On other hand we detected aldehydes which mainly inhibit mycelial growth as is case with Butanal, 3-methyl. Benzaldehyde plays important roles in chemical communications serving as a sex, aggregation and alarm pheromone as well as a defense compound in some insects and non-insect arthropods, and as a pollinator attractant, flavor volatile and antifungal compound in plants (Huang et al., 2022). Benzaldehyde also contributes to the green and fresh odor (Qian et al., 2019). Some of aldehydes as nonanal, decanal represent volatile signaling compounds and insect pheromone (Conchou et al. 2019). One more interesting VOC is phenol, who is emitted mainly for protection against stress (Naikoo et al.2019). Compounded as Cyclotrisiloxane, hexamethyl; m-Menthane and Cyclotetrasiloxane, octamethyl- usually formed as a product of hydrogenation or hydrogenolysis of various terpenoids (Madji et al., 2019).

Conclusions

Terpenoids are natural chemicals that are found everywhere in the living world. Research has shown that all living things release volatile organic compounds (VOCs). In our study, we looked at VOC release in soil, which is very important for how plants interact with their environment (Erb and Kliebenstein, 2020). Plants can release VOCs from both their parts above the ground and below the ground. Many VOCs are also caused by non-living (abiotic) or living (biotic) stresses

In our research, we discovered terpenes such as (+)-3-Carene, alpha-Pinene, Camphene, Cymene, and Eucalyptol, which clearly demonstrate the impact of non-living stress factors. We also identified aldehydes like 3-methyl Butanal, which primarily inhibit the growth of fungal strands. Benzaldehyde plays a crucial role in chemical communication, serving as a signal for mating, gathering, and warning, as well as providing a protective scent. Future research should focus on detailed quantifications of volatile compounds to produce sensors for their fast and accurate detection in real-time.

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APPLICATION OF MATHEMATICAL DECONVOLUTION ON FTIR SPECTRA FOR DETERMINATION OF PROTEIN STRUCTURE IN TOMATO *(Solanum lycopersicum L.)* TREATED WITH *TRICHODERMA SPP.*

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Abstract

This study investigates the impact of Trichoderma spp. on the protein structure of tomato (Solanum lycopersicum L.) seeds using FTIR spectroscopy. Tomato seed dormancy, which often leads to germination failure, poses a significant challenge for production. Trichoderma, a beneficial fungus, enhances plant growth, stress resistance, and seed quality. By applying T. harzianum and T. brevicompactum to tomato seeds, we monitored changes in protein content and structure through the Amide I (1600-1700 cm^{-1}) and Amide II (1510-1575 cm^{-1}) regions of the FTIR spectra. Mathematical decomposition of these bands allowed us to differentiate between ordered and disordered protein structures, as well as various secondary structures such as α-helices, β-pleated sheets, and β turns. Our analysis revealed that Trichoderma treatments increased the proportions of α-helices and β-pleated sheets while reducing β turns. Additionally, the treatments led to higher proportions of disordered structures in the proteins. This study demonstrates the efficacy of mathematical decomposition in interpreting spectroscopic results and highlights the potential of Trichoderma spp. to improve tomato seed quality and performance.

Keywords: *mathematical deconvolution, FTIR, proteins structure, tomato seed.*

Introduction

Tomato (*Solanum lycopersicum L.*) is one of the most economically important vegetables worldwide. Due to its short development cycle, diverse secondary metabolites, and small genome size that is suitable for genetic manipulation and transformation, the tomato is frequently used as a model organism. However, seed dormancy often results in germination failure, posing a significant challenge for production.

Trichoderma spp. are cosmopolitan filamentous fungi that inhabit soil and the rhizosphere [1]. They are mostly opportunistic, avirulent plant symbionts, characterized by rapid multiplication and efficient substrate colonization [2]. They have several beneficial effects on plants, which are manifested in the stimulation of plant growth and tolerance to abiotic and biotic stress factors [3]. In addition to the acquisition of resistance to abiotic stress and the control of plant pathogens, the stimulation of plant growth is also associated with various mechanisms. These include increased uptake of nutrients, influence on metabolic processes, photosynthesis, and synthesis of phytohormones. This trait is specific to selected strains and cannot be applied to the entire species. Over the past decade, *Trichoderma spp.* has been recognized as an effective seed germination enhancer. Consequently, seed treatments with *Trichoderma* can improve seed quality and plant performance both in the short and long term.

However, the early interactions between seeds and *Trichoderma spp*. remain poorly understood.
Using the Amid I (1600-1700 cm^{-1}) and Amid II (1510-1575 cm^{-1}) regions in FTIR spectra, researchers can monitor changes in the protein content and structure of tomatoes treated with *Trichoderma*. Mathematical decomposition of these bands allows the differentiation between ordered and disordered protein structures, providing detailed information on how fungal treatments affect the protein structures in tomatoes. The use of mathematical models can significantly support and assist in the monitoring of various biological experiments.

Material and Methods

Fungal strain and growth condition

The strains utilized in this study, T. harzianum (T4) and T. brevicompactum (T9), were sourced from the A horizon (5–30 cm) of agricultural soil and deposited at the Faculty of Ecological Agriculture, Educons University, Serbia, and the Department of Microbiology, University of Szeged, Hungary. Initially, pure cultures of the two Trichoderma isolates were revived from a 20% glycerol solution stored at −20 °C and propagated in potato dextrose broth (PDB) medium at 25 °C in darkness. After four days, the Trichoderma isolates were transferred from the PDB medium to the center of 9 cm diameter Petri dishes containing Murashige and Skoog medium (MS). Additionally, 30 seeds were evenly distributed around the periphery of each Petri dish for each treatment condition.

Plant material and growth conditions

Two medium-late tomato cultivars, Gruzanski zlatni (GZ) and Narvik (N) were investigated. The seeds of these tomato varieties were obtained from the Institute for Vegetable Crops, Smederevska Palanka, Serbia. At the same time, germination and plant material collection were conducted at Educons University, Sremska Kamenica, Serbia.
Tomato seeds were sterilized in a 2% NaClO solution for 20 minutes and then washed with distilled water. The seeds were germinated in a Petri dish (Ø 15 cm) on 0.5x MS medium for 48 hours in the dark at 25 °C. Tomato seeds of GZ and N were co-cultured with two Trichoderma species. The impact of Trichoderma spp. on the germination process of the two tomato cultivars was investigated over 48 hours before direct physical contact between the Trichoderma and the tomato seeds was established.

Fourier-transform infrared spectroscopy (FTIR) measurements

The influence of Trichoderma spp. on the germination process of tomato seeds was conducted using an FTIR spectrophotometer (IRAffinity-1, Shimadzu, Japan). Radicles of tomato cultivars GZ and N grown under control conditions as well as in co-culture with, two most perspective strains, T. harzianum and T. brevicompactum were collected from MS media. Radicles (length ~ 4 mm) were dried for five days at 60°C, after which they were homogenized. From each treatment, 250 mg of dry material was collected. Sample analysis was conducted utilizing the KBr tablet technique [4] (1 mg of sample in 200 mg of KBr) encompassing a wavelength range of 4000-600 cm^{-1}, 100 scans per spectrum and a resolution of 4 cm^{-1}.

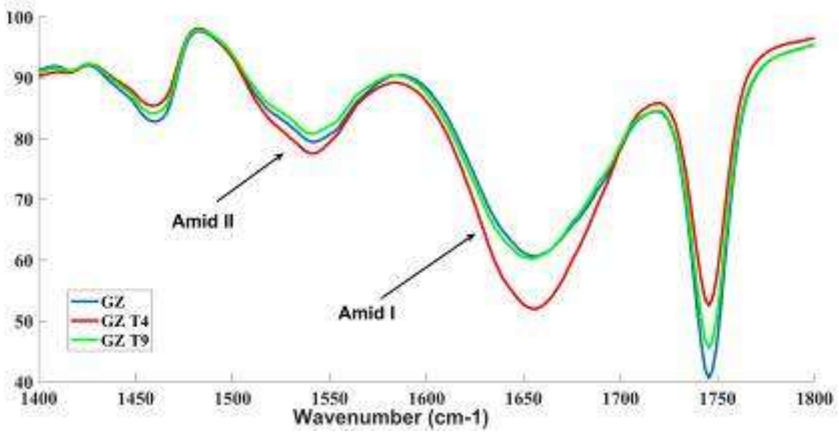
Mathematical deconvolution

All non-linear fittings were made using the Nelder–Mead simplex algorithm, performed in Matlab 6.5 [5]. The corresponding FTIR region was deconvoluted into four components for Amid II and three components for Amid I using the Gaussian model to obtain the optimal position and area for each component. To check their origin, the positions of all components were plotted as a function of the excitation wavelength. All spectra were normalized. The spectral components' areas obtained by deconvolution of normalized FTIR region for Amid I and Amid II region were determined.

Results and Discussion

The detection of differences in the structural organization or conformation of proteins in the radicles of tomato seeds was performed based on the parts of the spectrum where the deconvolution of the protein bands normalized to the unit area was performed. Obtain FTIR spectra for Gruzanski zlati (GZ) and Narvic (N) radicule are presented on Figure 1. An example of deconvolution, for the variety GZ, is given in Figure 2. Analysis was performed using a Gaussian deconvolution model, four-component for the Amide I band and three-component for the Amide II band using Matlab 6.5 (R2010a) software. The maxima positions of the components obtained by deconvolution of the Amide I band are assigned to the α-helix, β-pleated sheet, and turns, while the proportions of the ordered and unordered structure in proteins are obtained by deconvolution of the Amide II band [6-8]. The share of α helixes, β pleated sheets and turns in the protein structure was determined as the percentage share of the area of the components obtained by deconvolution and which correspond to these structures in the area of the entire Amide I band. The share of ordered and disordered structures in proteins was determined as the percentage share of the area of the corresponding components in the area of the Amide II band, with the band area taken as 100%.

Through deconvolution analysis of the Amid I protein band, which provides in-sights into the composition of various secondary structures within proteins, it was ascertained that in the presence of *T. harzianum* T4 and *T. brevicompactum* T9 treatments, there is a reduction in the proportion of twists and a simultaneously increase in the fractions of α-helixes and β pleated sheet. Notably, this effect is more pronounced in the GZ T4 sample compared to the GZ T9 (Figure 2A). The Amide II band is not so indicative of the share of types of secondary structure in the protein, but based on its deconvolution, the ratio of disordered and ordered structure, which includes all types of secondary structure, in plant proteins was estimated (Figure 2). The result shows that *T. harzianum* and *T. brevicompactum* do not change the ratio of ordered and disordered structure of proteins in seed radicle.



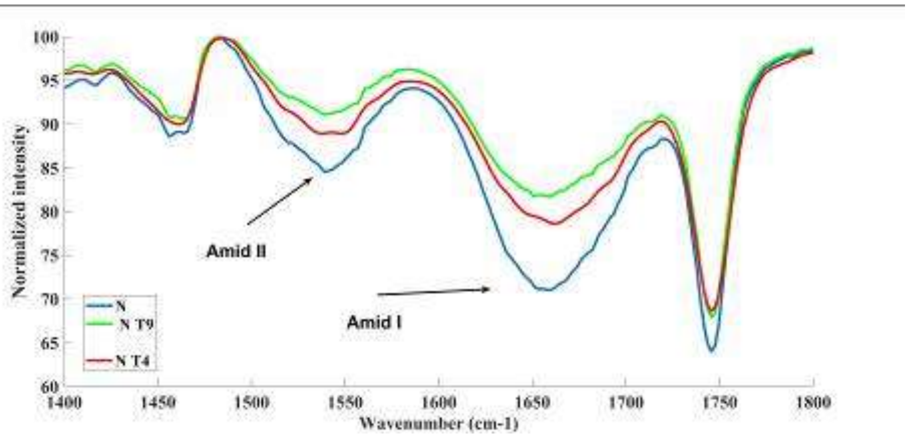


Figure 1 FTIR spectra of homogenized Gruzanski zlatni (A) and Narvik (B) radicles under different treatments: control (GZ, N), seeds treated with T. harzianum (GZ T4, N T4), seeds treated with T. brevicompactum (GZ T9,N T9)

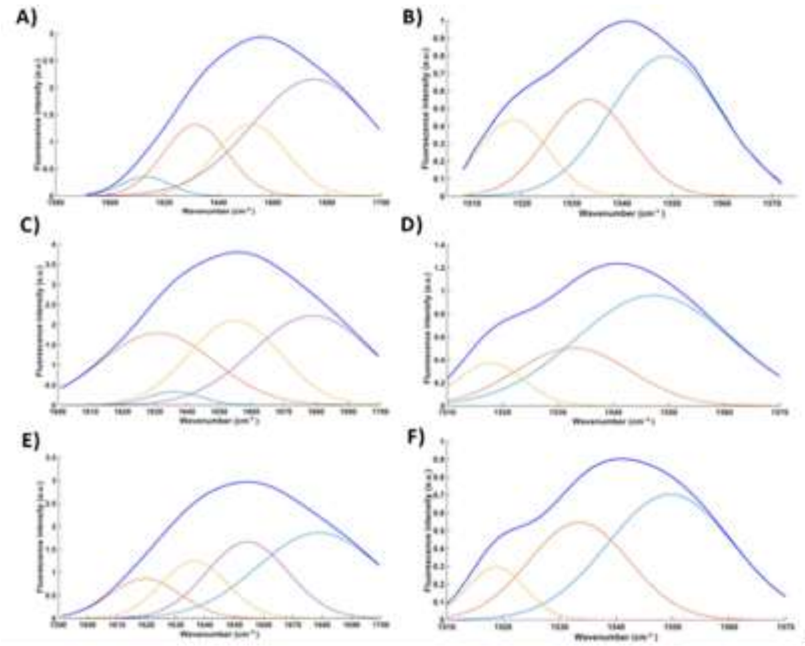


Figure 2. Examples of deconvolution of the Amide I band (A, C and E) and Amide II band (B, D and F) of the FTIR spectrum for tomato radicles: GZ control (A, B), GZ in co-culture with T. harzianum (C, D), GZ in co-culture with T. brevicompactum (E, F)

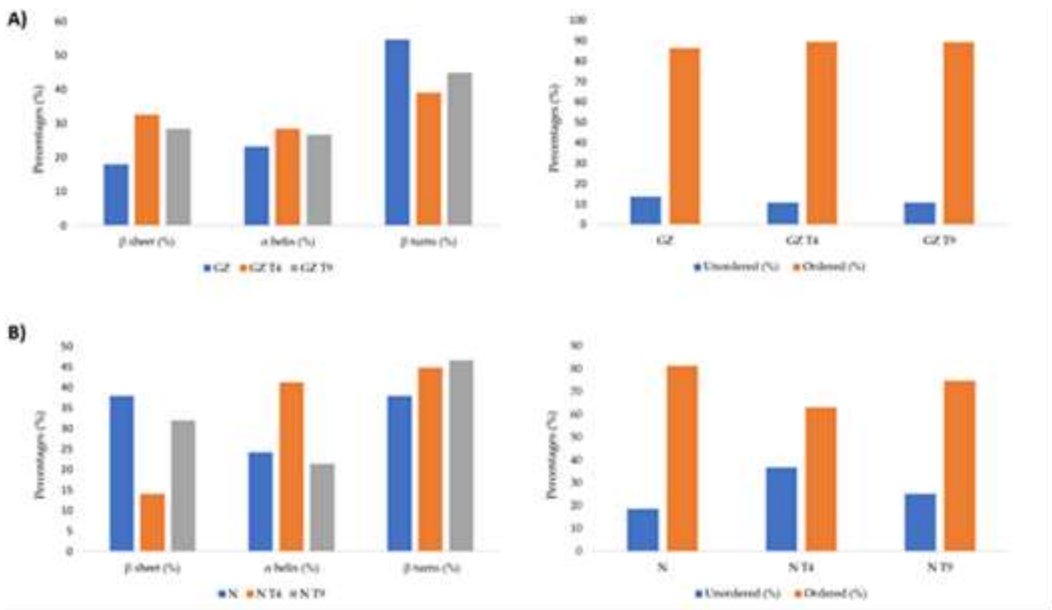


Figure 3. The proportional distribution of three distinct secondary structure types within seed radicles of GZ (A) and N (B), as determined through deconvolution of the FTIR band associated with Amide I (1600-1700 cm-1) (left column); The proportional distribution of ordered and disordered structure within seed radicles, as determined through deconvolution of the FTIR band associated with Amide II (1500-1580 cm-1) (right column).

Upon a comparative analysis of the spectra of GZ and N, distinct alterations in the protein structure are evident. Decomposition of the Amide I band (1600-1700 cm^{-1}) in N showed that both treatments resulted in an increased proportion of β turns compared to the control (Figure 3B). In the sample N T4, there is an observed increase in the proportion of α-helices, accompanied by a simultaneous reduction in β-pleated sheets, when compared to the control. Conversely, in the sample N T9, there is no significant alteration in the representation of these structural elements compared to the control group. Based on the Amide II band (1510-1575 cm^{-1}), the ratio of ordered to disordered structures was estimated. Compared to the control, the samples N T4 and N T9 have higher proportions of disordered structures in proteins compared to the ordered ones, which is more pronounced in the sample T4 (Figure 3).

Conclusion

Analysis using FTIR spectra, particularly the Amide I and Amide II bands, reveals that Trichoderma treatments lead to changes in protein secondary structures. Specifically, *T. harzianum* and *T. brevicompactum* treatments increase the proportions of α-helices and β-pleated sheets while reducing β turns. Although these treatments do not significantly alter the ratio of ordered to disordered protein structures in seed radicles, they do increase the proportion of disordered structures in other protein regions. These findings underscore the potential of *Trichoderma* spp. as an effective agents in improving tomato seed quality and plant performance.

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CONTACT TOXICITY OF THE *PINUS SIBIRICA* ESSENTIAL OIL AGAINST *DROSOPHILA SUZUKII*

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Abstract

Drosophila suzukii is a globally invasive pest that mostly targets soft-skinned fruits by laying eggs in ripening fruit, which leads to tissue damage by larval feeding on pulp. This pest is particularly damaging due to its unique ability, to attack healthy, ripening fruit with serrated ovipositor. Current control methods heavily rely on application of synthetic insecticides. Due to risk of pesticide residues in fruit, there is a growing effort for finding alternative, environmentally sustainable, control strategies. This study aimed to assess the insecticidal impacts of *Pinus sibirica* (Siberian pine) essential oil on *D. suzukii* adults in contact toxicity in three concentrations (1, 5 and 10%). The mortality, differentiated by sex, was observed after a 24-hour exposure period. Experimental units were equipped with moist tissue and a medium for water and feed supply, while medium also served for monitoring females' egg-laying ability under stressful environment. For better understanding of its potential mode of action, the chemical composition of *P. sibirica* essential oil was analyzed using Gas chromatography coupled with mass spectrometry (GC-MS). In this study, Siberian pine essential oil resulted in 35% mortality at 1% concentration, while concentrations of 5% and 10% caused 100% mortality of *D. suzukii* adults. Females exhibited higher resistance to treatments compared to males. At a concentration of 1%, the number of emerged adults was lower compared to the control treatments. Considering all above stated, essential oils offer a promising alternative management tool to chemical insecticides in various agriculture systems.

Keywords: *bioinsecticide, ecofriendly, D. suzukii, essential oil.*

Introduction

Drosophila suzukii (Matsumura, 1931) or spotted wing drosophila (SWD) is an invasive, highly adaptable pest that infests soft-skinned fruits, targeting a wide variety of stone and berry crops (raspberries, blueberries, strawberries, blackberries, cherries, and grapes). It can also infest wild plants across diverse genera. Females have unique serrated ovipositor, which allows them to penetrate the skin of undamaged and ripening fruits to deposit eggs (Walsh, 2011; Lee et al., 2011). Symptoms of *D. suzukii* infestation include the appearance of soft spots, structural collapse of berries, wrinkling, color changes, increased sugar level, and skin softening. Damage results from both egg-laying and larval feeding on the fruit pulp, leading to premature decay and fruit collapse before harvest. Infested fruits are more susceptible to secondary infections by other pests and pathogens such as bacteria, fungi, and yeasts, which accelerates decay of the infested fruit (Tait et al., 2018; Kirschbaum et al., 2020). This pest can produce up to 13 generations annually, facilitating its rapid and uncontrolled spread (Walsh et al., 2011; Kirschbaum et al., 2020). The spotted wing drosophila has a broad

distribution in moderate climate regions (Rota-Stabelli et al., 2013). The first record of *D. suzukii* flies in Serbia occurred in 2014, at several localities (Toševski et al., 2014).
Current control measures for *D. suzukii* mostly depends on synthetic insecticides. Given the pest's rapid and frequent reproduction cycles during the growing season, multiple applications of these insecticides are required to prevent significant fruit loss (Van Timmeren and Isaacs, 2013). However, this frequent use promotes resistance development within *D. suzukii* populations and leaves chemical residues on fruits. Consequently, there is an urgent need for alternative management strategies that are compatible with both integrated and organic crop production. Current research focuses on natural product-based methods, particularly essential oils-based botanical insecticides (Bošković et al., 2023). Natural pesticides are considered as alternatives to synthetic ones, with the goal of reducing reliance on artificial chemicals and promoting more sustainable and environmentally friendly pest control methods. Botanical insecticides are natural compounds derived from plants or plant components. Essential oils are biodegradable, environmentally safer, and have minimal impact on beneficial organisms, leaving little to no residues on fruits. With diverse modes of action compared to synthetic insecticides, essential oils help prevent the development of resistant insect populations (Isman, 2020; Vuković and Šunjka, 2021). These compounds, which consist of a complex mixture of volatile chemicals, can function as fumigants, contact insecticides, and influence pest behavior by deterring oviposition, repelling, or even attracting pests (Dam et al., 2019). By implementing biological control strategies, such as introducing natural predators or parasitoids, using microbial agents, or applying botanical bioinsecticides, a sustainable and eco-friendly approach can be taken to manage *D. suzukii* populations and minimize crop damage (Poveda et al., 2020).
The objective of this study was to assess the insecticidal and behavioral impacts of *Pinus sibirica* Du Tour essential oil on adults of *D. suzukii* through contact toxicity bioassay in three different concentrations (1, 5 and 10%). Effects of essential oils were evaluated on male and female adult flies. The emergence of adults from treated berries was also monitored and sex of newly emerged flies was also recorded.

Material and methods

Rearing of *D. suzukii* colony: The experimental part was conducted in the Insectarium facility of CIHEAM Bari, Italy. Adults in the Insectarium of CIHEAM has been maintained in a climatic chamber in Plexiglas cages under controlled conditions (22 °C ± 1 °C; 62% ± 4%, 12:12 h light:dark) with artificial diet (Schlesener et al., 2017). The genetic diversity of the colony was enhanced annually by introducing wild-caught flies from infested fruits, in order to avoid inbreeding. For the bioassay, adult flies aged 4 to 7 days were used and organic healthy blueberry purchased from the supermarket were selected as experimental fruits due to their year-round availability.
Essential oil solution: The essential oil tested in the multiple-choice test was anise oil (*P. sibirica*), obtained from Nature in Bottle, New York, SAD (Avena Lab – Farmadria ©). The essential oil was diluted in pure acetone at concentrations of 1%, 5%, and 10%, with water (marked as Control 1) and acetone (marked as Control 2) serving as control treatments.
GC–MS Analysis of the essential oil composition: For the characterization of *P. sibirica* essential oil chemical composition, gas chromatography with mass spectrometry (GC–MS Shimadzu QP2010) was used under conditions: fused silica HP-5 column; carrier gas He (1.0 mL/min); temperature programmed from 55 °C to 240 ° C with a temperature increase of 3 ° C/min; injection port temperature 250 ° C; and detector temperature 280 ° C. The constituents were identified by comparing their retention indices and MS spectra with those in the

databases available in the licensed MassFinder 4 software (EssentialOil 4a and Adams2205 databases). Prior to GC-MS analysis, the essential oil sample was dissolved in n-hexane.
Contact toxicity bioassay: In the experiment transparent polystyrene containers with a volume of 50 mL were used as the experimental units. Essential oil solutions were applied to the bottoms and sides of the containers with a spray finger. A fine mesh cover was placed over an opening to prevent saturation with essential oil volatiles and escape of flies. Paper discs were placed at the container bottoms, and the sprayed surface was allowed to dry before introducing ten *D. suzukii* flies (5 males and 5 females) into each test container. Each concentration (1%, 5%, and 10%) of the essential oil was tested in four replicates, along with control treatments. Control containers were treated with water and acetone, then allowed to dry completely. The test containers were maintained under controlled conditions (22°C ± 2°C, 62% ± 4% RH, 12-hour light:dark cycle) in a climate chamber. Flies mortality, taking into account the sex of the dead flies, was recorded after a 24-hour exposure period (Bošković et al., 2023). Each container contained a small wet tissue and an amount of medium to provide water and food for the adult insects, as well as to observe the females' egg-laying behavior in a stressful environment, when exposed to essential oil treatment. The counting of newly hatched flies began ten days into the experiment and continued until three consecutive days without any new emergence observed. The sex of the newly emerged flies was also documented.
Statistical analysis of the data was conducted using the statistical software "Statistica" version 14.0.0.15 (Tibco Software Inc., Palo Alto, CA, USA, 2021) for one-way ANOVA. Duncan's multiple range test was employed to compare treatment differences, while the chi-square test was utilized to assess the statistical significance of male and female emergence.

Results and discussion

In this study, *P. sibirica* essential oil caused 35% mortality in a contact test at a concentration of 1%, while at concentrations of 5% and 10%, mortality was 100% (Table 1). Statistically significant differences ($p<0.05$) in mortality was observed for all three oil concentrations compared to both controls. Using Duncan's multiple range test, statistically significant differences were determined between the 1% and 5% concentrations and between the 1% and 10% concentrations. The number of dead males at the 1% concentration was 11, while the number of dead females was 3, indicating that females exhibited greater resistance to the treatment compared to males, which can be possible attributed to the different immune molecular responses.

Table 1. Mortality of adult flies in the contact bioassay

Concentration	Mortality ± SD (%)	F	M
Control 1	0^{a}	0	0
Control 2	0^{a}	0	0
1%	35^{b} ± 0,57	3	11
5%	100^{c}	20	20
10%	100^{c}	20	20
F-test	1527		
p value	$p<0.05$		
χ^2	130.00		
df	31		
p value	$p<0.05$		

Control 1 - water; Control 2 - acetone; SD - standard deviation; F - females; M –males. Different indicate statistically significant differences.

The application of *P. sibirica* essential oil at a concentration of 1% resulted in a lower number of emerged adults compared to the control treatments, with an equal number of emerged females and males (Table 2). Statistically significant differences ($p<0.05$) in the number of emerged adults were observed for all three oil concentrations compared to both controls.

Table 2. Mean number of emerged adults after exposure to contact toxicity bioassay

Concentration	$\bar{x} \pm SD$ (%)	F	M
Control 1	$20^{a} \pm 6,58$	36	44
Control 2	$19,75^{a} \pm 3,5$	40	39
1%	$4^{b} \pm 1.15$	8	8
5%	0^{b}	0	0
10%	0^{b}	0	0
F-test	37.18		
p value	p<0.05		
χ^2	180.0		
df	120		
p value	p<0.05		

Control 1 - water; Control 2 - acetone; SD - standard deviation; F - females; M –males. Different indicate statistically significant differences

Siberian pine essential oil has a large number of compounds in its composition with a percentage of less than 5% (Table 3). The highest concentrations are found in β-himachlen (22.46%) and cis-thujopsene (17.19%). Additionally, α-cedrol is present at 11.08%, and α-cedren at 10.9%.

Table 3. Chemical composition of *Pinus sibirica* essential oil

Compound	**RI_{exp}**	**$RI^{a,b}$**	**Relative content (%)**	**IM^{c}**
			P. sibirica	
α-Kampinen	1394	1396	0.38	RI^{a}, MS
Longifolen	1402	1407	0.15	RI^{a}, MS
α-Cedren	1409	1410	10.9	RI^{a}, MS
β-Cedren	1417	1419	3.08	RI^{a}, MS
cis-Tujopsen	1428	1429	17.19	RI^{a}, MS
α-Himahlen	1446	1449	8.25	RI^{a}, MS
γ-Himahlen	1476	1481	5.47	RI^{a}, MS
Himahla-1,4-dien, 11-alfaH	1479	1485	0.82	RI^{a}, MS
β-Himahlen	1498	1504	22.46	RI^{a}, MS
Kuparen	1503	1504	1.99	RI^{a}, MS
α-Alasken	1511	1512	1.06	RI^{a}, MS
γ-Dehydro-ar-himahlen	1526	1530	0.21	RI^{a}, MS
γ-Kuprenen	1530	1532	0.27	RI^{a}, MS
α-Cedrol	1599	1600	11.08	RI^{a}, MS
β-Himahlen oksid	1611	1615	1.01	RI^{a}, MS
2-Himahlen-7b-ol (EO)	1646	1642	0.62	RI^{a}, MS
Allo himahalol	1660	1661	0.42	RI^{a}, MS
α-Bisabolol	1683	1685	0.27	RI^{a}, MS
γ-*cis*-Atlanton	1691	1694	1.55	RI^{a}, MS
Deodaron	1695	1698	0.87	RI^{a}, MS
γ-*trans*-Atlanton	1704	1706	1.7	RI^{a}, MS
α-*cis*-Atlanton	1715	1717	1.64	RI^{a}, MS
α-*trans*-Atlanton	1774	1777	5.48	RI^{a}, MS

t-trace (<0.1); RI a,b—retention index from the literature; a—Adams 2205 database; b—EssentialOil 4a database; RI—retention indices calculated from retention times in relation to those of a series of n-alkanes C8-C40 on a 30 m DB-5 capillary column; MS—mass spectra; IM c—identification method was MS based on a comparison with the Adams 2205 and EssentialOil 4a databases.

Pinus sibirica, has been poorly studied in the form of essential oil in biological tests for evaluating insecticidal effects, but its relative, *P. sylvestris* L., has been more extensively tested in biological assays using essential oils. The chemical composition of *P. sibirica* essential oil is very complex and largely depends on the plant part from which it is isolated (bark, trunk, needles) (Rogachev and Salakhutdinov, 2015). The most abundant compounds in the *P. sibirica* essential oil used in our study are *cis*-thujopsene, β-himachalene, and α-cedrol. The compound α-cedrol is also found in the essential oil of *Mosla soochowensis* Matsuda, which has been shown to exhibit fumigant and contact effects on two species of stored product pests, *Sitophilus zeamais* (Motschulsky, 1885) and *Tribolium castaneum* (Herbst, 1797) (Chen et al., 2017). In this research, Siberian pine essential oil demonstrated insecticidal contact effect at higher concentrations (5% and 10%) on the *D. suzukii* adults. The mechanism of action of botanical insecticides derived from essential oils is intricate and varies for each compound, often involving a synergistic interaction of multiple compounds at once. The specific components of essential oils play a vital role in determining their effectiveness against insects. Further research on the safety of botanical insecticides for non-target organisms is essential to ensure the practical application of plant essential oils, particularly in organic agriculture. Comprehensive studies are needed to assess the potential impact of these insecticides on beneficial insects, wildlife, and the environment as a whole. Understanding the broader implications of using botanical insecticides will help in developing sustainable and environmentally friendly pest management strategies in organic farming practices.

Conclusion

Siberian pine in this study has demonstrated significant efficacy against *D. suzukii* in laboratory experiments, that represent the first data on its effect on this pest. Essential oils, such as those from *P. sibirica*, offer several advantages as protective agents in agriculture. They are biodegradable, environmentally friendly, and exhibit multiple modes of action, which reduce the likelihood of pests developing resistance. Additionally, essential oils have minimal impact on beneficial organisms and leave little to no harmful residues on crops. Future research should focus on field trials to confirm the laboratory findings, investigate optimal application methods, and assess the long-term sustainability and economic feasibility of using essential oils in integrated pest management strategies. Given their chemical composition and insecticidal properties, *P. sibirica* essential oils has the potential to be a leading choice for biological control against *D. suzukii*.

Acknowledgements

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PRELIMINARY STUDY OF ESSENTIAL OILS EFFECT ON *BOTRYTIS CINEREA*

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Abstract

The causal agent of grey mold, *Botrytis cinerea*, attacks a wide range of crop species worldwide and leads to great economic losses. In fruits and vegetables, it causes pre- and post-harvest decay, reducing the quality and market value of the product. Controlling *B. cinerea* is challenging, due to its diverse modes of attack, numerous host plants, and ability to survive in various forms, including mycelia, conidia, and sclerotia for long periods. The current management strategy includes spraying fungicides during the flowering and fruiting stages. Intensive pesticide use leads to the development of resistance in target organisms, which makes it difficult to control. With the negative impact that conventional pesticides have on human health and the environment, it is necessary to implement alternative and eco-friendly methods against this pathogen. Essential oils are widely recognized for their antifungal effects, garnering significant interest among researchers nowadays. This study aimed to compare the inhibitory effects of star anise (*Illicium verum* L.), laurel (*Laurus nobilis* L.), and parsley (*Petroselinum sativum* Hoffm) essential oils on *B. cinerea in vitro*. Mycelial growth inhibition was assessed using a fumigation bioassay, with three replicates per essential oil. Star anise essential oil showed the highest efficacy, resulting in complete inhibition (100%) of *B. cinerea* mycelial growth. The efficacy of laurel and parsley essential oils decreased over seven days. The study showed that star anise essential oil has the potential to act as a biocontrol agent against *B. cinerea*.

Keywords: *Botrytis cinerea, essential oils, fumigation bioassay, eco-friendly, biocontrol.*

Introduction

Phytopathogenic fungi represent one of the major challenges in plant production. They lead to reduced crop yields and quality, resulting in significant losses in agricultural production. Synthetic fungicides are the main method for controlling fungal diseases. However, their irregular and excessive use increases concerns about residues in the environment, negative impact on human and animal health and developing resistance to pesticides prompting a search for alternatives. Essential oils are attracting growing interest due to their relative safety to nature and humans, worldwide production, and demonstrated antimicrobial and antifungal properties (Mohammadi et al., 2013). They are volatile liquids obtained from various parts of aromatic plants. These secondary metabolites protect plants from pests and diseases (Hanif et al., 2019). Essential oils often have a complex chemical composition, consisting of mono- and sesquiterpenes, aldehydes, ketones and phenols (Isman et al., 2011). They exhibit antifungal properties by inducing critical physiological and metabolic disruptions, leading to decreased mycelial growth and spore germination (Fincheira et al., 2023). They are lipophilic which leads to their rapid diffusion through the fungal cell wall and cytoplasmic membrane (Andrade-Ochoa et al., 2021). Affecting the leakage of ions such as K^+, Ca^{2+}, and Mg^{2+}, essential oils disrupt the integrity of the cell plasma membrane (Fincheira et al., 2023). Furthermore, they can interact with mitochondria, influencing membrane potential, proton pumps, and ATP synthase, thereby disturbing cellular pH and the electrochemical gradient across the plasma membrane (Maurya et al., 2021). However, research on essential oils for controlling phytopathogenic fungi primarily focuses on their ability to inhibit growth, with the mechanisms of action remaining unclear (Fincheira et al., 2023). *Botrytis cinerea* (teleomorph: *Botryotinia fuckeliana*) is a fungus from the class *Ascomycota* that causes gray mold disease in many plant species. It is found worldwide and can infect nearly every plant and plant part. This highly destructive pathogen can live both pathogenically and saprophytically, and can adapt to various environmental conditions, which significantly complicate its control in agriculture (Cheung et al., 2020; Rosslenbroich et al., 2000). It can remain in the latent phase and occur in the fruits in storage, where the infection can rapidly spread to adjacent fruits, leading to a subsequent rise in product loss (Álvarez-García et al., 2023). In the Republic of Serbia, fungicides based on 13 active ingredients are currently registered for controlling *B. cinerea* in various plant species. These active substances include boscalid, cyprodinil, dimoxystrobin, fludioxonil, fluopyram, fenhexamid, isofetamid, penthiopyrad, prothioconazole, pyraclostrobin, pyrimethanil, tebuconazole and trifloxystrobin, with specific usage varying depending on the plant species. Additionally, biopesticides based on *Bacillus subtilis*, *Bacillus amiloliquefaciens*, *Pythium oligandrum*, eugenol, geraniol, tea tree oil, thymol and sodium hydrogen carbonate are also registered (Group of authors, 2022). A challenging aspect in controlling *B. cinerea* is the lack of fungicides with short pre-harvest intervals, as well as the development of resistance to existing fungicides. *B. cinerea* is categorized among high-risk pathogens prone to developing resistance to conventional fungicides (Rosslenbroich et al., 2000). It has been extensively researched regarding the antifungal properties of vapor-phase essential oils, both in *in vitro* and *in vivo* (Fincheira et al., 2023). This research focused on assessing and comparing the effectiveness of essential oils from star anise (*Illicium verum* L.), laurel (*Laurus nobilis* L.), and parsley (*Petroselinum sativum* Hoffm) in inhibiting the growth of *B. cinerea* in a controlled laboratory conditions.

Materials and Methods

The experiment was carried out in 2024 in laboratory conditions. Three commercially available essential oils were tested to determine their vapor-phase antifungal activity against *B. cinerea*: star anise (*Illicium verum* L.), laurel (*Laurus nobilis* L.) and parsley (*Petroselinum sativum* Hoffm). *B. cinerea*, which is part of the permanent collection of microbiological cultures at the Laboratory for Biological Research and Pesticides, Faculty of Agriculture, University of Novi Sad in Serbia, was inoculated on potato dextrose agar (PDA) and kept at 23 °C for seven days. Mycelial growth inhibition was tested using a fumigation bioassay, with three replicates per essential oil. In each Petri dish, a mycelium slice of *B. cinerea* (5 mm) was placed onto the prepared PDA medium. Fifteen microliters of each essential oil were added to the inverted lid of the Petri dish, and then it was covered with the bottom part containing the mycelium. A sterile distilled water is placed on the lid of the control variant. The Petri dishes were sealed with parafilm and incubated in a thermostat at 23 °C for seven days (Figure 1).

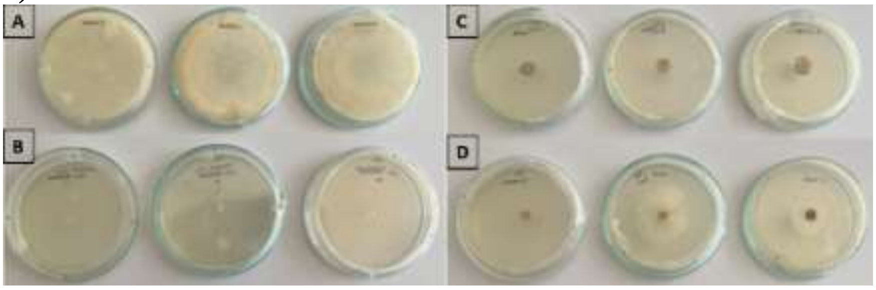

Figure 1. Petri dishes after seven days: A – control, B – star anise, C – parsley, D – laurel

Results and Discussion

The fungal growth was assessed daily for one week, measuring from the edge of the agar inoculum plugs. The mean value of *B. cinerea* mycelial growth of each treatment is given in Table 1.

Table 1. The mycelial growth of B. cinerea

Treatment	Mycelial growth (mm)						
	1st d	2nd d	3rd d	4th d	5th d	6th d	7th d
Control	11 ±2.94	35.67 ±0.47	55 ±1.63	79 ±1.41	82.33 ±2.05	82.67 ±2.05	85±0
EO of *I. verum*	0	0	0	0	0	0	0
EO of *L. nobilis*	0	0	0.67 ±0.94	3.67 ±2.36	7.33 ±3.30	15.67 ±8.99	18.67 ±8.96
EO of *P. sativum*	0	0	1.67 ±0.47	5.67 ±0.94	8.67 ±1.70	9±1.41	10.33 ±0.94

Using the data from Table 1, the daily efficacy of each essential oil was calculated by applying the following formula:

$$E, \% = \frac{\text{mean colony diameter of control sets} - \text{mean colony diameter of treatment sets}}{\text{mean colony diameter of control sets}} \times 100.$$

The efficacy of each essential oil is presented in Figure 2.

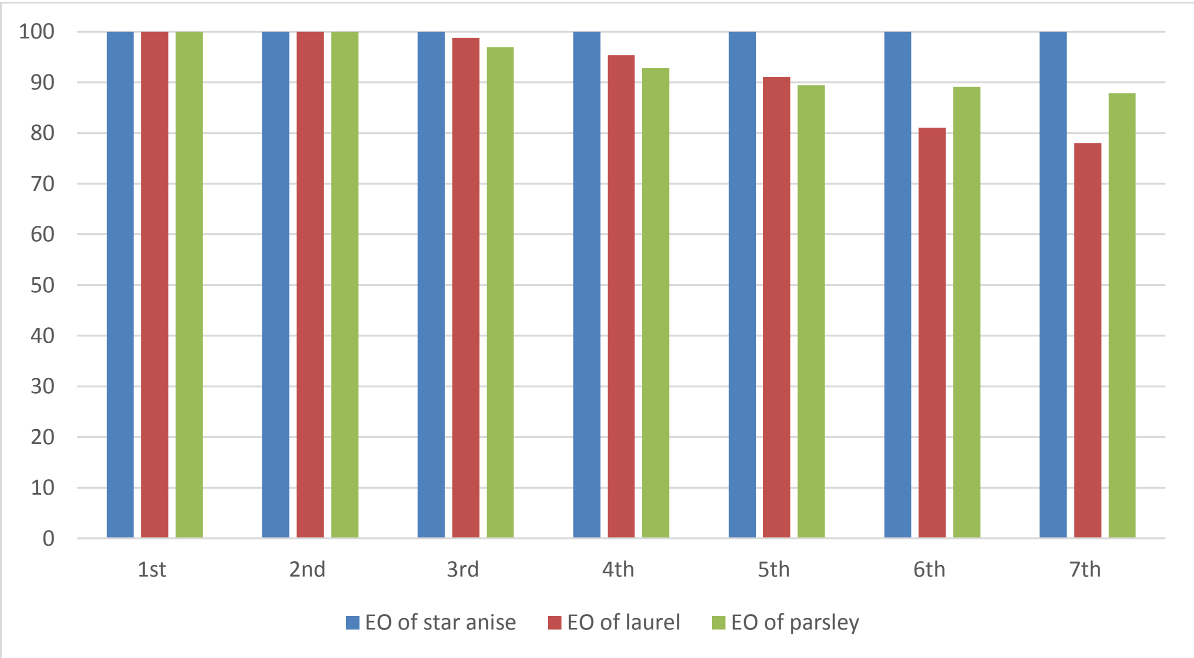

Figure 2. Efficacy (%) of essential oils against *B. cinerea* by days

This study indicates that analyzed essential oils have an inhibitory effect on the mycelial growth of *B. cinerea*. Among three tested essential oils, *I. verum* showed strong antifungal activity (100%) which was maintained throughout the seven days. The efficacy of laurel and parsley essential oils was 100% in the first two days, afterward gradually decreasing over

time. Parsley demonstrated better efficacy at the end of the seventh day (87.85%). The decrease in efficacy could be due to the fast volatility of parsley and laurel essential oils.
Several studies have tested the antifungal effect of star anise essential oil and obtained similar results. Lee et al. (2007) showed that this essential oil inhibits the mycelial growth of *B. cinerea* by about 90%. Huang et al. (2010) conducted experiments to evaluate the antifungal activity of star anise essential oil against several plant pathogenic fungi such as *Alternaria solani*, *Bipolaris maydis*, *Fusarium graminearum*, *Fusarium oxysporum* f. sp. *cucumerinum*, *Fusarium oxysporum* f. sp. *lycopersici*, *Pythium aphanidermatum*, *Rhizoctonia solani* etc. Their findings indicated a potent inhibitory effect against all tested fungi, suggesting that the presence of trans-anethole in the oil contributed significantly to its observed antifungal properties. Using gas chromatography-mass spectrometry, Lee et al. (2007) determined that *trans*-anethole is the main antifungal constituent of *I. verum* essential oil.
The fungicidal activity of the essential oil of *L. nobilis* was analyzed by De Corato et al. (2010). The authors investigated the inhibition of *B. cinerea*, *Monillinia laxa* and *Penicillium digitatum* mycelial growth depending on the applied concentration (200, 400, 600, 800 and 1000 µg/mL). *M. laxa* was completely inhibited by the oil at the lowest concentration, while *B. cinerea* was fully inhibited at the concentration of 1000 µg/mL. *P. digitatum*, was only partially inhibited across all concentration levels. Additionally, the authors examined the composition of the essential oil by gas chromatography analysis. Laurel essential oil was found to have a high content (over 10%) of 1.8-cineole, linalool, terpineol acetate, and methyl eugenol, and a low content (under 10%) of linalyl acetate, eugenol, sabinene, b-pinene, and a-terpineol.
Pineda et al. (2018) examined the effect of the essential oil of parsley against Collectotrichum acutatum, by the poisoned agar method. Results showed that essential oil significantly inhibits the radial growth of C. acutatum at concentrations higher than 100 µg/mL. Analysis by gas chromatography with mass spectroscopy (GC-MS) showed that the major components are phenylpropanoids myristicin (18.7%), parsley-apiole (27.6%) and 1,3,8-p-menthatriene (11.2%).

Conclusion

Nowadays, the focus in controlling postharvest diseases is towards more sustainable strategies. This can involve the use of various biological agents for plant protection, among which are essential oils. This study concludes that the essential oil of star anise exhibits significant antifungal properties and has the potential to be utilized as a natural fungicide to manage disease caused by *B. cinerea*. Due to the instability of essential oils in the environment, it is necessary to work to improve their persistence.

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MYCOPOPULATION ON RASPBERRIES IN SERBIA

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Abstract

Raspberry is a perennial deciduous plant, bushy or semi-shrubby, with annual and long biennial shoots, which belongs to the rose family. For many years, raspberries have been among the most economically important types of fruit in the Republic of Serbia. So far, there have been no systematic researches on raspberry mycopopulation in Serbia. In this paper, we present the results of preliminary research on raspberry mycopopulation originating from Serbia. A total of 28 randomly collected samples from three locations in Serbia were analyzed. Root and stem were analyzed. Plant fragments were carefully washed under running water. The samples thus prepared were disinfected with 1% sodium hypochlorite (NaOCl) for 1 minute and washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (KDA). In each Petri dish, five fragments taken per sample were placed in five repetitions, and incubated in a thermostat at 24°C. The examination was performed every three days, and on most samples mycelia developed around the plant fragment by the 14th day. Microscopic examination was performed using an Olympus CX31 microscope. The frequency of isolation was calculated in % according to the formula: number of colonized parts with fungi/total number of analyzed plant parts x 100. A total of 700 plant fragments were examined, from which six genera of fungi were isolated: *Didymella, Fusarium, Rhizoctonia, Verticillium, Alternaria* and *Epicoccum.*

Key words: *Frequency of isolation, mycopopulation, raspberry*

Introduction

Raspberry is a perennial deciduous plant, with bushy or semi-shrub growth, with annual and long biennial shoots, which belongs to the Rosaceae family. For many years, raspberries have been among the most economically important fruit species in the Republic of Serbia (Leposavić, 2023). The world production of raspberries in 2019 was 684,000 tons (Petrović *et al*., 2020; Leposavić, 2023). On the European continent, Russia, Poland, Serbia and Spain are the biggest producers. However, the largest commodity production is realized in Serbia, because more than 95% of processed and fresh raspberry fruits are placed on the world market.

The most common causes of raspberry diseases are phytopathogenic fungi, bacteria and viruses. Due to the characteristic habitus of plants and intense vegetative growth, microclimatic conditions are created in raspberry plantations that favor the development of pathogens and the emergence of infections. Phytopathogenic fungi represent a very numerous and economically harmful group of pathogens on raspberries. The most economically important fungal diseases on raspberry are: *Didymella applanata* (Niessl.) Sacc. – spur blight; *Elsinoe veneta* (Burkh.) Jenkins – anthracnose (cane spot); *Phragmidium rubi-idaei* de Candolle P. Karsten – raspberry rust; *Paraconiothyrium fuckelii* (Saccardo) Verkley &

Gruyter – raspberry blight; *Phytophthora rubi* Man in’t Veld – raspberry root rot; *Podosphaera macularis* (Wallroth) Braun & Takamatsu – powdery mildew; *Verticillium dahliae* Klebahn and *Verticillium albo-atrum* Reinke & Berthold – verticillium wilt; *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) – raspberry gray mold (Hai and Sutton, 1997; Munro *et al.,* 1988; Williamson, 2003; Williamson *et al.,* 2007; Mirković *et al.,* 2015). Among the bacterial disease-causing agents of raspberry, the most important are: *Agrobacterium tumefaciens* (Smith & Townsend) Conn – crown gall disease, *Pseudomonas syringae* pv. *syringae* van Hall. – pseudomonas blight disease and *Erwinia amylovora* (Burrill) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers & Smith – fire blight (Braun and Hildebrand, 2006; Ivanović *et al.,* 2012). More than 30 viruses that can infect raspberries have been described in the world literature. Viral diseases occur everywhere where raspberries are grown. Economically the most important some viruses of raspberries are: black raspberry necrosis virus, BRNV; Raspberry leaf mottle virus, RLMV; Rubus yellow net virus, RYNV; raspberry bushy dwarf virus, RBDV; raspberry leaf blotch virus, RLBV and others (Jevremović and Paunović, 2011; Jevremović *et al*., 2016; Jevremović *et al*., 2019).

Considering the great importance of raspberry as a fruit species, especially for less developed hilly and mountainous areas in Serbia, the aim of this work was the isolation and morphological determination of mycopopulation on raspberry in order to better understand the problems in raspberry production (extinction of plants and reduction of yield) as a consequence of the presence of phytopathogenic fungi. Despite the intensive effort to find alternative solutions for controlling phytopathogenic fungi on raspberry, chemical protection is the dominant method. However, the application of chemical measures is difficult. The biggest problem is the timing of fungicide application, because flowering and raspberry harvest often overlap, and the presence of pesticide residues in fruits can be a problem. Also, the significant problem is the fact that a small number of fungicides are registered for the control of phytopathogenic fungi on raspberries.

Materials and methods

The samples were collected from 2019 to 2022 in the period June - August. A total of 28 samples from three localities in Serbia were analyzed (Čačak 8, Ivanjica 10, and Arilje 10). The experiment was conducted in the phytopathological laboratory of the Faculty of Agriculture in Kruševac, University of Niš. The plant parts were carefully washed under running water. After washing, parts of the stem and roots were cut into pieces measuring 0.5–1 cm. The samples thus prepared were disinfected with 96% ethanol for 30 seconds, 1% sodium hypochlorite (NaOCl) for 1 min and washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. In each Petri dish, 5 plant parts were placed, in five repetitions. They were kept in a thermostat at 24°C and a light regime of 12 hours day/12 hours night. Examination was performed every 3 days, and on most samples, mycelia developed up to two weeks. The developed mycelia were screened on a new PDA substrate and, after the initial growth, the top part of the mycelium was screened again on PDA. Microscopic examination was performed using an Olympus CX31 microscope. Morphological identification of fungi was performed using standard keys (Dhingra and Sinclair, 1995). The frequency of isolation in % was calculated according to the formula (Vrandečić *et al*., 2011):

$$(\%)\ \text{Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

A total of 700 plant parts were examined, from which 6 genera of fungi were isolated: *Didymella, Fusarium, Rhizoctonia, Verticillium, Alternaria* and *Epicoccum* (Table 1). Macroscopic symptoms of infection were clearly visible on the plants from which the fungi were isolated. On all plants from which the fungi were isolated, clearly expressed symptoms on the stems in the form of necrotic spots and lesions were observed. From those plants *Alternaria* fungi were isolated. In plants where purple-brown necrosis was observed on the stems, in the lower third of the stem, the fungi from the genus *Didymella* were isolated. Fungi from the genera *Fusarium* and *Rhizoctonia* were isolated from the plants with symptoms in the form of light to dark brown necrosis on the root system. Discoloration of the conducting tissue was also observed in some plants on the root system, and fungi from the genus *Verticillium* were isolated from those plants.

Table 1. Frequency of fungal isolation on raspberry

Location	Number of samples Plant part	Fungi species	(%) Isolation frequency
Čačak 1	25	*Alternaria* sp., *Fusarium* sp., *Didymella* sp., *Verticillium* sp.	15, 10, 25, 10
Čačak 2	25	*Didymella* sp., *Alternaria* sp., *Epicoccum* sp.	25, 10, 5
Čačak 3	25	*Alternaria* sp., *Didymella* sp., *Verticillium* sp.	25, 20, 40
Čačak 4	25	*Alternaria* sp., *Didymella sp.*, *Verticillium sp.*	15, 35, 15
Čačak 5	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp., *Epicoccum* sp.	25, 10, 5, 5
Čačak 6	25	*Didymella* sp., *Verticillium* sp.	60, 10
Čačak 7	25	*Didymella* sp., *Alternaria* sp., *Epicoccum* sp.	40, 15, 5
Čačak 8	25	*Didymella* sp., *Rhizoctonia* sp.	35, 20
Ivanjica 1	25	*Didymella* sp., *Fusarium* sp., *Epicoccum* sp.	20, 5, 5
Ivanjica 2	25	*Didymella* sp., *Alternaria* sp., *Fusarium* sp., *Rhizoctonia* sp.	60, 20, 10, 5
Ivanjica 3	25	*Didymella* sp., *Epicoccum*sp.	30, 35
Ivanjica 4	25	*Didymella* sp., *Alternaria* sp., *Fusarium* sp.	15, 35, 5
Ivanjica 5	25	*Didymella* sp., *Epicoccum* sp., *Rhizoctonia* sp.	25, 5, 15
Ivanjica 6	25	*Didymella* sp., *Verticillium* sp.	25, 15
Ivanjica 7	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp.	35, 10, 5

Ivanjica 8	25	*Didymella* sp., *Epicoccum.* sp., *Fusarium* sp., *Rhizoctonia* sp.	35, 5, 20, 5
Ivanjica 9	25	*Didymella* sp., *Verticillium* sp., *Alternaria* sp., *Epicoccum* sp.	10, 15, 5, 5
Ivanjica 10	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp.	25, 10, 15
Arilje 1	25	*Didymella* sp., *Rhizoctonia* sp., *Alternaria* sp., *Epicoccum*.sp.	25, 15, 5, 10
Arilje 2	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia sp.*	25, 10, 10
Arilje 3	25	*Didymella* sp., *Fusarium* sp., *Alternaria* sp., *Epicoccum* sp.	25, 10, 15, 15
Arilje 4	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp.	30, 5, 15
Arilje 5	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia sp.*	15, 10, 5
Arilje 6	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp., *Alternaria* sp.	5, 10, 5, 10
Arilje 7	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp.	25, 10, 10
Arilje 8	25	*Didymella sp.*, *Fusarium* sp., *Rhizoctonia* sp., *Alternaria* sp.	30, 15, 20, 5
Arilje 9	25	*Didymella* sp., *Fusarium* sp., *Alternaria* sp., *Epicoccum*.sp	30, 5, 10, 20
Arilje 10	25	*Didymella* sp., *Fusarium* sp., *Rhizoctonia* sp.	15, 20, 5

D. applanata is widespread in all raspberry growing regions in Europe, America, Africa, Asia and Australia (Mirković, 2018). In the Fraser Valley, British Columbia in the 1930s, several phytopathogenic fungi were isolated from infected raspberry plants: *Leptosphaeria coniothyrium* (*Coniothyrium fuckelii*), *Ilyonectria destructans* (*Cylindrocarpon radicicola*), *Fusarium* spp., *Cylindrocladium* spp., *Pythium* spp., *R. solani*, *Rhizoctonia* spp. After artificial inoculations of healthy raspberry plants, obtained with fungal isolates, it was determined that all isolated species can cause necrosis on the roots of healthy plants (Berkeley, 1936). Raspberries are the third most popular berry in the United States and a growing specialty crop for both the wholesale industry. Postharvest susceptibility to gray mold (*B. cinerea*) drastically reduces the shelf life of this delicate fruit (Hai and Sutton, 1997; Williamson, 2003; Williamson *et al.*, 2007; Harshman *et al*., 2014). *D. applanata* causes spur blight of raspberry was isolated from canes of diseased raspberries in a plantation in Novosibirsk, Russia (Shternshis *et al.,* 2006). *Alternaria tenuissima* has been reported on raspberries in China, in 2014 and 2015, as a leaf spot disease was observed on cultivated raspberry. The disease incidence averaged approximately 75%. Some small, circular, light brown spots appeared on the leaves in the early stage of disease development. The spots gradually enlarged and became round or irregular, dark brown, and developed concentric rings with dark brown margins normally surrounded by light yellow halos measuring 0.5 to 4.0 mm. Finally, the necrotic spots often coalesced to large blights of 11.0 to 15.0 mm. Severely infected leaves aged early and some fell off (Cong *et al.,* 2016). Raspberry cane disease is caused by various phytopathogenic fungi, including *Fusarium avenaceum* (Fusarium wilt), *D. applanata* (spur blight), *L. coniothyrium* (cane blight) and *B. cinerea* (cane *Botrytis*) with *F. avenaceum* being the major cause of cane diseases in Northern

Germany (Girichev *et al.,* 2018). Soilborne pathogens are a major concern to red raspberry and strawberry producers throughout the Pacific Northwest. Red stele, caused by *Phytophthora fragariae* var. *fragariae*, and raspberry root rot, caused by *P. fragariae* var. *rubi*, can cause serious economic losses in cool, wet production areas (Pinkerton *et al*., 2002). Soilborne fungal pathogens (such as *Phytophthora* spp., *Verticillium* spp., *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp.), lesion nematode (*Pratylenchus penetrans*) and weeds are common pests of Mexican strawberry and raspberry nurseries. Phytophthora root rot caused by *P. fragariae* var. *rubi* is the most serious root disease of red raspberries in many growing regions Mexico. Raspberry roots are also very sensitive to excessive moisture and lack of oxygen in the soil for extended periods of time. These conditions cause decay and root death. Best roots are produced in non-infested soils with good drainage (Lopez-Aranda *et al.,* 2016).

Conclusion

Growing raspberries is a very important and economically significant activity in Serbia. One of the most important factors that limits the profitability of raspberry cultivation are phytopathogenic fungi, the cause of economically important diseases. In rainy and humid conditions, diseases develop more intensively, and yields in the following year may be significantly reduced. The use of pesticides, i.e., fungicides, is the dominant method for phytopathogenic fungi control in raspberries. The reason why raspberry protection is based on the use of fungicides is the lack of raspberry varieties that show tolerance to economically significant disease agents. Such as *Didymella, Rhizoctonia, Verticillium, Phytophthora* and *Botrytis*. However, the implementation of these measures is difficult. The biggest problem is the limited time of pesticide application because the flowering and harvesting of raspberries can take a very long time, over 60 days. As a problem in the chemical protection of raspberries, a fairly poor range of fungicides is cited as a result of chemical companies' lack of interest in registering new active substances and formulations because the market is small and the registration of preparations is expensive. However, the application of chemical agents to protect raspberries from pathogenic fungi is the only measure that is necessary for profitable production. Without the application of all other available measures, primarily agrotechnical and mechanical, their full effect will be absent.

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PHYTOPATOGENIC FUNGI OF ALFALFA IN SERBIA

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Abstract

Alfalfa (*Medicago sativa* L.) occurs in nature as a cultivated and wild species. As one of the oldest and most important perennial forage crops, alfalfa has the potential to produce large amounts of high-quality forage. This paper presents the preliminary results of the population study of phytopathogenic fungi on alfalfa. A total of 625 samples of alfalfa plants were collected between 2002 and 2019 in Serbia from 17 locations. Standard phytopathological isolation was performed from the transition of healthy to diseased tissue on potato dextrose agar (PDA) with streptomycin. After incubation at 22°C and a light regime of 12 hours day/12 hours night, the obtained cultures were examined microscopically and the morphological identification of fungi to the genus level was carried out using standard keys. Checking the pathogenicity of the obtained isolates was done through artificial inoculation with damage to plant parts. Inoculation was performed with 7-day-old fungi isolates, and the inoculated plants were stored at a temperature of 20 to 25°C. Based on the morphological and pathogenic characteristics of the obtained isolates, it was determined that alfalfa pathogens were dominant in Serbia *Colletotrichum*, *Fusarium, Verticillium, Rhizoctonia, Sclerotinia* and *Phoma*. As the causative agents of alfalfa diseases are not sufficiently studied in Serbia, the conducted research represents a significant contribution to defining the role of phytopathogenic fungi in the symptoms they cause on alfalfa, all with the aim of contributing to finding adequate measures for their successful suppression.

Key words: *alfalfa, phytopathogenic fungi.*

Introduction

The genus *Medicago* has more than 60 species, of which about 70% are annuals. Among the field plants for feeding cattle, alfalfa has the most important place, so many call it the "queen of fodder plants". After destroying the areas under alfalfa, large amounts of nitrogen and organic matter remain in the soil, the decomposition and mineralization of which improve the physical, chemical, and microbiological properties of the soil (Đukić *et al*., 2009).
At least 62 parasitic fungi that cause alfalfa diseases have been described in the world, of which only 19 types of disease-causing fungi have been described in our country. According to the part of the plant that primarily endangers, parasites on alfalfa are classified as those that inhabit the above-ground parts of alfalfa with the manifestation of symptoms on the leaves and stems, and parasites that inhabit the ground part of the tree, the crown, and the root (Balaž and Popović, 2005; Vasić, 2013). The most widespread and harmful parasitic fungi that threaten alfalfa are: *Colletotrichum trifolii*, *C. destructivum*, *C. truncatum* and *C. dematium* – causes of anthracnose; *Pseudopeziza medicaginis* and *Stemphylium botryosum* – causes of alfalfa leaf spotting; *Peronospora trifoliorum* – the causative agent of alfalfa blight, *Uromyces striatus* – the causative agent of alfalfa rust, *Cercospora medicaginis* – the cause of leaf

spotting; *Leptotrochila medicaginis* – the cause of yellowing of alfalfa leaves; *Fusarium oxysporum* f. sp. *medicaginis*, *Verticillium albo-atrum* and *V. dahliae* – causes of wilting of alfalfa; *Rhizoctonia solani*, *Sclerotinia trifoliorum* and *S. rolfsii* – causative agents of root and ground rot (Mackie *et al*., 2003; Ivanović, 2005; Krnjaja *et al*., 2005a; Vasić, 2007; Vasić *et al*., 2010; Vasić *et al.,* 2011a, 2011b; Vasić, 2013). In addition to the aforementioned pathogenic fungi, alfalfa root and crown rot is caused by *Phytophthora megasperma*, *Phoma medicaginis* – the cause of the blackness of the tree and leaf spotting of alfalfa, as well as species from the genus *Pythium* – the cause of seedling blight and rotting of alfalfa seeds. The most commonly isolated *Fusarium* species that cause root rot and root neck are *F. oxysporum*, *F. solani* – the causes of fusarium wilt of alfalfa (Stuteville and Erwin, 1990; Krnjaja *et al*., 2005a; Krnjaja, 2005b). In addition to fungi, bacteria also cause significant damage to alfalfa production, including *Pseudomonas syringae* pv. *syringae* – the cause of bacterial stem spotting; *Pseudomonas marginalis* pv. *alfalfae* – the cause of bacterial root rot; *Clavibacter michiganensis* susp. *insidiosum* – the cause of bacterial wilt; *Xanthomonas campestris* pv. *alfalfae* – the cause of bacterial leaf spot and others (Stuteville and Erwi1990; Arsenijević, 1997; Balaž and Popović, 2005). Numerous phytopathogenic viruses that cause diseases have been described on alfalfa, such as Alfalfa mosaic virus, AMV; Cucumber mosaic virus, CMV; Clover yellow mosaic virus, CYMV and others (Stuteville and Erwin, 1990; Šutić, 1995; Jasnić, 2005). Damages resulting from the presence and development of pathogenic agents are expressed through a reduction in the quantity and quality of green mass from 10 to even 70% depending on the alfalfa variety, pathogen type, climatic and edaphic conditions. Reduction of the assimilation surface, falling of leaves, immaturity of seeds, the presence of harmful pathogen metabolites are also consequences of the presence of pathogens on alfalfa leaves. Diseases of the crown and roots cause a weakening of the plant's vitality, an increase in sensitivity to frost, premature decay, an increase in weediness, and thus a decrease in hay quality (Vasić, 2013). During a longer period of monitoring (2002-2019), it was determined that in some locations in Serbia there were large losses in alfalfa fields, with typical symptoms of root rot, necrosis, chlorosis and wilting of alfalfa plants. The phytopathogenic fungi affecting alfalfa have not been extensively studied in Serbia. Therefore, a research initiative was undertaken to identify and characterize the fungal species responsible for alfalfa diseases. The objective of this study is to contribute to the development of effective management strategies for the successful control of these fungal pathogens.

Materials and Methods

The isolates analyzed in this study were obtained from diseased alfalfa plants collected over the period from 2002 to 2019. The collection of samples was carried out in the main production areas of alfalfa in the territory of the Republic of Serbia, including a total of 17 localities: Čurug, Ašanja (Southern Bačka District), Srpska Crnja, Farkaždin (Southern Banat District), Trnavci (Nišava District), Vraneši (Raška District), Banatsko Karađorđevo, Aleksandrovo (Middle Banat District), Banovci (Srem District), Markovac (Danube District), Selo Varvarin, Kobilje, Globoder, Bela Voda (Rasina District), Kloka (Šumadija District), Davidovac (Pčinj District) and Dobrichevo (Pomeranian District). During field sample collection, plants exhibiting symptoms of chlorosis, necrosis, rot, and withered tops were selected. In total, 625 samples were collected for analysis. The pathogen was isolated from the stem and roots of alfalfa. After bringing the samples to the laboratory, the samples were first washed with running water, and after washing, parts of the stem and roots were cut into pieces measuring 0.5-1 cm. The samples thus prepared were disinfected with 96% ethanol for 10 seconds, 1% sodium hypochlorite (NaOCl) for 1 min. and washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar

(PDA) with streptomycin. In each Petri dish, five plant parts were placed, in two repetitions. They were kept in a thermostat at 22°C and a light regime of 12 hours day/12 hours night. The developed mycelia were transplanted onto a new PDA medium (Dhingra and Sinclair, 1995). A microscopic examination was performed using an Olympus CX31 microscope. Morphological identification of fungi to genus was performed using standard keys.
In order to determine the pathogenic properties of selected isolates obtained from infected alfalfa plants, a pathogenicity test was performed by inoculating injured alfalfa plants (Vasić, 2007). Before inoculation, all plants were wounded at the base of the stem with a sterile spear needle. In this way, the injured plants were inoculated by placing small fragments of the colony of studied isolates in the wounds. Inoculation was performed with isolates of the obtained mushrooms, 7 days old, grown on PDA medium. Plants inoculated in this way were stored at a temperature of 20-25°C. The experiment was set up in two replicates with five plants per isolate. Alfalfa plants that were injured in the same way served as a control, after which they were inoculated with small fragments of the substrate without mycelia.

Results and discussion

After checking the alfalfa crop, several alfalfa fields showed signs of wilting. Plants with symptoms were collected from 17 locations in Serbia. All the plants from which the fungi was isolated clearly showed symptoms in the form of necrotic spots and lesions on the stem. Fungi of the genus *Fusarium* were isolated from these plants. Similarly, necrosis with white aerial mycelium in the lower third of the stem was observed in some plants, and fungi of the genus *Sclerotinia* were isolated from these plants. On some plants, black fruiting bodies-pycnidia belonging to the genus *Phoma* were observed on the stem. When fungi of the genus *Colletotrichum* are isolated from alfalfa, the tips of the stems are usually bent down. Fungi are also distinguished based on morphological and pathogenic characteristics, in addition to the symptoms of plants present in the field, the causative agents of alfalfa diseases were determined (Table 1).

Table 1. Morphological and pathogenic characteristics of the obtained isolates on alfalfa plants

Types of fungi	**Morphological characteristics**	**Pathogenic characteristics**
Fusarium spp.	52.09–84.35 x 0.86–9.25µm	On the roots, the alfalfa plant test causes typical symptoms of root system rot
Rhizoctonia spp.	12.87–85.8 x 3.6–8.56 µm	Causes root neck rot on alfalfa test plants
Sclerotinia spp.	6–9 x 10–20 µm	On the test plants, it causes rot from the roots towards the root neck and stem
Phoma spp.	5.45–7.14 x 2.98–4.18 µm	Black fruiting bodies develop on the test plants
Verticillum spp.	6–12 x 2.5–3 µm	The pathogen on the test plants causes symptoms in the form of discoloration of the vascular tissue of the roots
Colletotrichum destructivum *C. trifolii* *C. linicola*	10–25 x 2.5–7.4 µm 12.5–17.5x 5.75 µm 12.5–25x 2.5–7.5 µm	On the test plants, lesions were observed in the ground part of the tree, most often on the lower third, light to dark brown in color, irregular in shape, with dark brown edges

The roots of the plants showed symptoms in the form of light brown necrosis, and fungi of the genera *Fusarium* and *Rhizoctonia* were isolated from these plants. Discoloration of the conducting tissue has also been observed in some root system plants and fungi belonging to the genus *Verticillium* have been isolated from these plants.

Based on the morphological and pathogenic characteristics of the obtained isolates, it was determined that fungi of the genus *Colletotrichum* are prevalent on alfalfa in Serbia, followed by fungi belonging to the genera *Fusarium*, *Verticillium*, *Rhizoctonia*, *Sclerotinia* and *Phoma*. The obtained results (Table 1) of morphological and pathogenic characteristics of our isolates are in agreement with the results obtained by Harvey, 1965; Graham *et al*., 1979; Latunde-Dada *et al*., 1997; Boland and Hall, 1994; Krnjaja *et al*., 2005a; Krnjaja, 2005; Rhodes and Sulch, 2005; Cedeño *et al*., 2006; Castell-Miller *et al*., 2007; Vasić, 2013; Reich *et al*., 2017; Xu *et al*., 2019; Ling *et al*., 2019. Species of the genus *Fusarium* are soil fungi and are common wherever alfalfa, red clover and other perennial forage legumes are grown. In the presence of other stress factors, *Fusarium* spp. caused by root rot, often followed by root neck rot or a combination thereof, causing the death of diseased plants. Symptoms on the upper parts of the plant are similar to other types of root rot. *F. oxysporum*, *F. solani* and *F. roseum* are most often isolated from diseased alfalfa roots (Graham *et al*., 1979; Krnjaja *et al*., 2005a; Ling *et al*., 2019). In Serbia, 11 different species of the genus *Fusarium* were isolated from diseased alfalfa plants (Krnjaja, 2005b). Root of the root, tree and root neck caused by *Rhizoctonia solani* is a soil fungus, the most destructive phase of the disease occurs when *R. solani* infects the root neck and as a result of this development the plants die and the yield decreases. Although it can cause root neck rot alone, it is most often associated with other fungi (*Fusarium* spp., *Phoma* spp. and *Colletotrichum* spp.), which also cause root neck tissue death (Mijušković, 1993; Rodiso and Sulc, 2005; Krnjaja *et al*., 2005a).

Root neck and stem rot is a disease of alfalfa caused by *Sclerotinia trifoliorum* (syn. *Sclerotinia sclerotiorum*) - the damage caused by this fungus on alfalfa is significantly less than with *Trifolium* species, especially red clover (Graham *et al*., 1979). Disease symptoms appear most often in early spring, and hard winter frosts promote the spread of rot from the roots to the root neck and trunk (Graham *et al*., 1979; Reich *et al*., 2017).

Alfalfa root disease caused by the *Phoma sclerotioides* was discovered in the United States in 1996. Symptoms of the disease most often appear in three-year-old alfalfa. In addition to alfalfa, this fungus also attacks *Trifolium* species. The fungus in the form of pycnosclerotia maintains vitality in plant residues in the soil. Crop rotation with non-host plant species is balanced, with fertilization, early mowing and winter cultivation of cultivars less sensitive to low temperatures being the most common pathogen control methods (Gray *et al*., 2004; Cedeño *et al*., 2006). Verticillium wilt caused by *Verticillum albo-atrum* is the most economically important alfalfa disease in the world. If this disease is detected in alfalfa, it means that 50% of the plants are already infected. The pathogen causes symptoms in the form of discoloration of the vascular tissue of the roots, burning and wilting of individual leaves, and chlorosis of the leaf tip in the shape of the letter "V". The fungus maintains vitality in soil, plant debris and seeds (Graham *et al*., 1979; Krnjaja *et al*., 2005a; Xu *et al*., 2019). Symptoms of anthracnose caused by fungi of the genus *Colletotrichum* (*C. destructivum*, *C. trifolii* and *C. linicola*) occurred most often after the second cutting, regardless of weather conditions. The tops of the stems are usually bent down, the leaves dry, turn yellow and then turn pink, which gives the whole field a pink appearance in case of strong attack (Latunde-Dada *et al*., 1997; Lantunde-Dada *et al*., 1999; Johnston, 2000; Latunde-Dada and Lucas, 2007; Vasić, 2013). In temperate regions *C. trifolii*, *C. destructivum* and *C. linicola* persist in alfalfa stems and crowns (Vasić, 2013).

Conclusion

The obtained results indicate that alfalfa is susceptible to attack by a large number of phytopathogenic fungi that can significantly affect the reduction of its yields and quality.
Since the pathogenic species of fungi that cause diseases on alfalfa have not been sufficiently studied in Serbia, research was carried out with the aim of determining the species that cause diseases on alfalfa, as well as their more detailed morphological and pathogenic determination, all with the aim of contributing to finding adequate measures for their successful suppression.

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INFLUENCES OF DIFFERENT DRYING METHODS ON THE CHEMICAL COMPOSITION OF BURLEY TOBACCO

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Abstract

Drying is the first technological operation during tobacco processing, which is carried out by the growers, that significantly affects the quality, flavour and aroma of tobacco. Therefore, knowledge of drying methods that are particularly suitable for large-leaf tobacco varieties is of the utmost importance. This paper reviews previous research on the possibilities of using different drying methods (freeze-drying, air-curing, sun-curing and flue-curing) and their influence on the chemical composition of burley tobacco. Burley is a large-leaf tobacco that is normally air/shade cured. The chemical parameters that have the greatest influence on the quality characteristics of tobacco, i.e. carbohydrates and nitrogenous substances, and the changes in their content were investigated. With freeze-drying as a control method, there is no loss of dry matter due to the low temperatures, which inactivate all enzymes, so that the carbohydrates and nitrogenous substances were also at their highest. By using atypical drying methods for burley tobacco, acceptable results can be achieved in terms of changes in certain chemical characteristics. After drying in a solar dryer, the pH, nicotine, total nitrogen and ash content were lower, but the total soluble sugar content was higher than air-cured burley. Drying process changes a chemical profile of the tobacco. None of the methods tested met all the requirements for the most favourable chemical properties of the tobacco after drying. It therefore makes sense to focus further research on the possibilities of combining different drying processes, taking into account both the quality of the dried tobacco and the costs of the drying process.

Key words: *Burley tobacco, drying methods, carbon metabolites, nitrogen metabolites, usability of tobacco.*

Introduction

The drying method is the most important factor for the quality of tobacco products (Zong et al., 2020). According to the technological classification, burley tobacco is categorized as a light, air-drying tobacco variety. Original burley is characterized by thick, dark leaves and accounts for about 10 % of the total production in the Republic of Serbia. Burley tobacco leaves make up a considerable proportion (up to 30 %) of cigarette blends, i.e. American-style cigarettes. Burley has a high protein (up to 10 %) and nicotine (more than 2 %) content, which is responsible for the alkaline reaction of the smoke and the bitter taste. When dried correctly, the carbohydrates are almost completely broken down, which is characteristic only of this type of tobacco. An important characteristic for the utility value of burley tobacco is its high absorption capacity. The leaf can absorb up to 25 % of its weight in water/additives, which is why it is the most important carrier of additives in the cigarette blend (Radojičić, 2011; Radojičić, 2016).

The drying of tobacco is a process of heat and mass exchange between heated air and the material. This process involves complex physical and chemical changes that occur as the tobacco leaves slowly lose moisture. It is divided into three phases: the yellowing phase, the colour fixation phase and the final drying phase. Freshly picked, technologically mature

leaves contain 80-90% water and 10-20% dry matter. The first phase of drying is a physiological-biochemical process that takes place in the living cells of the tobacco leaves. Hydrolysis of chlorophyll, starch, proteins, pectin substances, glycosides etc. takes place. Once the biochemical processes in the tobacco leaf are complete, only the water needs to be removed from the tobacco leaves (Radojičić, 2016; Đulančić, 2014).
Burley is a large-leaf tobacco that is dried in the air and in the shade (air curing - AC). Existing roofed rooms or specially constructed drying rooms can be used for air curing. For burley grown on an area of 1 hectare, a drying room with a volume of 800 m^3 is required (Radojičić, 2016).

Material and Methods

This paper summarizes some previous researches on the possibility of using different drying methods and their influence on the chemical composition of burley tobacco. The basic characteristics of burley dried by the air-curing, sun-curing, flue-curing and freezing tobacco leaf methods are presented.
The most important chemical parameters of tobacco quality, namely carbohydrates, nitrogenous substances, and the changes in their content from the beginning to the end of the drying process were investigated. It was found that differences in the chemical composition of tobacco significantly affect its quality and usability (Schwanz et al., 2019). Several studies (Tricker et al., 1991; Talhout et al., 2006; Roemer et al., 2012) show that the main factor determining the sweetness and flavour of the smoke is the total soluble sugar. The physical intensity and smoke concentration of the tobacco varieties is determined by the total nitrogen and nicotine.

Results and Discussion

The quality of tobacco depends on its chemical composition, which is influenced by the type of tobacco and the way it is dried. Traditionally, each tobacco type is characterized by a specific drying process. To determine whether the quality of the tobacco can be changed/improved, the effects of using different drying methods on the chemical composition of burley were investigated.
To increase the productivity of the burley tobacco drying process, an experiment was conducted using a bulk drying system. The changes in the chemical composition of the leaves during drying were monitored and compared with those of the leaves during conventional air drying. Burley tobacco leaves were dried in a controlled temperature and humidity chamber under the following conditions: 35 °C, 83 % relative humidity in the yellowing and browning phase; 45 °C, 50 % relative humidity in the stem drying phase. There were some differences in the changes in solanone and norsolanadione content and free amino acid content compared to those in air drying. The changes in other chemical constituents in the leaves during drying were similar to those in conventional air drying (Tomita et al., 1995).
Chen et al. (2021) investigated the influence of different drying methods on the chemical composition of burley tobacco. They carried out four drying methods: freeze-drying, air-curing (AC), sun-curing (SC) and flue-curing (FC). Freeze-drying: The tobacco leaves were dried in a freeze-dryer for 150 hours. Although AC is a typical method for drying burley, the authors used freeze-dried tobacco as a control sample in this study.
Air-curing: The tobacco leaves were fixed on drying racks and covered with plastic sheets. The drying racks were placed in rooms without light during air drying. The temperature in the rooms was 23 - 30°C and the relative humidity was 75 – 85%. The process was completed when the midrib of the leaves was dry. Sun-curing: The tobacco leaves were placed on drying

racks and covered with plastic foil. The drying racks were placed outside to expose them to the sun. The temperature was 25 - 38°C and the relative humidity was 60 – 70%. Flue-curing: The drying method includes the yellowing stage, the leaf-drying stage and the midrib-drying stage under controlled conditions of temperature and relative humidity for each of the phases mentioned. Figure 1 shows the morphology of burley tobacco leaves treated with different drying methods.

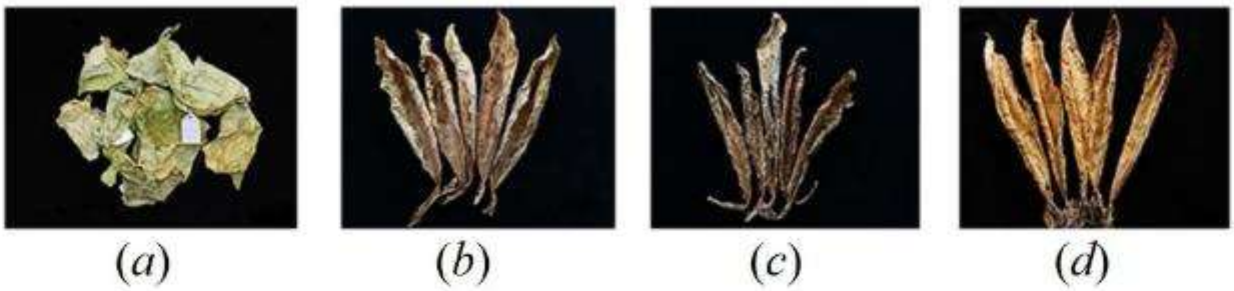

Figure 1. Morphology of burley tobacco leaves treated with different drying methods: (a) freeze-dried; (b) air-cured; (c) sun-cured; (d) flue-cured (Chen et al., 2021)

The burley tobaccos treated with the three drying methods were compared separately with the freeze-dried burley tobacco as a control group. Most leaf dry matter is lost when burley is dried in the shade (AC). The loss of dry matter was 16.15 % for the AC burley tobacco, 7.78 % for the SC burley tobacco and 12.06 % for the FC burley tobacco (Chen et al., 2021).
In freeze drying, there is no loss of dry matter due to the low temperatures, which inactivate all enzymes and slow down or stop all chemical and biochemical processes.
The authors (Chen et al., 2021) compared the content of of carbon and nitrogen metabolites between freeze-dried and air-cured, sun-cured and flue-cured burley tobacco (Table 1 and Table 2).

Table 1. The effects of different drying methods on the content of carbon metabolites in burley tobacco (Chen et al., 2021)

Drying method	Starch (%)	Total sugars (%)	Reducing sugars (%)	Fructose (%)	Sucrose (%)	Total carbon content (%)
Freeze - drying	16.21	7.20	5.43	2.70	1.37	41.76
AC	0.85	6.53	4.38	2.47	0.28	38.37
SC	0.72	4.93	3.05	1.77	0.23	41.04
FC	1.47	9.66	3.19	2.48	1.40	37.94

Table 2. The effect of different drying methods on the content of nitrogen metabolites in burley tobacco (Chen et al., 2021)

Drying method	Proteins (%)	Nicotine (%)	Total nitrogen (%)
Freeze - drying	9.69	5.29	2.87
AC	6.68	4.84	2.51
SC	6.79	4.82	2.79
FC	6.18	4.86	2.49

They found a significant decrease in starch, total sugar and reducing sugar content compared to freeze-dried tobacco used only as a control group. Freeze-drying yielded the highest starch content (16.21 %) and generally all carbon metabolites. This is to be expected considering that hydrolytic enzymes are not active at temperatures below 0 °C, so the starch is not degraded. For all types of tobacco, it is desirable that the starch is reduced as much as

possible, as it has a negative effect on the sensory properties of the tobacco during the smoking process. In the dried leaf, the permissible amount of starch that does not affect the quality of the tobacco is 2-5 %. In addition to the traditional AC method for burley tobacco, the SC method has also proven its worth. The total sugar content is highest with the FC method (9.66 %), while the content of reducing sugars, which are carriers of the flavour, was highest with AC burley. The losses in total carbon content were significantly higher in FC burley (9.15 %) than in AC burley (8.12 %) and (1.72 %) in SC compared to freeze-dried control tobacco. Burley is the tobacco variety that naturally contains the most nitrogenous substances, so in this case the changes associated with this group of compounds will have the greatest impact on the quality of the dried leaf. Table 2 shows that the greatest loss of protein (36.22%) and total nitrogen (13.24%) is seen in FC burley compared to freeze-dried tobacco. The differences in nicotine content are not significantly pronounced.
Chen et al. (2021) concluded from this experiment that it is possible to achieve acceptable results with atypical drying methods for burley tobacco in terms of changing certain chemical characteristics. Radojičić et al. (2017) investigated the possibility of drying burley tobacco in a solar dryer. They compared the chemical properties of burley tobacco dried in a solar dryer with those of air-cured burley tobacco (Table 3). The results showed that the pH, nicotine, total nitrogen, protein nitrogen and ash content were lower in solar-dried burley tobacco. However, the positive result of this work was that the total soluble sugar content of solar-dried burley tobacco was higher than that of AC tobacco. As already mentioned, the main factor for the sweetness and flavour of the smoke is the total soluble sugar. Since burley contains a very small amount of sugar (literally in trace), the amount of 3.96% is significant (Radojičić et al., 2017). Also, the duration of the drying process in a solar dryer was 16 days shorter compared with the standard AC process.

Table 3. The effect of different drying methods on the chemical composition of burley tobacco (Radojičić et al., 2017)

Drying method	pH	Nicotine (%)	Total nitrogen (%)	Protein nitrogen (%)	Starch (%)	Soluble sugars (%)	Ash (%)
Solar dryer	5.71	2.71	3.88	1.52	2.97	3.96	17.49
AC	5.80	2.91	3.96	1.77	0.60	0.21	21.53

From this one could conclude that drying burley in a solar dryer leads to burley with good chemical properties. However, the authors investigated both the physical and sensory properties of the tobacco. The changes in the physical properties of the tobacco such as tissue fullness, leaf thickness and specific gravity were higher compared to the standard, which can be considered as negative characteristics. Also, based on the classification criteria for burley tobacco according to its sensory properties, it was classified in quality group III (Radojičić et al., 2017). None of the methods presented here met all the conditions to achieve the most favourable chemical properties of burley tobacco after drying, so further development should focus on combining different drying methods.

Conclusions

The drying of tobacco is a very important step in the production process that significantly affects the quality, flavour and aroma of the tobacco. The final choice of drying method depends on the desired quality of the tobacco, the effectiveness of the drying method itself and the economic aspects of the process. The chemical profile of the tobacco is changed by the drying process, which also affects its usability.
An important conclusion from this study is that it is possible to try other methods in addition to the traditional AC methods for burley tobacco or to combine several drying methods. The

conversion of chemicals in burley tobacco with different drying methods will be further investigated. Tobacco growers need to study and understand the different drying methods, apply the best practices and adapt to specific conditions and production goals. The growing awareness of sustainability and environmental protection will also have an impact on approaches to drying tobacco and the development of new ways to apply existing methods, as well as the development of entirely new methods for drying burley tobacco.

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CHEMICAL TRANSFORMATIONS IN FLUE-CURED TOBACCO DEPENDING ON THE DRYING METHOD: A REVIEW

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Abstract

Drying tobacco is an important step in the production of high-quality tobacco leaves. The usual method of drying Virginia tobacco is the flue-cured method (FC), which is carried out under controlled conditions in specially designed dryers. In poorer countries, however, this drying method is not practical, both because of the high energy costs for operating the dryer and because of the high prices of the dryer itself. In addition, the existing drying process needs to be improved by using heat pumps or non-fossil fuels as energy sources. For these reasons, it is necessary to find an alternative, economically acceptable method for drying Virginia tobacco. The aim of this work was to investigate the possibility of using a different/more modern drying process that can simultaneously achieve favorable chemical properties. The usual drying methods, the variables that influence their efficiency and the resulting effects on the tobacco leaves are considered. Alternative methods are also presented, highlighting their unique characteristics, challenges and benefits. Air-cured (AC) and sun-cured (SC) Virginia produces a dark-colored tobacco that is atypical of Virginia-cured leaves. The nitrogen and starch content are highest in freeze-cured tobacco and the leaf is green in color. The chemical profile of the tobacco has completely changed, and with it its usability. The FC method gives the best results, especially when working on improving and automating the process. Replacing coal with tobacco waste briquettes offers great potential for improving energy efficiency and reducing carbon dioxide emissions by up to 95% without compromising product quality.

Keywords: *Flue-cured, air-cured, sun-cured, freeze-dried, chemical properties.*

Introduction

The curing of tobacco is often a decisive process that determines its usability and thus the selling price (Zong et al., 2020). It is a controlled process in which freshly picked tobacco leaves are transformed into a final product that is ready for consumption or further processing. Parameters of the drying process, such as temperature and relative humidity, have a decisive influence on the development of tobacco quality (Gong et al., 2005; Xiang-Yang et al., 2007; Zhang, 2015; Zhu et al., 2016; Zou et al. al., 2019; Popova et al., 2020). For this reason, it is very important to apply the optimal drying regime, which for Virginia tobacco is drying in a dryer under controlled conditions (Radojičić, 2016). Virginia tobacco is one of the three most important types of tobacco grown in different countries around the world. In the Republic of Serbia, it accounts for around 90% of total production. It is indispensable for the manufacture of the English-type cigarettes and is widely used in blends for the American-type blend cigarettes. It is therefore extremely important to know the drying methods specifically suitable for this type of tobacco. The standard Virginia method of drying in a special dryer has been practiced for years and has proven its worth.

It is important to note that the energy costs for this type of drying account for the largest share of the cost structure. They account for 26-28% of total costs (Radojičić, 2011), which is understandable given the high price of energy sources and the high consumption per unit of product (1 m^3 of natural gas is used to dry 1 kg of tobacco). In addition, the high price of dryers represents a major financial expense for an average farm (Kulić et al., 2017). For these reasons, it is necessary to find an alternative, more economically acceptable method of drying Virginia. The aim of this work is to investigate the possibility of using a different drying method while maintaining favorable technological properties of tobacco. Alternative methods have emerged that offer unique characteristics and potential advantages in terms of efficiency and tobacco properties.

Material and Methods

The paper refers to a review of scientific works in which the possibility of drying Virginia by other means has been investigated and the results obtained in these studies. The paper presents the basic properties of Virginia dried by the standard flue-cured (FC) method, followed by air-cured (AC) and sun-cured (SC) methods and freezing of the tobacco leaves. It also discusses the possibilities for improving the standard FC method with the aim of automating the process more and/or saving energy.

Results and Discussion

Drying Virginia under natural conditions

In developing countries, the flue-cured (FC) drying method is not practical because of the high energy costs of operating the dryer and the high price of the dryer itself. It is generally known that Virginia tobacco can also be dried in the sun (sun-cured, SC). This drying method produces a darker colored leaf, which is mainly used for the production of pipe or cigar blends (Voges, 1984).

Kulić et al. (2017) investigated the possibilities of the air-cured method (AC) for Virginia tobacco by comparing the properties with the standard method (FC). It was found that the drying methods used have an influence on the duration of the process as well as on the sensory and chemical properties of the dried leaves. The drying process lasted 6 days under controlled conditions (FC) and 30 days in the air/shade (AC). The leaves dried in a T-42 dryer under controlled conditions had a light yellow color (light Virginia) with fine and thin stems. The air-dried (in shade) leaves had a dark color (dark Virginia), with pronounced and coarse stems, especially the midrib. The values of the eight parameters observed (nicotine, total nitrogen, nitrogen proteins, total proteins, reducing sugars, ash, sand and pH) are higher in AC tobacco (Table 1). In contrast, the soluble sugar content and pH are lower. As a general conclusion of this study, it was found that air/shade drying of Virginia (AC) completely changes its sensory and chemical properties, which is why it approaches dark tobaccos in terms of properties (Kulić et al., 2017).

Table 1. Mean value of the chemical properties of Virginia treated with two different drying methods (Kulić et al., 2017)

Drying method	Nicotine (%)	Total N (%)	N proteins (%)	Total proteins (%)	Reducing sugars (%)	Ash (%)	Sand (%)	pH
FC	2.03	1.80	0.87	5.46	19.39	11.61	1.48	5.19
AC	2.25	2.73	2.06	6.60	1.96	15.43	8.91	4.92

Chen et al. (2021) conducted an experiment in which they investigated the extent to which the chemical properties of Virginia tobacco change when it is subjected to different drying processes: Freeze-drying, air-curing (AC), sun-curing (SC) and flue-curing (FC). The sensory properties of the leaves and the values of the following parameters were monitored: loss of dry matter, carbohydrates (starch and reducing sugars), proteins, total nitrogen and nicotine. The tobacco cured by the standard FC method had a recognizable golden yellow color typical of Virginia. When cured in the shade and in the sun, leaves with a much darker color were obtained (Figure 1). Tobacco that is frozen has a greenish color because at temperatures below 0 ℃ most of the enzymes that influence the decomposition of leaf pigments lose their activity.

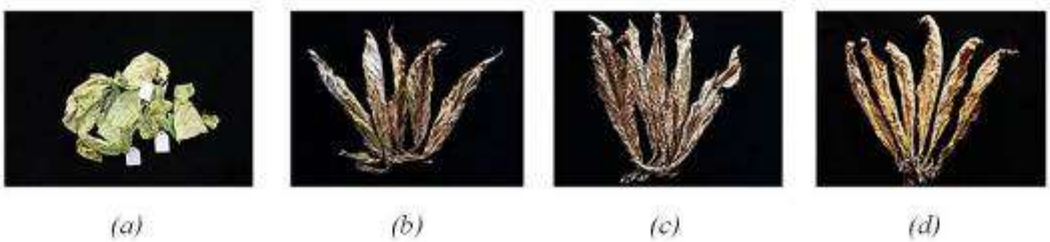

Figure 1. Morphology of Virginia tobacco leaves treated with different drying methods: (a) freeze-dried; (b) air-cured; (c) sun-cured; (d) flue-cured (Chen et al., 2021)

The greatest loss of leaf dry matter is observed in shade- dried (AC) tobacco (up to 25.72 %), as this process takes the longest. The loss of dry matter when drying in the sun - SC (14.76 %) and under conditioned conditions - FC (18.97 %) is much more acceptable. There is no loss of dry matter during freeze-drying, as the low temperatures inactivate the enzymes and slow down or stop the chemical and biochemical processes. The content of carbohydrate components varies considerably. The freezing process results in the highest starch content (46.25 %) and by far the lowest total sugar content (8.08 %). Both data can be explained by the fact that the enzymes show no activity at temperatures below 0 ℃. As it is desirable to degrade the starch as much as possible, the SC and AC methods also did not give good results (12.57% and 8.95%). However, there were no significant deviations with regard to the content of reducing sugars. The highest content of reducing sugars, which are responsible for the taste of Virginia tobacco, was achieved during sun drying (19.08 %). The content of nitrogen substances is highest in freeze-dried tobacco, which in turn is related to the inhibition of enzymes. The FC drying method gave the best results. Not only does it provide the lowest protein content (3.65 %), which is desirable for a traditionally mild and light Virginia tobacco, but it also has the nicotine content typical of Virginia (2.06 %). SC and AC Virginia contain more proteins (4.67 % and 4.50 %, respectively), but the nicotine content (1.71 % and 1.73 %, respectively) is low to satisfy the average cigarette consumer. Chen et al. (2021) concluded that it is possible to achieve acceptable results in terms of changing certain chemical properties by using atypical drying methods. The authors emphasize that it is necessary to additionally investigate the combination of methods in order to obtain a product of the highest quality.

Digitization of the drying process using modern bulk-curing ovens

Condorí et al. (2018) conducted an experiment in which they investigated the possibility of improving the FC method of tobacco drying by increasing the automation of the dryer and thus reducing the possibility of human error during the process. They designed a prototype of the Virginia drying oven and added a sensor to monitor the color of the leaf in real time, after which the critical parameters of the process, the drying and wet-bulb temperatures, would be

changed. Leaf color is one of the fundamental parameters of tobacco quality, as it has a direct impact on the commercial value of the tobacco. The factors monitored in this test are temperature, relative humidity and moisture content (mass of moisture in moist air/mass of dry air). The authors considered that the degree of humidity is the most important indicator of the quality of the drying process. They observed a sudden increase in moisture content during the yellowing phase, which is probably related to the high air circulation during this period. The final key value for the moisture level was 0.045 kg/kg, and they considered that any value between 0.04 and 0.05 kg/kg could be considered acceptable.
In the prototype oven, the humidity drops below the lower acceptable value of 0.04 kg/kg after 85 hours of drying. Therefore, it was found that this type of improvement of the standard FC drying method is only possible during the first 85 hours of the process, while the yellowing phase continues (Condorí et al., 2018).

Replacing a traditional coal stove with a heat pump

Bao and Yang (2016) investigated the possibility of using a heat pump (the working fluid is Freon R134a) as an alternative to a conventional coal-fired kiln in drying in Virginia to reduce energy consumption and combustion products. The authors determined the drying and wet bulb temperatures experimentally and by calculation using a mathematical model over 4 phases: the yellowing, wilting, color fixation and midrib drying phases (Table 2).

Table 2. Temperatures in different stages of drying (Bao and Yang, 2016)

		Yellowing		Wilting		Color fixation		Drying of the midrib	
		TST	TVT	TST	TVT	TST	TVT	TST	TVT
Upper limit	Experimental values	38	35.8	42.4	37.6	53.9	38	67.5	41
	Theoretical values	37	34.2	34.8	35.4	54.6	37.5	67.8	39.5
Lower limit	Experimental values	36.3	35.6	39.5	37.5	53	41.9	65.4	40.3
	Theoretical values	33	37.8	41	41	54	43.6	67.1	45.2

*TST and TVT indicate the temperature of the dry and wet bulb thermometer

Bao and Yang (2016) concluded in this study that although the mathematical model does not provide the same values for the dry and wet bulb temperatures as the experimentally determined values, an average error of 4 %, which takes all temperatures into account, is considered acceptable. The authors state that the mathematical model should be further improved and that this test is an indication that the drying process of tobacco can be carried out with a heat pump in the future. In China (Xiao et al., 2015) and Italy (Bortolini et al., 2019), studies are underway with the aim of greening the drying process for the Virginia tobacco variety. Results studies in China, Yunnan Province, have shown that biomass briquettes pressed from tobacco waste behave in the same way as coal, but better than coal in the high-temperature drying phases due to the higher combustion rate. The energy yield when burning biomass briquettes was between 39 and 42 %, which is higher than the 36 % achieved when burning coal in parallel tests. Replacing coal with tobacco waste briquettes, together with improving energy efficiency, offers the greatest potential for the Chinese tobacco industry to achieve the national target of reducing greenhouse gas emissions by 40-45%. Research in Italy shows that switching to non-fossil fuels for tobacco curing leads to annual cost savings of up to 13% and a reduction in carbon dioxide emissions of up to 95 without compromising product quality.

Conclusions

Based on the research results presented in this paper on the possibility of using different ways of drying Virginia, certain conclusions can be drawn. Drying Virginia in the sun (SC) is the least favorable for the degradation of starch and total proteins, which has a negative effect on quality. Shade-drying Virginia (AC) produces a dark-colored tobacco that is atypical for a dried Virginia leaf. The content of soluble sugars has decreased tenfold, while the content of nitrogen components has increased. The nicotine content increased by 10.8 %, the protein nitrogen by 2.36 times, the total nitrogen by 1.52 times and the total protein by 21 % as a result of this type of drying, which also lowered the pH value. The ash content has increased by 33 % and the sand content by a factor of 6. The chemical profile of the tobacco has changed completely, and with it its utility value. The FC drying method delivers the best results, especially when working on improving and automating the process.
The drying of tobacco is an important step in the production process that affects the quality and usability of the tobacco. The final choice of curing process depends on the producer's preferences, the desired tobacco quality, efficiency, environmental impact and cost. It is important that tobacco growers study and understand the different curing methods, apply the best practices and adapt to specific production conditions and objectives. In the future, research and innovation will likely lead to the development of new drying methods. The growing awareness of sustainability and environmental protection will also influence approaches to tobacco drying. Combining traditional methods with new alternatives can provide opportunities for the production of diverse and high quality tobacco products.

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MICROBIOLOGICAL QUALITY OF HOMOLJA WHITE CHEESE IN BRINE

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Abstract

Microbiological contamination of food, which leads to frequent illness and even death, is one of the main concerns of consumers. For this reason, the food industry and services that take care of food control and the protection of public health face the important task of monitoring each stage of the food chain and applying measures that will prevent microbiological contamination of food. White brine cheeses are traditionally produced in the Balkans and the northeastern Mediterranean~~,~~ and are characterized by fermentation in brine in anaerobic conditions. Given that the microbiological quality and safety of these cheeses are affected by a large number of factors (quality of raw milk, use or not of pasteurization, hygiene during the process of making, ripening, storing and placing the cheese, % of salt, packaging), it is very important to control the microbiological correctness of such cheeses, both during production and at the point of sale. This paper presents the results of the microbiological evaluation of the quality of Homolj white cheese in brine from cow's milk, which is produced in the area of Homolje (Serbia). Cheese samples were taken in April, May, and June 2023, from five producers selling their cheese at the market of the municipality of Žagubica. A total of 15 samples were analyzed for the presence of *Escherichia coli*, *Coagulase-positive staphylococci* and *Listeria monocytogenes.* Microbiological analysis showed that 12 samples met the quality criteria of the Rulebook, and the presence of *Escherichia coli* was registered in three samples. The defective samples were from the same manufacturer.

Kewords: *Homoljski cheese, quality, hygiene, microbiological analysis, pathogenic microorganisms.*

Introduction

Losses of the world's food supply, which are caused by contamination by microorganisms, amount to approximately one quarter of the total food losses and represent a major economic and ethical problem throughout the world. Dairy products on their way from farm to table can be exposed primarily to microbiological and chemical hazards. They represent a suitable medium for the growth of a wide range of microorganisms and therefore have a reduced shelf life. Ensuring the safety of milk and dairy products starts on the farm with animal health, food quality, sanitation and hygiene measures in the area, and then with processing conditions, subsequent heat treatment, storage conditions and storage of finished products (Motarjemi et al., 2014; Asselt et al., 2016).

Dairy products, especially cheeses, have a high nutritional value and a high level of consumption worldwide. They are a good source of protein and essential amino acids that have improved digestibility and bioavailability. They are one of the favorite foods in the world, for which the demand for the last two decades has been growing, and thus the production of cheeses, especially cheeses with the protection of geographical origin. All this is accompanied by a greater emphasis on improved quality and consistency when it comes to measures to control biological, chemical and physical hazards and to standardize production processes. But despite all the measures implemented, food-borne pathogenic microorganisms still pose significant risks to public health. Bacteria are the most common foodborne pathogens of public health importance. The key to cheese safety are: the quality and safety of milk, the initial flora of the milk, together with the conditions of processing and subsequent heat treatment, and careful control and monitoring of the processing steps during the conversion of milk into different cheeses. Proper handling, storage, distribution, retail and home use by the consumer are also important to ensure and maintain the safety of the cheese, thereby preventing contamination, re-contamination and/or cross-contamination (Rashtchi et al., 2021; Crippa et al., 2022).

Microbiological hazards are more commonly encountered in dairy products than chemical and physical hazards. *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella*, and the human pathogen *Escherichia coli* have been identified as the most important microbiological hazards in dairy products. Soft and semi-soft cheeses are most often associated with enterotoxins *L. monocytogenes* and *S. aureus*, while raw milk is most often associated with human pathogens *E. coli* and *Campylobacter spp. Cronobacter spp*. and *Salmonella spp*. are the microbiological hazards of greatest concern in powdered milk formulas intended for infants (Asselt et al., 2016). White brine cheeses are widely produced in the northeastern Mediterranean and Balkans. They are traditionally produced as artisanal cheeses. They are produced from cow's milk, sheep's and/or goat's milk or from a mixture of these milks. Feta (Greece), Domiati (Egypt), Beyaz peynir (Turkey), Halloumi (Cyprus), Brinza (Bulgaria), Homoljski cheese (Serbia), Vlasic cheese (BiH) are some of the most famous of this group of cheeses. White cheeses in brine belong to the group of semi-hard or soft cheeses, without or with few mechanical cavities, acidic (pH ~ 4.5), salty and some of them, spicy taste, which are stored and matured in brine, and therefore the dominant microflora gives a significant contribution to the ripening process and regulates the quality of the final product. Due to long-term brining, the cheeses acquire a salty, slightly sour taste. Salt concentration is important in preventing the growth of harmful microorganism except for *Staphylococcus aureus* which can grow in the presence of 6.5% salt, and *Listeria monocytogenes* which grows in a concentration of 10% salt. The action of lactic acid bacteria during ripening and NaCl in the brine (10-18%) are the most important parameters for the preservation of these types of cheese. Homoljski cheese is autochthonously produced from sheep's milk, cow's milk and goat's milk. Since 1996, this cheese has protected geographical origin (Radovanović et al., 1996a; Radovanović et al., 1996b; Radovanović et al., 1996c; Maćej et al., 2006; Sikimić et al., 2006; Feng et al., 2022; Osek et al., 2022). The basic process of traditional production of Homoljsk cheese consists of the following stages:

- Preparation and curing of milk - Milk is filtered through cheesecloth and curing is carried out at a temperature of 30 - 32 °C. After adding the rennet, the milk is continuously stirred for 2 - 3 minutes, then the stirring is stopped and the milk is left for 1.5 - 2 hours.
- Self-pressing and pressing - The formed curd is transferred to strainers where the cheese curd is formed by the process of self-pressing, which lasts 1.5 - 5 hours, and then pressing is carried out, so that the curd is loaded with 0.5 - 2 kg/kg of cheese mass. lasting 2 - 4 hours.
- Cutting and salting the cheese - The cheese lump is cut into slices of uniform size and shape 15×15×5 cm. The cut slices stand for 10 min, to separate the whey better. Salting is done with dry salt of each layer of cheese, when stacking them in containers for ripening. The salt content in cheese should be 2-2.5%. Load the cheese when the container is full and then pour in the salty whey.
- Storage and care of cheese - Ripening lasts 3-4 weeks, in a dark room at a temperature of 14-18 °C. In the beginning, grooming is done every 3-4 days, and later on every 7-

10 days. After ripening, the cheese is stored at 4-8 °C (Dozet et al., 2004; Dozet et al., 2006; Maćej et al., 2006; Sikimić et al., 2006).

Given that white cheeses mature for a long time in brine, the dominant microflora affects the ripening process, as well as the shelf life of the cheese itself. The possibility of survival and/or growth of pathogenic bacteria is influenced by pH, aw, the temperature at which ripening takes place, the quality of raw milk, the thermal treatment of milk, the activity of starter cultures, the process of salting, i.e. the absorption of salt and its final concentration in the water content, and in addition, staff health and cleanliness, staff training, water control, sanitation, equipment handling and production and control processes. Not only do they affect the safety of the cheese, but these factors also affect the metabolic processes that lead to the development of the final sensory characteristics (Bintsis et al., 2002; Dozet et al., 2006). In addition to non-dairy microflora (yeasts and molds), micrococci and coliform bacteria can be found in white salty cheese. Coliform bacteria usually disappear during the cheese ripening process and participate in the production of CO_2 and H_2. Yeasts also generate CO_2 from lactose, which is responsible for the early flatulence that commonly occurs in cheeses made from unpasteurized milk with poor hygiene, as well as cheeses exposed to high temperatures. The active acidity of cheese is the main factor for the development of pathogenic microflora (it has optimal growth in the range of pH 6-7.5) (Alichanidis et al., 2007; Makarijoski B., 2019; Makarijoski B., 2023).

Homoljski cheese is traditionally produced from raw milk, but today thermalized or pasteurized milk is used to produce this cow's milk cheese. Although thermalization destroys some pathogenic bacteria, certain pathogens (*Listeria monocytogenesi, E. coli*) can survive and contaminate the final product. The survival of these bacteria depends on the initial number of contaminants, heat, tolerance to an acidic environment and salt, present strains, competitive microflora, length of ripening, cheese composition, and production conditions. With white cheese in brine, the cheese is often left at a high temperature (16 - 20 °C) before ripening and immersion in the brine, and then pathogens can reach high numbers, which is a critical point in the production of this cheese. Commission Regulation (EC) no. 2073/2005 and no. 1441/2007 uses *Coagulase-positive staphylococci* as index organisms to assess the probability of staphylococcal enterotoxins in cheese made from raw or pasteurized milk, and *E. coli* is used as an indicator of the hygiene level of production in cheese made from milk subjected to heat treatment. *Listeria spp*. is used as an index organism for the probable presence of *L. monocytogenes* in food (Crippa et al., 2022; EZ br. 2073/2005; EZ br. 1441/2007).

Material and Methods

A total of 15 cheese samples were taken for this test, which were taken in three time intervals, during the spring and summer of 2023 (April 20, 2023, May 21, 2023, and June 24, 2023). The cheeses selected for testing were produced in different villages of the municipality of Žagubica (Suvi Do, Laznica, Jošanica, Vukovac, Žagubica) in Serbia. The cheese was purchased at the market, in the salesroom for dairy products (kg of cheese in brine (5x200g)), in the original packaging provided by the manufacturer (plastic container). The samples were transported to a certified laboratory for testing the quality of milk and milk products (Public Health Institute Požarevac), in hand coolers (at a temperature of 3.5 °C). At the time of receipt, the temperature of the sample was 4 °C, which is an acceptable state of the sample. It was sampled with sterile equipment (gloves, sterile knife and fork, disposable containers), and then stored in a refrigerator at a temperature of 4 °C. The samples were analyzed within 4 hours from the moment of sampling. Microbiological tests were conducted in accordance with the regulations of the Rulebook on general and special conditions of food hygiene at any stage of production, processing, and circulation (Official Gazette of the Republic of Serbia No. 72/2010 and 62/2018). The presence of *Escherichia coli*, *Coagulase-positive staphylococcus,* and *Listeria monocytogenes* was examined. The methods used for the microbiological analysis of Homoljski cheese are as follows:

- *Escherichia coli*: SRPS ISO 16649-2:2008 - horizontal method for determining the number of ß-glucuronidated positive *Escherichia coli* colony counting technique at 44°C using 5-bromo-4-chloro-3-indolyl ß-D-glucuronide);
- *Coagulase-positive staphylococci* (*S. aureus* and other species): SRPS EN ISO 6888-1:2009 - horizontal method for determining the number of *Coagulase-positive staphylococci* (colony counting technique using Baird-Parker agar after aerobic incubation at 35 °C or 37 °C);
- *Listeria monocytogenes*: SRPS EN ISO 11290-2:2017 - Horizontal method for number detection and determination *Listeria monocytogenes* and *Listeria spp*.

According to the current Rulebook, the microbiological criteria for cheese produced from raw milk are:

- *Escherichia coli*: m = 100 cfu/g, M = 1000 cfu/g;
- *Coagulase-positive staphylococci*: m =10^4 cfu/g, M = 10^5 cfu/g;
- *Listeria monocytogenes*: m = M = 100 cfu/g or absence in 25g sample,

and for cheese produced from milk processed at a temperature lower than the pasteurization temperature:

- *Escherichia coli*: m = 100 cfu/g, M = 1000 cfu/g;
- *Coagulase-positive staphylococci*: m = 100 cfu/g, M = 1000 cfu/g;
- *Listeria monocytogenes*: m = M = 100 cfu/g or absence in 25g sample.

Results and Discussion

A total of fifteen samples were tested in three terms during the spring and summer of 2023, and the results of the tested microbiological parameters are shown in Table 1, 2 and 3.

Table 1. Presentation of test results for the month of April

Samples	Parameter	Unit measures	Reference value	The resulting value
sample 1	*E.coli*	cfu/g	≤100	**1100**
sample 2	*E.coli*	cfu/g	≤100	<100
sample 3	*E.coli*	cfu/g	≤100	<100
sample 4	*E.coli*	cfu/g	≤100	<100
sample 5	*E.coli*	cfu/g	≤100	<100
sample 1	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 2	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 3	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 4	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 5	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 1	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 2	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 3	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 4	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 5	*Listeria monocytogenes*	cfu/g	≤100	<100

Table 2. Presentation of test results for the month of May

Samples	Parameter	Unit measures	Reference value	The resulting value
sample 1	*E.coli*	cfu/g	≤100	**1500**
sample 2	*E.coli*	cfu/g	≤100	<100
sample 3	*E.coli*	cfu/g	≤100	<100
sample 4	*E.coli*	cfu/g	≤100	<100
sample 5	*E.coli*	cfu/g	≤100	<100
sample 1	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 2	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 3	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 4	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 5	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 1	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 2	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 3	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 4	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 5	*Listeria monocytogenes*	cfu/g	≤100	<100

Table 3. Presentation of test results for the month of June

Samples	Parameter	Unit measures	Reference value	The resulting value
sample 1	*E.coli*	cfu/g	≤100	**1700**
sample 2	*E.coli*	cfu/g	≤100	<100
sample 3	*E.coli*	cfu/g	≤100	<100
sample 4	*E.coli*	cfu/g	≤100	<100
sample 5	*E.coli*	cfu/g	≤100	<100
sample 1	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 2	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 3	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 4	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 5	*Coagulase-positive staphylococci*	cfu/g	≤10	<10
sample 1	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 2	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 3	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 4	*Listeria monocytogenes*	cfu/g	≤100	<100
sample 5	*Listeria monocytogenes*	cfu/g	≤100	<100

In none of the controlled samples was the presence of *Listeria monocytogenes* found in the 25 g sample. The number of *Coagulase-positive staphylococci* did not exceed the limit of 100 cfu/g, so there was no need for further testing for the presence of staphylococcal enterotoxins. And *E. coli* was detected in sample number 1, in all three sampling periods (April, May, June). In April in the amount of 1100 cfu/g, in May in the amount of 1500 cfu/g and in June in the amount of 1700 cfu/g. Sample No. 1 (Suvi Do village), in which *E. coli* was found in all three sampling periods, is an indicator of poor microbiological quality of milk, poor hygiene measures during production, inadequate processing and/or contamination of cheese after the production process. The presence of coliforms is often used to assess hygienic conditions during the production of dairy products. The existence of coliforms in pasteurized dairy products shows a lack of proper hygiene practices on farms and milk processing facilities. It may be associated with milking cows with mastitis, cleanliness of the milking machine, and poor sanitation practices during milking. *E. coli* strains can survive thermization (heating of milk at sub-pasteurization temperatures for a short time) at 65 and 67.5 °C. They can show moderate to high heat resistance and sometimes the ability to withstand the high temperatures applied during heat processing, such as pasteurization. During cheese production, the levels of indicator bacteria of the microbiological quality of the cheese usually increase in the initial stages, but decrease during ripening and ripening. The exception is fresh cheeses that have not been aged. So this may also indicate insufficient ripening of this cheese.

Conclusion

The results of the microbiological evaluation of the quality of Homolje white cheese (a total of 15 samples) showed that 12 samples met the quality criteria of the Rulebook, and the presence of *E. coli* was registered in three samples. The defective samples were from the same manufacturer. These results emphasize the need to implement and maintain good hygiene practices~~,~~ and study risk factors to prevent contamination and bacterial growth. Constant focus on milk quality and good hygiene in the premises where the production process takes place, personal hygiene of people involved in production must be a priority, because some of these pathogens can cause serious illness and even death. Constant education of producers on the implementation of sanitation and hygiene measures is also necessary.

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CORRELATION BETWEEN TOTAL POLYPHENOLS CONTENT AND TOTAL FLAVONOIDS CONTENT IN RED GRAPEFRUIT WITH ANTIOXIDANT ACTIVITY

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Abstract

Red grapefruit (*Citrus paradisi* Macf.) is a worldwide consumed tropical fruit that belongs to the *Rutaceae* family. Due to phytochemicals in its composition this fruit has health benefits. The aim of this work is to show correlation between total polyphenols content (TPC) as well as total flavonoids (TFC) content of fruit extract and antioxidant activity determined by four different methods. Fresh fruits originating from Turkey were bought in a local supermarket in Niš, Serbia. The quantity 10 g was added to a mixture consisting of organic solvent mixture and 0.05% (w/v) butylated hydroxytoluene. The mixture was stoppered and mixed on an orbital shaker at 180 rpm for 15 minutes at 5°C. The upper (non-polar) layer was separated from the lower (polar) phase. The non-polar phase was analyzed using UV-Vis spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan) to determine the TPC and TFC. By calculation Pearson’s correlation coefficient correlation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP, and CUPRAC methods was calculated. Pearson’s coefficient indicates the strongest correlation between TPC and CUPRAC, ABTS, and FRAP (0.99, 0.99, 0.96, respectively). The correlation between TFC and CUPRAC, ABTS, and DPPH (0.84, 0.74, 0.59, respectively) is high. The Pearson’s correlation coefficient showed a direct correlation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP, and CUPRAC methods. The correlation coefficient differs depending on the method used for antioxidant activity measurement.

Keywords: *Red grapefruit, Pearson’s correlation coefficient, total polyphenols content, total flavonoids content.*

Introduction

Red grapefruit (*Citrus paradisi* Macf.) is worldwide known tropical fruit consumed in everyday diet that belongs to *Rutaceae* family. This one is the second largest produced citrus on global market with average production of 60 million tons per year. With its red color, as a result of carotenoids presence, it stands out with its appearance and mild sour to semi sweet taste. Many different health benefits of this fruit are experimentally confirmed (Ukom *et al*. 2022).

Besides the vitamin C, this fruit is rich source of flavonoids as reported in one of previous research (Miljković *et al*., 2023). The flavonoids have found usage in a treatment of: inflammation, cardiovascular diseases, and in cancer prevention (Andersen and Markham, 2006). The presence of carotenoids is responsible for the fruits color and they are valuable phytochemicals in human diet. Lycopene is a natural red-orange carotenoid that belongs to the group of strongest natural antioxidants, as a constituent of oranges, tomatoes, grapefruits, etc. It is importance to consume it in everyday diet because only 6 mg per day reduce the chance getting prostate cancer by 10-20% (Harini et al., 2016).

The grapefruit juice has antiseptic potential, but also promote bone growth and weight loss. High attention should be made on grapefruit juice consumption because one up to two glasses of grapefruit juice inhibits presystemic drug metabolism via CYP3A isoforms in the small bowel and changes drug concentration (Kirby and Unadkat, 2007). This interaction is reported with more than 40 different drugs (Saito *et al.*, 2005).
Bearing in mind worldwide consumption of this fruit and the effects on human health that it can provide, this work is written to show the correlation between total polyphenols content (TPC) as well as total flavonoids (TFC) content of fruit extract and antioxidant activity determined by four different methods.

Material and methods

Plant material: The red grapefruits were bought at the local market in Niš, Serbia in March of 2022. Firstly, they were washed with deionized water, sliced and homogenized in Brown® blender.
List of chemicals used: acetone was purchased from Fisher Scientific (Loughborough, United Kingdom). Hexane, butylated hydroxytoluene, sodium acetate, ethanol and glacial acetic acid were purchased from Sigma-Aldrich (Steinheim, Germany).
10 g of fruit flesh sample was mixed with a solution consisting of 250 ml hexane, 125 ml of acetone, 125 ml of ethanol (2:1:1, v/v/v), and 0.05 % (w/v) butylated hydroxytoluene (BHT). The mixture was mixed on an orbital shaker to mix at 180 rpm for 150 minutes (temperature of mixing was 5 °C). Afterwards, 75 ml of cold deionized water was added and the mixture was agitated for another 5 min. In order to allow the separation of polar and non-polar layers, the suspension was left at room temperature for 10 minutes. The extract was re-dissolved in hexane.
The Pearson correlation coefficient express the strength and direction of the linear relationship of correlation. It is the most often recommended test for such relation examination (Puth *et al.*, 2014). In this work, calculation of Pearson’s correlation coefficient was done to find relation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods.

Results and Discussion

Results for Pearson’s correlation coefficient for relation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods are presented in Table 1.

Table 1. Pearson’s correlation coefficient

	Total polyphenols	Total flavonoids	ABTS	DPPH	FRAP	CUPRAC
Total polyphenols	1.00	0.99	0.99	0.99	0.97	0.95
Total flavonoids		1.00	0.99	0.98	0.93	0.91
ABTS			1.00	0.98	0.92	0.89
DPPH				1.00	0.98	0.96
FRAP					1.00	0.99
CUPRAC						1.00

Pearson’s correlation measures the linear relationship between two continuous sets of data and is the most used for this type of researches. The coefficient is in range from −1 to 1, with a value of −1 indicating a perfect negative correlation, 0 indicating no correlation, and 1 indicating a perfect positive correlation. The ABTS test includes free radicals, the DPPH test also, with practically color changing in the presence of antioxidants. These two tests showed the highest positive correlation between antioxidant activity of *Citrus paradisi* extract and the TPC and TFC. High positive correlation between antioxidant activity and TPC and TFC was also calculated for the results obtained with methods involving the reduction of Fe^{2+} and Cu^{1+} ions, that is FRAP and CUPRAC. The TPC showed to be highly responsible for antioxidant activity obtained with all tests. Similar is for the contribution of TFC in antioxidant activity.
These results are of importance because they identify the class of chemical compounds that are in direct correlation with antioxidant activity of *Citrus paradisi*, that is, the phytochemicals that contribute to health benefits. However, further analysis should be performed in order to identify concretely each compound and its present/contribution.

Conclusions

Red grapefruit (*Citrus paradisi*) is a worldwide consumed tropical fruit that have numerous health benefits for human health. One should be known, that due to interaction with liver enzymes it has inhibitory effect on metabolism of different drugs and that is why the recommendation is to be avoided during the medical therapy. The extract of red grapefruit showed antioxidant activity that is in direct correlation with the TPC and TFC in it. The results are recommending this not so expensive fruit in everyday diet as a rich source of health beneficial phytochemicals that contribute to human health.

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PEARSON’S CORRELATION COEFFICIENT FOR TOTAL POLYPHENOLS AND TOTAL FLAVONOIDS WITH ANTIOXIDANT ACTIVITY IN TOMATO JUICE

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Abstract

Tomato juice is among the healthiest and most popular health-promoting drinks. This tomato based product produced by cooking of fresh tomatoes is rich in polyphenols which are known as health-beneficial biocompounds. This work is written with the aim to find correlation between total polyphenols content (TPC) as well as total flavonoids (TFC) content of fruit extract and antioxidant activity determined by four different methods calculated as Pearson’s coefficient. Mature tomato Hector-F1 cultivar fruits bought in a market in Niš, Serbia were washed and sliced. Afterward, they were milled before cooking according to traditional recipe until boiling point when salt was added. The quantity of 10 g was extracted with a mixture of organic solvents, of which the (upper) non-polar phase was analyzed using UV-Vis spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan) to determine the TPC and TFC. Calculation of Pearson’s correlation coefficient was done to find a relation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods. Pearson’s coefficient shows highest possible correlation between TPC with CUPRAC (1) and ABTS (0.99), and a little bit lower with FRAP (0.97). The correlation between TFC with CUPRAC (0.84), ABTS (0.74) and DPPH (0.59) is high. The Pearson’s correlation coefficient showed direct correlation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods. The obtained values differs with method used for antioxidant activity determination.

Keywords: *Tomato juice, Pearson’s correlation coefficient, total polyphenols content, total flavonoids content.*

Introduction

The tomato (*Solanum Lycopersicum* L.) and tomato based products have multiple health beneficial properties (Lenucci *et al*., 2006). They present a healthy food consumed in everyday diet. In addition to cholesterol absence, they are low in fat and total calories, and present rich source of fibers and proteins. The vitamins A, C, *β*-carotene, potassium and lycopene are just making tomato based products are even more recommending. The carotenoids are class of pigments present in vegetables and fruits. More than 700 of them have been identified and their function as a contributors of human health in biochemical reactions is well described (Britton *et al*., 2004). The lycopene is a phytochemical considered as a measure of ripeness and total quality of tomatoes, with antioxidant activity due to the extended system of conjugated double bonds. The tomatoes and tomatoes based products are primary dietary source of lycopene in everyday nutrition, and considered as healthy foods (Clinton, 1998; Mangels *et al*., 1993). The main sources of lycopene are dishes prepared with tomato sauce (Dragon *et al*., 2000). It was found that reduced risk of cancer is related with the amount of lycopene daily intake from food (Gerster, 1997). The chemical composition of tomato and tomato based products is depending on: genetic diversity, cultivation technology, climate, geographic site of production, fruit ripeness, techniques of harvesting and processing, as well as storage of the final products (Daood et al., 2014). During food processing happens chemical changes of the compounds that result to the formation of new chemical compounds or isomers and derivatives. This is why the food products are getting analyzed on chemical composition. For that purpose, different analytical methods are available to determine the content of chemical compounds such as high-performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), optothermal and photothermal methods, Raman, infrared and near-infrared spectroscopy, and UV-Vis spectrophotometric methods (Jaramillo *et al*., 2018). Food processing is getting performed to prolong food shelf life and has a long history. It can be done on house and industrial levels. There are numerous studies on the effect of food processing (increased temperature and exposition to light and oxygen), and it is not gratef to generalize the outcome of this process. The increase in total phenolics content (TPC) and total flavonoid content (TFC) can be reached because of better extraction and exposition to higher temperatures, oxygenation, and light. The decrease in phytochemicals is most commonly the result of phenolic compound degradation (Ozkan et al., 2022). This study aims to investigate the correlation between the total polyphenol content (TPC) and total flavonoid content (TFC) of tomato juice extract, and its antioxidant activity. The antioxidant activity was determined using four different methods and the correlations were calculated using Pearson’s coefficient.

Materials and Methods

The Hector-F1 tomato fruits were purchased from a local market in Niš, Serbia, in September 2021. Country of their origin was Turkey. Firstly, they were washed with deionized water, and afterwards sliced with a knife and homogenized in a blender, the seeds were removed. The tomato juice was prepared according to a traditional recipe, that is, the milled tomato was heated up to boiling point, stirred for additional 5 min, salted, packed into glass flasks and kept into a shade. In one of previous articles chemical composition and antioxidant activity of tomato juice grown in Serbia is reported (Miljković *et al*., 2022). The Pearson correlation coefficient express the strength and direction of the linear relationship of correlation. It is the most used test for such relation examination (Puth *et al*., 2014). Calculation of Pearson’s correlation coefficient was done to find relation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods.

Results and Discussion

Results for Pearson’s correlation coefficient for relation between TPC and TFC with antioxidant activity determined by ABTS, DPPH, FRAP and CUPRAC methods are shown in Table 1.

Table 1. Pearson’s correlation coefficient

	Total polyphenols	Total flavonoids	ABTS	DPPH	FRAP	CUPRAC
Total polyphenols	1.00	0.82	0.99	0.019	0.97	0.99
Total flavonoids		1.00	0.74	0.59	0.65	0.84
ABTS			1.00	-0.11	0.99	0.99
DPPH				1.00	0.23	0.06
FRAP					1.00	0.96
CUPRAC						1.00

Pearson’s correlation coefficient is the most often used calculation method for finding such correlations as the relation between TPC and TFC and the antioxidant activity of tomato juice extract. It measures the linear relationship between two continuous sets of data and is the most used for this type of researches. The coefficient is in range from −1 to 1, with a value of −1 indicating a perfect negative correlation, 0 indicating no correlation, and 1 indicating a perfect positive correlation.
The positive correlation is calculated between TPC and antioxidant activity measured by ABTS, FRAP and CUPRAC method. That means that higher TPC contribute directly to higher antioxidant activity measured by these two method which use different mechanisms (color changing of free radicals in presence of antioxidant, reduction of Fe^{2+} and reduction of Cu^{1+} ions, respectively). The relation between TFC measured by these three method was found to be high. The direct correlation was almost none for TPC and antioxidant activity measured by DPPH method, although it is positive and strong in a relation with TFC.

Conclusions

The tomato juice is a worldwide consumed tomato based product that have numerous health benefits for human health. It is very popular among older population and the results obtained in this study are also giving recommendation for it consumption as health beneficial food product. It is constituent of popular food Pizza and can be served on many different ways, as during winter times so during summer times as refreshment. The extract of tomato juice showed antioxidant activity that is in direct correlation with the TPC and TFC in it.

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CORDYCEPS SINENSIS MUSHROOMS, THEIR ANTIOXIDANT, ANTIMICROBIAL ACTIVITY AND COMPLEXATION WITH SILVER IONS

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Abstract

Cordyceps sinensis is coming to public attention primarily as a dietary supplement. In addition to its culinary use, it has also found its application among pharmacy clients. Its immunomodulatory and anti-inflammatory effect is coming to the fore. It is also interesting in terms of reducing bacterial resistance to treatment, which is caused by the overuse of antibiotics. It is confirmed that cordycepin, as a major bioactive molecule, inhibits the growth of cancer cells by multiple mechanisms, which serves as a supportive therapy for cancer. There is a great interest in natural *Cordyceps sinensis*, but harvesting it is quite difficult and devastating for the environment. For this reason, solutions for its artificial cultivation are also being sought. It turns out that even artificially cultivated *Cordyceps sinensis* contains a number of important substances for human health. The thesis deals with the cultivation of *Cordyceps sinensis* fungi on different substrates, where pea and maize were used as substrates. Maceration and ultrasound extraction were used to prepare the extracts. Four solvents were used for both types of extractions: water, methanol ethanol and ethyl acetate. The yield of extracts ranged from 1.41% to 10.41%. The highest yield was observed in the extract obtained by maceration in distilled water, as the substrate was a pea. The antioxidant activity of all obtained extracts was subsequently determined using DPPH radical. The highest antioxidant activity was determined in the sample of *Cordyceps sinensis* that was cultivated on maize. Methanol was used as the extraction solvent and the IC_{50} value was 2.94 mg/mL. The infrared spectra of the prepared extracts were subsequently measured and analyzed. In the five samples with the highest antioxidant activity, the antimicrobial activity on the bacteria strain *Staphylococcus aureus* and *Escherichia coli* was determined by the disk diffusion method. In the tested extracts, antimicrobial activity was not detected.

Keywords: *mushrooms, Cordyceps sinensis, antioxidant activity, extracts, complexation.*

Introduction

Fungi are interesting for their diversity, as evidenced by more than 1200 entemopathogenic fungi (Humber, 2000). *Cordyceps* is one of the largest genera, with more than 500 species (Hodge,1998; Hywel-Jones, 2002; Muslim, 2010). Mushrooms of the *Cordyceps* species have been an integral part of traditional Chinese medicine for centuries, which has described it as sweet and warming (Panda, 2011). The basis of this Chinese medicine is not shamanism, or some form of occultism, but the collection and use of medicinal plants, their parts, and the collection of mushrooms and other gifts of nature. Many ancient herbal recipes have fallen into oblivion due to the negative influences of then and now. It should be added, however, that even traditional Chinese medicine is not a panacea for every existing ailment. However, in the case of diseases such as oncological and autoimmune diseases, it can have a significant impact on the quality of people's lives.

Cordyceps sinensis (*CS*) is found in the wild only in the Himalayas, at altitudes higher than 3500 m above sea level, especially on the Tibetan Plateau. Their collection is difficult and

hence availability is low, which has resulted in high price. It is therefore one of the most expensive medicinal mushrooms in the world. Because of its high price, it has become almost exclusively reserved for members of the imperial family and nobility.
The genus *Cordyceps* (Sung, 2007) has a rich history. It begins to be written more than 2000 years ago. The discovery of this genus occurred in an unusual and interesting way. The vitality, energy and strength of the cattle reared on the Tibetan grasslands became the basis. The herders noticed a fungus growing in the pastures, and by eating it, the cattle were brimming with strength and vitality. This discovery gave rise to long-term research by scientists and doctors that is still ongoing today. *Cordyceps* was first introduced to the Western scientific community in 1726 by Perennin Jean Baptiste du Halde, a monk of the Jesuit order, at a scientific symposium held in Paris. This Jesuit came into contact with this fungus during his stay at the imperial court (Panda, 2011).
Due to its broad spectrum effects, it is also of interest in Western medicine. Positive effects of *Cordyceps* have been reported in the treatment of the liver, kidney (Guan, 1992; Holliday, 2004; Holliday, 2008) and immune system (Yamaguchi, 1990). Positive effects have been described treating civilization diseases such as cancer (Lin, 1984; Yamada, 1984), metabolic diseases and cardiovascular diseases (Xiang, 2016). With long-term use, it has a positive effect on lowering cholesterol and low density lipoprotein levels, improving glucose metabolism, and reducing muscle fatigue (Koh, 2003). Antibacterial, antifungal, anti-inflammatory properties are also not negligible (Shahed, 2001; Kim, 2003). Also based on these scientific findings, the demand for *Cordyceps* has increased disproportionately. This in turn has also affected its price. Thus, the resulting shortage of natural *Cordyceps* forced scientists to look for ways to cultivate it artificially (Ni, 2009) and to provide suitable laboratory conditions. Last but not least is the determination of the content of active substances in the products thus obtained. An interesting fact in this case is that *CS*, as a drug, is listed as a pharmaceutical in the Chinese Pharmacopoeia (Lin, 2011).
Currently, biotechnological processes have been able to approach the natural conditions in which *CS* grows to such an extent that cultivated *Cordyceps sinensis* becomes a full-fledged substitute for natural *Cordyceps*. Currently, it is possible to grow *CS* of very high quality. Our aim was to investigate the antioxidant and antimicrobial activity of artificially grown *CS*. It was cultivated on peas and maize substrate. In addition, the ability of *CS* to complex silver ions was monitored. The obtained results will be used for comparison with natural *Cordyceps*.

Material and Methods

Extracts were prepared from five grams of the powder form of *CS* mushrooms. During the maceration, the mushroom samples were allowed to stand in the given solvent 50 mL (water, methanol, ethanol, ethyl acetate) for 24 hours in the dark at room temperature. Subsequently, the given solutions were filtered and concentrated to dryness on a rotary vacuum evaporator. In the case of ultrasound extraction, the extract preparation procedure was the same, but *CS* mushroom extracts were prepared using a Bandelin Sonorex Digitec device with a frequency of 35 kHz, P 140/560 W, DT 103 H (Germany) at a temperature of 65°C for one hour. After extraction, the samples were filtered and evaporated to dryness on a vacuum evaporator.
The DPPH method (1,1-diphenyl-2-(2,4,6-trinitrophenyl)hydrazyl radical) was used to determine the antioxidant activity of the extracts. The progress of the stable radical scavenging reaction was monitored spectrophotometrically on a Lasany LI-722 Spectrophotometer (Germany) at a wavelength of 517 nm. A fresh DPPH radical solution with a concentration of 2.0 mM was used for testing. The antioxidant activity of the measured samples was calculated from the equation I (%) = (A_{BL}-A_{SL}/A_{BL}) ×100, where I (%) represents the inhibition of DPPH radical activity, A_{BL} is the absorbance of the blank DPPH

solution, and A_{SL} is the absorbance of the sample after 30 minutes of incubation in the dark at room temperature from the addition of the DPPH solution. Infrared (IR) spectra were measured on a Thermo Scientific Nicolet 6700 FTIR device in the range of 4000-400 cm^{-1}.
The proton spectrum of the selected extract was measured on a 400 MHz Varian Mercury Plus spectrometer (Palo Alto, California, USA). The extract (20 mg) was dissolved in 0.6 mL of deuterated water D_2O-d_2. The process of complexation of the extracts with silver ions was monitored spectrophotometrically in the range of 200-900 nm (Wang, 2016). Samples for measurements were prepared from 10 mg of crude extract to which 2 mL of distilled water and 422 μL of 10.0 mM/L $AgNO_3$ solution were added. The mixture was heated in a water bath and samples for measurements were taken at time intervals of 20, 40 and 60 minutes. The samples were diluted with distilled water in a cuvette before the actual measurement. According to the method of the Rojas 2006, was determining the antibacterial activity. Testing was carried out on agar plates by the disc diffusion method on bacterial strains of *Staphylococcus aureus* and *Escherichia coli*.

Results and Discussion

The extraction of powderd *CS* material was carried out in four different solvents (water, methanol, ethanol and ethyl acetate) by two different methods, maceration and ultrasound extraction. The yield of the prepared extracts varied between 1.41-10.41%. The highest yield 10.41% was obtained from the extract of the *CS* sample cultivated on peas and prepared by maceration in distilled water. When determining the antioxidant activity by the DPPH method, the IC_{50} values were in the range of 2.94-66.68 mg/mL. The most effective extract, which had the highest ability to scavenge the stable DPPH radical, had the lowest IC_{50} value (2.94 mg/mL). It was a methanol extract prepared by maceration of a *CS* sample cultivated on maize. IR spectra were measured for all eight extracts. In Fig. 1 are IR spectra of selected extracts.

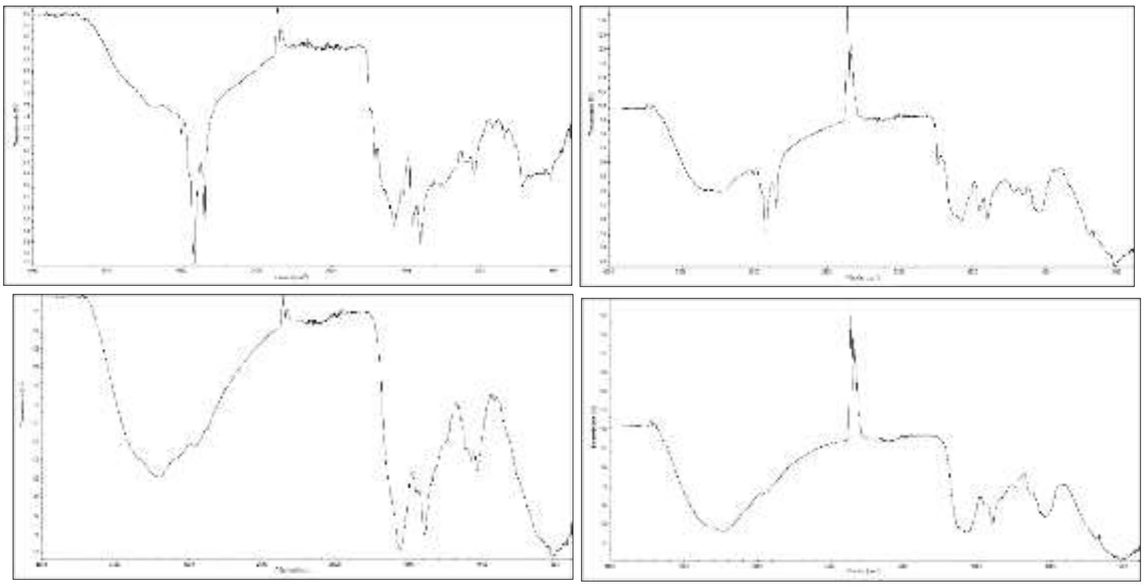

Fig. 1. IR spectrum of a sample of CS cultivated on maize, obtained by maceration in methanol (upper left); IR spectrum of a CS sample cultivated on pea, obtained by ultrasound extraction in methanol (upper right); IR spectrum of a CS sample cultivated on maize, obtained by maceration in distilled water (bottom left); IR spectrum of a CS sample cultivated on pea, obtained by ultrasound extraction in distilled water.

A broad band in the region around 3500-3000 cm^{-1} is attributed to hydroxyl groups, and a sharp band present in some IR spectra of the extracts at 1415 cm^{-1} belongs to the bending vibration of the O-H group. The presence of bands in the region below 3000 cm^{-1} can be assigned to C-H bond vibrations. Although these bands have a low intensity in some spectra of the extracts, their position in the spectra is characteristic and therefore easily identifiable. The band around 1650 cm^{-1} can be assigned to the glucose ring. A characteristic band around 1090 cm^{-1} indicates the existence of a pyran skeleton and an absorption band at 825 cm^{-1} indicates the presence of an α-glycosidic bond. Based on the presence of bands in this areas, we can assume that polysaccharides are present in the extracts.

A polysaccharide were found in the ^{1}H NMR spectrum of the *CS* extract cultivated on maize, obtained by maceration in distilled water (Fig. 2). This is evidenced by the presence of complicated multiplet signals. These are chemical shifts of proton signals at 5.41 ppm and a broad multiplet signal in the region of 3.96–3.63 ppm. In the saccharide region, a triplet signal belonging to C–H protons can be identified, which are bound near the hydroxyl –OH group at 3.97 ppm with an interaction constant J = 12.0 Hz. By comparing the chemical shifts and the multiplicity of signals with the works of authors who dealt with the issue of determining the chemical structure of polysaccharides, we can conclude that the extracted polysaccharide has a structure similar to maltodextrin. (Pavia, 2013)

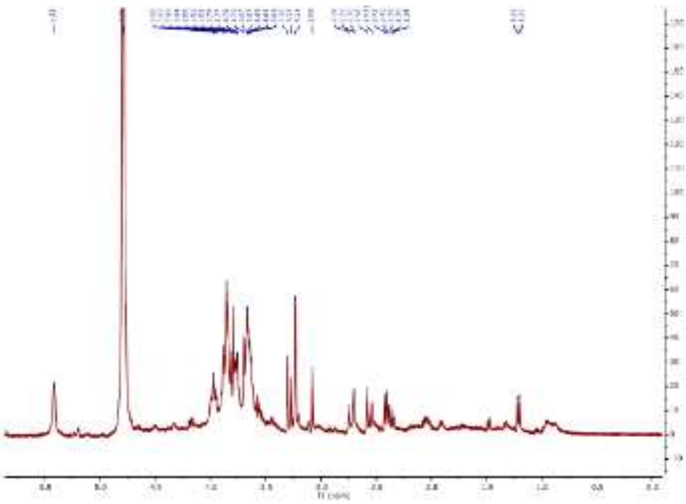

Fig. 2. ^{1}H NMR spectrum of *CS* extract cultivated on maize, obtained by maceration in distilled water.

Chemically, maltodextrin is an oligosaccharide composed of D-glucose units. Glucose units are primarily connected by (1→4) glycosidic bonds. By comparing our proton spectrum (Fig. 2) with the spectrum of maltodextrin (Pavia, 2013), we can see the agreement in the chemical shifts of the proton signals, as well as in their multiplicity.

The use of UV/Vis spectrophotometry showed that upon addition of $AgNO_3$ to the extract of *CS* (cultivation on peas, aqueous extract, extraction using ultrasound), an increase of absorbance was observed in the 400-450 nm region (Fig. 3), indicating the formation of complexes between the extracts and $AgNO_3$. The region from 400-500 nm represents the wavelength range with maximum absorbance for silver nanoparticles. Gradual heating of the extracts increased the absorbance value in that wavelength range. According to the Lambert-Beer law, increasing concentration causes increasing absorbance, and this represents an increase in the ability of the extracts to form complexes with $AgNO_3$. Interestingly, the silver ion complexes with *CS* extract were stable even after 1 hour of heating at 90 °C.

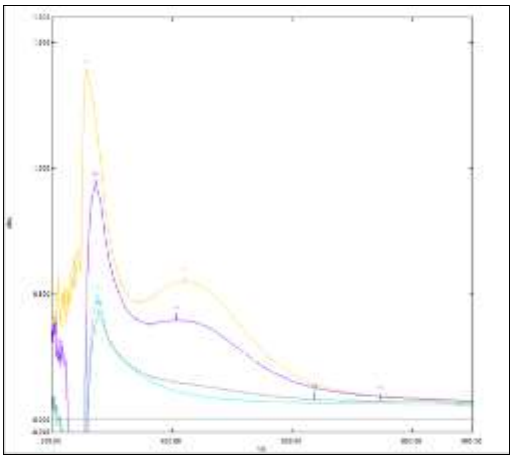

Fig. 3. UV/Vis spectrum of complexation of *CS* extract with silver ions. Turquoise color - pure extract, after adding 10 mM $AgNO_3$: dark green color - 20 minutes heating, violet color - 40 minutes heating, yellow color - 60 minutes heating. Absorbance increase upon complexation of *CS* extract after 1 hours heating.

The antibacterial effects of the extracts were tested on agar plates by disc diffusion method on two bacterial strains, *Staphylococcus aureus* and *Escherichia coli*. Five extracts were selected which were found to have the highest antioxidant activity. These extracts were hypothesized to be able to exhibit antibacterial activity. These were mainly four methanol extracts obtained by ultrasonic extraction and maceration (cultivation on maize and pea) and one ethanol extract (cultivation on pea, maceration). Even after 24 hour of cultivation no antibacterial activity was observed for any of the analysed extracts.

Conclusions

In this work, eight extracts of *CS* fungi cultured on two substrates (pea and maize) prepared by maceration and ultrasonication were analyzed. The extracts were prepared using four solvents: methanol, ethanol, water and ethyl acetate. The antioxidant activity of the prepared extracts was determined by DPPH method. The most effective extract in radical scavenging with IC_{50} value of 2.94 mg/mL was the methanolic extract prepared by maceration (culture on maize). The presence of hydroxyl functional groups was confirmed in the analyzed extracts by using infrared spectroscopy. Also, analysis of the ^{1}H NMR spectrum of *CS* extract confirmed the presence of polysaccharides in the samples. UV/Vis spectrophotometry was used to monitor the formation of complexes between *CS* extract and $AgNO_3$. The antibacterial testing of the extracts by disc diffusion method on two strains of *Staphylococcus aureus* and *Escherichia coli* did not show any effect.

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SURVEILLANCE OF *RHIZOCTONIA SOLANI* STRAIN PATHOGENICITY ON DIFFERENT CROP SPECIES AND BIOCONTROL POTENTIAL OF CANDIDATE AGENTS

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Abstract

Sustainable food production necessitates a safe and secure food system. Cultivating tomatoes, melons, and watermelons can enhance agricultural viability, food security, and their integration into diverse intercropping systems. However, *Rhizoctonia solani*, a necrotrophic plant pathogen with a broad host range, poses a significant threat to these crops, causing blights, damping-off, and rots. This study aimed to develop an eco-friendly strategy for managing *R. solani* damping-off (Rs26, Rs94, Rs13, Rs57, and Rs123). Following a pathogenicity assay to assess disease incidence in tomato, melon, and watermelon plants, an *in vitro* assay evaluated the efficacy of biological agents (*Trichoderma harzianum*, *T. viride*, *Metarhizium* sp., and *Gliocladium* sp.) in controlling *R. solani*. All *R. solani* isolates displayed varying degrees of pathogenicity across all crops compared to the control. Isolates Rs94 and Rs13 caused the most severe disease in watermelon (3.80 and 3.83, respectively), while Rs26 displayed the highest pathogenicity in tomato (3.84). Disease severity scores exceeding 2.59 across all *R. solani* isolates on melon indicate high susceptibility in this crop. All antagonistic fungi inhibited the growth of *R. solani* isolates to varying degrees. Notably, *Trichoderma* spp. displayed the most consistent and significant inhibition across all *R. solani* isolates, with reductions in mycelial growth ranging from 82.97% (Rs13/*T. viride*) to 94.67% (Rs26/*T. harzianum*). These findings hold promise for the development of an integrated and eco-friendly approach to managing *R. solani* damping-off.

Keywords: *Trichoderma* spp., *Metarhizium* sp., *Gliocladium* sp., *Tomato, Melon, Watermelon.*

Introduction

Rhizoctonia solani JG Kühn (teleomorph: *Thanatephorus cucumeris* (AB Frank) Donk) is a multifaceted fungal plant pathogen in the soil (Li *et al.,* 2021). This necrotroph, deriving sustenance from dead or dying host tissues, possesses a prolonged soil persistence achieved through sclerotia formation, a hardened structure enabling survival during unfavorable conditions. *R. solani* isn't a single entity but rather a complex encompassing over 100 distinct species (Abbas *et al.,* 2023). This complex wreaks havoc on a vast array of economically significant agricultural and horticultural crops and trees globally (Erper *et al.,* 2021). The extensive host range and distribution of this pathogen allow it to cause numerous diseases, including sheath blight observed in key field crops like corn and rice (Dubey *et al.,* 2012). *R. solani* exhibits varying degrees of pathogenicity towards a range of crops. It is particularly destructive to seedlings and seeds of eggplant, pepper, lettuce, and zinnia (Abbas *et al.*, 2023). In potatoes, this fungus causes both stem canker and black scurf, leading to significant reductions in tuber yield and quality (Naqvi *et al.,* 2024). However, the most severe disease caused by *R. solani* is the root rot of cotton, posing a substantial threat to this economically vital crop. In greenhouse settings, it is a primary pathogen responsible for root and crown rot in tomatoes. This fungus further extends its destructive reach to numerous vegetables, including cucurbits and tomatoes, causing a spectrum of seedling diseases encompassing seed rot, root rot, pre-emergence damping-off, and post-emergence damping-off. The economic impact of *R. solani* is substantial, causing significant yield losses across more than a hundred crop and horticultural species annually. Furthermore, its emergence as a significant problem is ongoing, with recent observations highlighting its ability to cause stem rot in sweet potato (Abbas *et al.*, 2023; Naqvi *et al.*, 2024).

R. solani exhibits a remarkable ubiquity and cosmopolitan distribution, existing in the soil as both a saprophyte (decomposing dead organic matter) and a plant pathogen (Naqvi *et al.*, 2024). This multifaceted fungus is further categorized into fourteen genetically distinct anastomosis groups (AG1 to AG13 and AGBI) (Li *et al.*, 2021). These groups display a unique level of host specificity, meaning they preferentially infect particular plant species and are reproductively incompatible with each other (Abbas *et al.*, 2023). Collectively, the *R. solani* species complex boasts a vast host range encompassing numerous plant species critical to agriculture, forestry, and the bioenergy industry (Erper *et al.*, 2021). This includes but is not limited to, prominent crops like tomato, wheat, rice, barley, potato, melon, watermelon, and sugar beet. The extensive host range and diverse lifestyles within the *R. solani* complex highlight its significant role as a plant pathogen with a broad economic impact (Dubey *et al.*, 2012; Yang *et al.*, 2024). This increasing pathogenicity underscores the need for effective control strategies to mitigate the widespread damage caused by *R. solani* (Abdelghany *et al.*, 2022).

R. solani reigns as a major fungal culprit behind the devastating damping-off and root rot diseases plaguing numerous vegetables (Yang *et al.*, 2024). Unfortunately, for most vegetables, there's a dearth of effective fungicides to combat *Rhizoctonia* infections, although some chemicals like chlorothalonil, thyophanate methyl, and iprodione have been occasionally recommended (Agrios, 1988). However, the escalating environmental concerns associated with chemical pest control necessitate alternative solutions (Hajji-Hedfi *et al.*, 2023). Here, the development of a biocontrol program emerges as a crucial undertaking. The scientific literature overflows with research on biological control of soil-borne pathogens utilizing fungal mycoparasites (Ruiz-Cisneros *et al.*, 2018; Mohamed *et al.*, 2020). Several fungi, most notably *Trichoderma* spp., have garnered significant attention for their antagonistic properties against *R. solani* (Behiry *et al.*, 2023). The precise mechanisms employed by *Trichoderma* spp. to achieve biocontrol are antibiotic and hydrolytic enzyme production, mycoparasitism, and hyphal disruption (Hajji-Hedfi *et al.*, 2023). These biocontrol mechanisms likely exhibit specificity towards particular antagonists and plant pathogens, with the possibility of multiple mechanisms operating independently or synergistically during microbial interactions (Almaghasla *et al.*, 2023). Furthermore, *Trichoderma* spp. may potentially influence the viability of *R. solani* sclerotia, offering an additional layer of control (Behiry *et al.*, 2023). Overall, the exploration and development of biocontrol agents like *Trichoderma* spp. present a promising and environmentally friendly approach to combating the detrimental effects of *R. solani* on vegetable crops (Shalaby *et al.*, 2022).

This research aimed to assess the pathogenic potential of five *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) on three distinct crop species: tomato, melon, and watermelon. Furthermore, the investigation evaluated the *in vitro* efficacy of *Trichoderma* spp. as a potential biocontrol agent against *R. solani*.

Materials and Methods

Pathogenicity test

An investigation into the pathogenic potential of five *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123) was conducted using three distinct crop species: tomato (cv. Firenze), melon (cv. Badii), and watermelon (cv. Crimson Sweet). The experiment aimed to determine variations in virulence among the *R. solani* isolates. Each isolate originated from a different rhizosphere (watermelon, tomato, melon, tomato, and watermelon, respectively). Sterilized pots (20 cm diameter) were filled with disinfested soil (clay:sand, 2:1 v/v) at 2.5 kg per pot. Inoculum for each fungal isolate was prepared by culturing them in sterilized sorghum grain medium for 15 days at 25±2°C. To infest the soil, inoculum was mixed with the upper soil layer at a rate of 2% (w/w). The infested soil was thoroughly mixed and irrigated every other day for a week before planting to stimulate fungal growth and ensure proper distribution throughout the soil. Five viable seeds, sourced from the Regional Centre of Agricultural Research in Sidi Bouzid, Tunisia, were planted in each pot. Three replicate pots were used for each isolate-crop combination, with an additional three un-infested pots serving as controls (negative control). Plants were grown in a greenhouse chamber under a 16 h/8 h light/dark cycle at 23–25°C with regular irrigation (Matrood and Rhouma, 2021; Rhouma *et al.*, 2024). Disease severity (DS) was evaluated after 60 days using a 0-4 scale adapted from Carling *et al.* (1999): 0 - no visible damage, 1 - minor hypocotyl discoloration, 2 - discoloration with small necrotic lesions (<1 mm diameter), 3 - discoloration with larger necrotic lesions (≥1 mm diameter), and 4 - plant death.

Antagonistic action of antagonistic fungi toward *Rhizoctonia solani*

An *in vitro* dual culture assay was employed to assess the antagonistic interaction between four potential biocontrol agents (*Trichoderma harzianum*, *T. viride*, *Metarhizium* sp., and *Gliocladium* sp.) and five *R. solani* isolates (Rs26, Rs94, Rs13, Rs57, and Rs123). The assay utilized potato dextrose agar (PDA) plates. Two agar plugs, each 0.5 cm in diameter, were obtained: one containing a four-day-old culture of the biocontrol agent and the other containing the target *R. solani* isolate. These plugs were positioned on opposite sides of a single 9 cm diameter PDA plate, following a standardized placement protocol: the antagonist plug was placed 2 cm from the plate's edge towards the center, and a 5 cm gap was maintained between the two plugs. A control plate included only a blank PDA plug on one side and the *R. solani* isolate plug on the opposite side. Each treatment was replicated three times, with each replicate consisting of five plates. All plates were incubated for seven days at 28°C ± 2°C following established protocols (Hajji-Hedfi *et al.*, 2023; Rhouma *et al.*, 2024). After incubation, the percentage inhibition of *R. solani* radial growth was determined using a formula established by Matrood and Rhouma (2021). This formula calculates the percent inhibition (I) as: $I (\%) = (1 - Cn/C0) \times 100$, where Cn represents the radial growth of the *R. solani* colony in the presence of the biocontrol agent and C0 represents the radial growth of the *R. solani* colony in the control plate without an antagonist.

Results and Discussion

Pathogenicity test

Table 1 offered valuable insights into the pathogenicity of five *R. solani* isolates towards three economically important crops: tomato, melon, and watermelon. The data reveal that all isolates caused varying degrees of disease on all three crops compared to the control treatment, which showed no disease development. This indicates the inherent pathogenic potential of all *R. solani* isolates tested. However, a closer examination of the disease severity scores (ranging from 0 to 3.84) highlights interesting patterns. Isolates Rs94 and Rs13 emerged as the most aggressive pathogens on watermelon, with disease severity scores of 3.80 and 3.83, respectively. Conversely, isolate Rs26 displayed the highest pathogenicity on tomato (3.84), demonstrating potential variations in virulence between *R. solani* isolates. It's noteworthy that all isolates caused significant disease on melon (disease severity scores above 2.72), suggesting a high level of susceptibility for this crop to all *R. solani* isolates tested (Table 1).

Table 1. Pathogenicity of five isolates of *Rhizoctonia solani* (Rs26, Rs94, Rs13, Rs57, and Rs123) on three crops (tomato, melon, and watermelon).

Treatments	Tomato	Melon	Watermelon
Control	0±0d[a]	0±0c	0±0d
Rs26	3.84±0.07a	2.72±0.11b	1.91±0.06c
Rs94	2.59±0.04b	3.70±0.03a	3.80±0.04a
Rs13	2.02±0.01c	3.68±0.01a	3.83±0.05a
Rs57	1.90±0.02c	3.83±0.05a	3.55±0.14ab
Rs123	1.74±0.09c	3.59±0.06a	3.42±0.04b
P-value[b]	< 0.01	< 0.01	< 0.01

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at $P \leq 0.01$.
[b]Probabilities associated with individual F tests.

This research investigated the pathogenicity of various *R. solani* isolates, supporting intraspecific strain diversity within this fungus (Abdelghany *et al.*, 2022). All tested isolates exhibited virulence, causing varying degrees of damping-off and root rot diseases across all tested crops. These findings align with previous studies by Dubey *et al.* (2012), Erper *et al.* (2021), Abbas *et al.* (2023), and Yang *et al.* (2024), who demonstrated the pathogenicity of *R. solani* isolates from diverse sources on various crops. All isolates in these studies caused seed rot, pre-emergence damping-off, post-emergence damping-off, and root rot diseases, highlighting the broad host range of *R. solani* and its significant economic impact (Dubey *et al.*, 2012; Erper *et al.*, 2021; Abbas *et al.*, 2023; Yang *et al.*, 2024). Susceptibility to *R. solani* is potentially linked to the action of polygalacturonase enzymes, which might degrade plant cell wall pectate, facilitating infection (Naqvi *et al.*, 2024). *R. solani* typically targets seedling hypocotyls at the soil line before progressing downwards into the root system. Meristematic tissues in seedlings are particularly susceptible due to their vulnerability to the fungus' polygalacturonase. As these tissues mature, they gain resistance by converting pectin to calcium pectate, a form unaffected by *R. solani*'s enzymes. Additionally, increased cuticle thickness with maturity can limit exudation, hindering the formation of infection cushions by the fungus (Naqvi *et al.*, 2024).

Antagonistic action of antagonistic fungi toward *Rhizoctonia solani*

Table 2 showed into the potential application of four fungal antagonists (*T. harzianum*, *T. viride*, *Metarhizium* sp., and *Gliocladium* sp.) for controlling the growth of *R. solani*. The results revealed promising antifungal activity from all four antagonists. All fungal antagonists significantly inhibited the mycelial growth of most *Rhizoctonia* isolates compared to the control ($P < 0.05$). The inhibition by *T. harzianum* ranged from 83% for isolate Rs57 to 94.67% for isolate Rs26, demonstrating its broad-spectrum potential for *R. solani* control. Interestingly, the effectiveness of the other antagonistic fungi varied. While *T. viride* displayed consistent inhibition across all *R. solani* isolates (although statistically significant only for Rs26 and Rs123). *Metarhizium* sp. (65.57-75.57% for Rs26 and Rs94, respectively) also exhibited significant inhibition for most isolates, while *Gliocladium* sp. (51.35-66.75% for Rs13 and Rs26, respectively) showed a more variable effect, with significant suppression for some isolates but less consistent results for others. The variation in effectiveness among the antagonists and *Rhizoctonia* isolates suggests potential differences in their interactions (Table 2).

Table 2. Effect of four isolates of antagonistic fungi (*Trichoderma harzianum*, *T. viride*, *Metarhizium* sp., *Gliocladium* sp.) on mycelial growth of five isolates of *Rhizoctonia solani* (Rs26, Rs94, Rs13, Rs57, and Rs123) under laboratory conditions.

Treatments	***Trichoderma harzianum***	***T. viride***	***Metarhizium* sp.**	***Gliocladium* sp.**
Rs26	94.67±±0.92a[a]	89.55±1.01a	65.57±0.62b	66.75±1.86a
Rs94	90.67±0.67ab	86.46±0.96a	75.57±0.54a	53.51±1.19b
Rs13	91.80±0.21a	82.97±0.72a	75.17±0.38a	51.35±1.27b
Rs57	83±0.76b	83.60±1.17a	71.75±1.51ab	51.90±1.35b
Rs123	86.34±0.75ab	89.48±0.86a	74.11±0.69ab	59.78±1.73ab
P-value[b]	< 0.05	≥ 0.05	< 0.05	< 0.05

[a]Duncan's Multiple Range Test, values followed by various superscripts differ significantly at $P \leq 0.01$.
[b]Probabilities associated with individual F tests.

R. solani exhibited diverse symptomology depending on the host's phenological stage at infection. Early stages typically incur damping-off, while later stages may display necrosis and sclerotium formation. This soil-borne pathogen persists through saprotrophic decomposition of organic matter or spreads via infected host tissues through translocation processes (Dubey *et al.*, 2012; Erper *et al.*, 2021; Abbas *et al.*, 2023; Yang *et al.*, 2024). Lewis and Lumsden (2001) and Abbas *et al.* (2017) explored a biocontrol formulation for damping off in greenhouse pepper and cucumber, utilizing a mixture of vermiculite, powdered wheat bran, and biomass of *Trichoderma* spp. and *Gliocladium* spp. The study investigated the *in vivo* disease control efficacy of *T. harzianum* strain SQR-T37 and a bio-organic fertilizer (BIO) against *R. solani* in potted plants. Results suggest that mycoparasitism is the primary mechanism underlying SQR-T37's antagonistic activity. In one experiment, the presence of SQR-T37 significantly reduced the *R. solani* population in soil, from 10(6) to 10(4) internal transcribed spacer (ITS) copies per gram. This experiment achieved a 45% control efficiency with the application of 8 g SQR-T37 hyphae per gram of soil. A second experiment demonstrated superior control efficiency (81.82%) when using BIO compared to SQR-T37 alone (27.27% control efficiency with 4 g SQR-T37 hyphae per gram soil). These findings indicated that while SQR-T37 effectively suppresses *R. solani* populations through mycoparasitism, BIO application offers a more robust control strategy, potentially by stabilizing the antagonist population (Huang *et al.*, 2011). Kobori *et al.* (2015) highlighted the potential of fermenting *T. harzianum* cultures to generate microsclerotia and submerged conidia. Amending pots with dried microsclerotia from these cultures effectively reduced or eliminated damping-off in melon seedlings caused by *R. solani*. Furthermore, greenhouse experiments using *T. harzianum* at concentrations of 5 g/kg and 10 g/kg seed resulted in infection rates of 3.33 and 16.67%, respectively, compared to the untreated control group (30-40% infection). These findings collectively support the potential of *T. harzianum*, particularly when formulated in BIO, as a biocontrol agent against *R. solani*-induced damping-off disease (Ali and Taha, 2016).

The management of *R. solani* presented a significant challenge due to several factors. First, the fungus boasted a high sclerotia survival rate, allowing for long-term persistence in soil. Second, its extremely broad host range allowed it to infect a vast array of plants. Third, its ecological behavior makes control strategies difficult, as no known cultivars exhibit complete resistance (Behiry *et al.*, 2023). Consequently, control methods rely heavily on agronomic practices such as crop rotation. However, the polyphagous nature of some *R. solani* isolates can render crop rotation ineffective if commonly rotated crops are also susceptible. Broad-spectrum fungicides offer an alternative but pose environmental concerns due to their high toxicity (Matrood and Rhouma, 2021). Additionally, chemical controls may not always be economically viable for managing soil-borne pathogens. Therefore, biocontrol strategies emerge as a promising and eco-friendly approach for plant protection against *R. solani* (Rhouma *et al.,* 2024). Numerous studies demonstrated the efficacy of biological control using *Trichoderma* species, which not only suppress *R. solani* but also promote plant growth and stimulate defense responses (Mohamed *et al.,* 2020; Hajji-Hedfi *et al.*, 2023). *Trichoderma* spp. are a ubiquitous group of filamentous fungi, often found in high numbers within agricultural soils and decaying organic matter. These versatile fungi exhibit a range of lifestyles and interactions with *R. solani*, making them well-suited for biocontrol applications in plant disease management (Mohamed *et al.,* 2020).

Trichoderma species hold promise as biocontrol agents or biological fungicides for plant disease management. These fungi can exert their effects through both direct and indirect mechanisms. One approach involves the production of extracellular metabolites, including volatile and water-soluble compounds as well as low molecular weight secondary metabolites. For instance, a study identified a *T. viride* isolate as a prolific producer of inorganic phosphate, indole-3-acetic acid, and siderophores, all of which correlated with its strong antagonistic activity against *R. solani* (Lewis and Lumsden, 2001). *Trichoderma* spp. also possess proteins crucial for various functions, including the synthesis of deleterious secondary metabolites; completion and recognition processes; signal transduction; genetic reprogramming of gene expression; and mycoparasitism of *R. solani* (Hajji-Hedfi *et al.*, 2023).

Numerous studies have documented the efficacy of *Trichoderma* species against *R. solani* in diverse agricultural settings. For example, research has screened *T. viride* and *T. harzianum* strains for their ability to antagonize *R. solani*, the rice sheath blight pathogen. Biocontrol of *R. solani* in greenhouse and field tomatoes has been achieved using *T. harzianum*. Similarly, *T. asperellum* has been shown to control *R. solani*-induced rice sheath blight in tropical lowland rice ecosystems. Formulations containing various *Trichoderma* spp. have demonstrated success in controlling *R. solani*-mediated damping-off in greenhouse crops. Furthermore, *T. virens* exhibited promising results in controlling potato stem canker, suggesting potential for improved biocontrol efficacy with this species. A study investigating *Trichoderma* isolates from sugar beet fields harbouring *R. solani* disease patches identified *T. gamsii* strain T30 as the most antagonistic strain, exhibiting various mechanisms of antagonism. These collective findings highlight the broad applicability and diverse mechanisms employed by *Trichoderma* species in biocontrol strategies against *R. solani* (Lewis and Lumsden, 2001; Huang *et al.,* 2011; Kobori *et al.,* 2015; Ali and Taha, 2016; Abbas *et al.,* 2017; Ruiz-Cisneros *et al.,* 2018; Mohamed *et al.,* 2020; Shalaby *et al.,* 2022; Almaghasla *et al.,* 2023; Behiry *et al.,* 2023).

Trichoderma spp., particularly *T. asperellum* and *T. harzianum*, demonstrate biocontrol potential against *R. solani* through various mechanisms, including the production of water-soluble and volatile metabolites. A novel approach involves the use of liquid culture fermentation to generate microsclerotia and submerged conidia from *T. harzianum* strain T-22. Formulations containing these microsclerotia effectively reduced or eliminated damping-off in bean seedlings caused by *R. solani*. Furthermore, several *Trichoderma* species (*T. harzianum*, *T. viride*, *T. virens*, *T. hamatum*, *T. koningii*, and *T. pseudokoningii*) have been shown to control root rot disease in various crops caused by *R. solani*. Recent research has identified *T. saturnisporum* as a promising new biocontrol agent with potential applications against *R. solani*. Studies suggested that *T. harzianum*'s biocontrol activity is mediated by multiple mechanisms. These include the induction of plant defense-related genes in host plants and the production of high ergosterol levels, enabling faster growth in soil. This enhanced growth likely contributes to the positive effects observed on bean plant growth and defense against *R. solani*-induced root rot. Additionally, *Trichoderma* spp. are known to produce a diverse array of antibiotic substances, including gliotoxin, gliovirin, viridian, and trichoviridin, further contributing to their antagonistic activity against *R. solani* (Lewis and Lumsden, 2001; Huang *et al.,* 2011; Kobori *et al.,* 2015; Ali and Taha, 2016; Abbas *et al.*, 2017; Ruiz-Cisneros *et al.*, 2018; Mohamed *et al.,* 2020; Shalaby *et al.,* 2022; Almaghasla *et al.,* 2023; Behiry *et al.*, 2023).

Conclusions

Trichoderma harzianum has emerged as a promising candidate for biocontrol and biostimulant applications within the agricultural sector. This versatile fungus holds the potential to not only suppress plant diseases but also promote crop growth and yield. Prior research has established *T. harzianum*'s capacity to exert antifungal activity against the five isolates of *R. solani*. It is hypothesized that this inhibitory effect stems from the production of secondary metabolites by *T. harzianum*, which possess antibiotic properties. The current study delves deeper into elucidating the mechanisms employed by microbial biocontrol agents and biostimulants like *T. harzianum*, contributing to a more comprehensive understanding of their role in integrated plant disease management strategies.

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EFFECTIVENESS OF *PSEUDOMONAS* SP. CULTURE FILTRATE IN THE MANAGEMENT OF *ALTERNARIA SOLANI* CAUSING TOMATO EARLY BLIGHT

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Abstract

Climate change is exacerbating fluctuations in environmental conditions, leading to increased plant disease severity and risk in agricultural production. Tomatoes are particularly susceptible to the airborne fungal pathogen *Alternaria solani*, which causes early blight disease and significant yield losses. This study investigated the potential of *Pseudomonas* sp. culture filtrate as a biocontrol alternative to traditional pesticides for managing early blight in tomatoes. The antifungal activity of the filtrate was assessed using *in vitro* dual culture techniques and *in vivo* assay on tomato fruits. Laboratory experiments evaluated different concentrations (10, 20, and 40%) of the culture filtrate for their ability to inhibit *A. solani* mycelial growth, spore germination, and growth rate. Disease severity and lesion diameter were measured on tomato fruits to determine the filtrate's efficacy. The 40% concentration demonstrated the strongest antagonistic effect, significantly inhibiting mycelial growth (64.31%), spore germination (0.80 x 10^4 spores mL^{-1}), and mycelial growth rate (1.17 mm h^{-1}). Furthermore, *in vivo* application of the filtrate on tomato fruits led to a substantial reduction in disease severity (0.40) and lesion diameter (0.70 cm) compared to the positive control (4 and 5.15 cm, respectively). These findings suggest that *Pseudomonas* sp. culture filtrate has significant potential as an eco-friendly biofungicide for controlling early blight disease in stored tomatoes. This approach offers a valuable alternative to traditional chemical controls, which can have negative environmental consequences. Further research is warranted to investigate the efficacy of this biocontrol method under field conditions and explore its potential for broader application in tomato production.

Keywords: *Alternaria solani, Biological control, Early blight, Pseudomonas, Tomato.*

Introduction

Tomato (*Solanum lycopersicum* L.), the world's second most important vegetable crop (189 million tons produced in 2021), faces a significant challenge from over 200 potential diseases (Sallam *et al.,* 2023). Among these, early blight caused by the fungal pathogen *Alternaria solani* (Sorauer) is a major concern. *A. solani* readily invades tomato fruits through compromised tissues, such as wounds or areas of weakness (Sallam, 2011). This infection can occur in the field, during harvest, or even during post-harvest handling and storage. The high moisture content, abundant nutrients, and thin exocarp (outer skin) of tomato fruits make them particularly susceptible to colonization by *A. solani* (Adhikari *et al.,* 2017). Notably, ripe fruits are far more vulnerable compared to those at earlier developmental stages (Lanna-Filho *et al.,* 2017). Further complicating the issue, several *Alternaria* species are known to produce a diverse array of mycotoxins, including tentoxin, alternariol, altertoxin I, and tenuazonic acid. These mycotoxins can contaminate tomato fruits, posing a potential health risk to consumers as they exhibit carcinogenic, mutagenic, genotoxic, and cytotoxic effects in humans and animals (Chen *et al.,* 2021). Traditionally, early blight has been controlled by dipping tomato fruits in fungicides (Rhouma *et al.*, 2023). However, growing public concern regarding the negative impacts of synthetic pesticides has led to a preference for alternative management strategies (Eljounaidi *et al.,* 2016). One such approach involves the use of antagonistic microorganisms, particularly bacteria naturally present in soil and on the surfaces of vegetables and fruits (Fouda *et al.,* 2021). These bacteria offer a promising avenue for the control of postharvest diseases like early blight disease (Sallam, 2011).

Public health concerns and environmental considerations have fueled a global movement towards sustainable management strategies for plant diseases (Montealegre *et al.,* 2003; Grata, 2016). In this light, biological control agents are emerging as a viable alternative to synthetic chemical fungicides (Sharifi and Ryu, 2016). These bioagents exhibit antagonistic properties that target plant pathogens (Montealegre *et al.,* 2003). One promising approach involves inducing crop resistance using *Pseudomonas* species (Jayaraj *et al.*, 2007; Grata, 2016). Studies have demonstrated the potential of developing biocontrol products from *Pseudomonas* bacteria. These biocontrol treatments, often delivered in the form of *Pseudomonas* sp. culture filtrates, have proven effective against a wide range of plant pathogens in an eco-friendly manner (Sallam *et al.,* 2021; Sallam *et al.,* 2023). This strategy holds promise as a long-term solution for plant disease management, as evidenced by research by Kaur *et al.* (2016) who reported a 39-46% reduction in tomato early blight using a *Pseudomonas*-based formulation.

Arid environments like those found in Tunisia pose unique challenges for plant disease management. In this context, researchers are exploring the potential of harnessing native, habitat-specific antagonistic microorganisms for biological control of tomato early blight caused by *A. solani*. These locally adapted microbes may exhibit superior performance under prevailing climatic conditions compared to foreign strains. This study investigates this possibility through a two-step approach. First, the laboratory phase will evaluate the antagonistic activity of culture filtrates derived from *Pseudomonas* sp. *In vivo* assay will be employed to quantify the efficacy of these filtrates in inhibiting *A. solani* growth. Secondly, the study will assess the filtrates' impact on tomato fruit inoculated with *A. solani*.

Materials and Methods

In vitro antagonistic activity of *Pseudomonas* culture filtrate against *Alternaria solani*

An *in vitro* dual culture assay was employed to assess the antifungal activity of *Pseudomonas* sp. filtrates against the mycelial growth of *A. solani*. 25 mL aliquots of each filtrate concentration (10%, 20%, and 40%) were aseptically incorporated into separate flasks containing 200 mL of molten potato dextrose agar (PDA) medium. The bacterial filtrates were prepared by inoculating King's B medium with a loopful of a previously cultivated *Pseudomonas* sp. colony. These cultures were incubated in an electric shaker at 32°C and 200 rpm for 48 hours. Following incubation, the filtrates were harvested and stored in a refrigerator until used in the bioassay (Rhouma *et al.,* 2024).

Mycelial growth inhibition (MGI) of *A. solani* was evaluated 7 days post-inoculation using the following formula adapted from Rhouma *et al.* (2024): MGI (%) = (1 - Ce/Ct) x 100; where Ce represents the radial growth diameter of *A. solani* in the presence of the *Pseudomonas* sp. filtrate, and Ct represents the radial growth diameter of *A. solani* in the control plate lacking filtrate (Rhouma *et al.,* 2024).

Mycelial growth rate (MGR) of *A. solani* was determined using the following formula reported by Hajji-Hedfi *et al.* (2023): MGR (mm/h) = Σ [(Dn - Dn-1) / T_{en}]; where Dn represents the radial growth diameter of *A. solani* at day n (n = 1, 2, 3, 5, 6, and 7) after

inoculation, and T_{en} represents the corresponding incubation time at day n (Hajji-Hedfi *et al.*, 2023).
Spore production (SP) of *A. solani* was determined on the seventh day of incubation following a method adapted from Guzmán-de-Peña and Ruiz-Herrera (1997). Three mycelial plugs (Ø 8 mm) were collected from the central, intermediate, and peripheral regions of each colony. These plugs were transferred to test tubes containing 10 mL of 0.05% Tween 80 solution and homogenized using a vortex for 2 minutes. Spore counts were performed using a Neubauer chamber under light microscopy and expressed as spores per milliliter (spores mL^{-1}) (Guzmán-de-Peña and Ruiz-Herrera, 1997).

In vivo evaluation of *Pseudomonas* sp. and salicylic acid on tomato fruits inoculated with *Alternaria solani*

An *in vivo* bioassay was conducted to assess the antifungal activity of *Pseudomonas* sp. filtrates and salicylic acid against *A. solani* on tomato fruits. Uniform, ripe tomatoes (cv. Firenze) were harvested from a field in Sidi Bouzid, Tunisia, and disinfected with sterile water, 2.5% NaClO, and sterile water rinses under a sterile flow cabinet. Each fruit received a 5 mm wound on the blossom end. Treatments (20 µL each) included: T1 (negative control, sterile water), T2 (positive control, *A. solani* spore suspension, 10^6 spores mL^{-1}), T3 (40% *Pseudomonas* sp. filtrate + *A. solani*), and T4 (salicylic acid + *A. solani*). Following a 2-hour incubation, fruits were inoculated with 20 µL of the *A. solani* spore suspension (10^6 spores mL^{-1}). A randomized complete block design with three blocks of ten containers each was employed. Each container held an average of six fruits on sterile wet paper and was maintained in a sealed plastic bag (>90% humidity) within a growth chamber (21°C, 16 h light/8 h dark photoperiod) for seven days. The experiment was repeated twice. Disease severity (DS) (0-4 scale) and lesion diameter (LD) were evaluated after seven days to determine the efficacy of each treatment against *A. solani* infection (Matrood and Rhouma, 2021; Hajji-Hedfi *et al.*, 2023; Rhouma *et al.*, 2024).

Statistical analysis

Statistical analysis was performed using the mean values of the replicates. The data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA). Differences between treatments, homogeneity of variances, and normality were checked by applying Duncan’s Multiple Range Test. All statistical tests were performed with a significance level of 1% ($P \leq 0.01$).

Results and Discussion

In vitro antagonistic activity of *Pseudomonas* sp. culture filtrate against *Alternaria solani*

Table 1 showcased the effectiveness of *Pseudomonas* sp. culture filtrate in controlling *A. solani* and presented the impact of different filtrate concentrations (10, 20, and 40%) on MGI, MGR, and SP of *A. solani* under laboratory conditions. The results revealed a clear dose-dependent response ($P < 0.01$). All three concentrations of the filtrate significantly inhibited the growth of *A. solani* compared to the control where the fungus grew unrestricted. The highest concentration (40%) exhibited the strongest antifungal activity, achieving a remarkable 64.31% inhibition of mycelial growth. This translates to a significantly slower growth rate (1.17 mm h^{-1}) than control (2.16 mm h^{-1}). Furthermore, the culture filtrate significantly reduced spore production of *A. solani* ($P < 0.01$). At the highest concentration (40%), spore production was drastically reduced to 0.80 x 10^4 spores mL^{-1}, compared to a whopping 5.50 x 10^4 spores mL^{-1} in the control (Table 1).

Table 1. Effect of *Pseudomonas* sp. culture filtrate at different concentrations (10, 20, and 40%) on mycelial growth inhibition (MGI), mycelial growth rate (MGR), and spore production (SP) of *Alternaria solani* under laboratory conditions.

Treatments	**MGI (%)**	**MGR (mm h^{-1})**	**SP (10^4 spores mL^{-1})**
Pseudomonas sp. (10%) + *A. solani*	44.60±0.16c[a]	1.49±0.05b	2.44±0.04b
Pseudomonas sp. (20%) + *A. solani*	53.52±0.24b	1.35±0.04bc	2.22±0.01c
Pseudomonas sp. (40%) + *A. solani*	64.31±0.37a	1.17±0.02c	0.80±0.01d
A. solani	-	2.16±0.12a	5.50±0.14a
P-value[b]	< 0.01	< 0.01	< 0.01

[a] Duncan’s Multiple Range Test, values followed by various superscripts differ significantly at P ≤ 0.01.
[b] Probabilities associated with individual F tests.
Average values ± standard deviation.

In vivo* evaluation of *Pseudomonas* sp. and salicylic acid on tomato fruits inoculated with *Alternaria solani

Building upon the promising results from Table 1, Table 2 investigated the efficacy of *Pseudomonas* sp. culture filtrate (at 40% concentration) and salicylic acid in a more practical setting - *in vivo* on tomato fruits infected with *A. solani*. Table 2 evaluated the impact of these treatments on LD, a measure of disease progression, and DS, a broader indicator of plant health. Treatments significantly (P < 0.01) reduced disease severity compared to the positive control where *A. solani* caused extensive damage (lesion diameter of 5.15 cm and disease severity of 4). Notably, *Pseudomonas* sp. filtrate demonstrated superior disease control. It significantly reduced lesion diameter by over 86% (to a mere 0.70 cm) and disease severity by a remarkable 90% (to 0.40). This suggests the filtrate effectively restricts lesion formation and minimizes overall disease impact on the tomato fruit. Salicylic acid also provided significant disease control, with a reduction in lesion diameter of nearly 47% (2.73 cm) and disease severity of 58% (1.69) compared to the positive control. However, its effectiveness was considerably lower compared to the *Pseudomonas* sp. filtrate (Table 2).

Table 2. Effect of *Pseudomonas* sp. culture filtrate (40% concentration) and salicylic acid on lesion diameter (LD) and disease severity (DS) in tomato fruits inoculated with *Alternaria solani in vivo*.

Treatments	**LD (cm)**	**DS**
Negative control	0±0d[a]	0±0c
Positive control	5.15±0.11a	4±0a
Pseudomonas sp. (40%) + *A. solani*	0.70±0.03c	0.40±0.01c
Salicylic acid + *A. solani*	2.73±0.09b	1.69±0.02b
P-value[b]	< 0.01	< 0.01

[a] Duncan’s Multiple Range Test, values followed by various superscripts differ significantly at P ≤ 0.01.
[b] Probabilities associated with individual F tests.
Average values ± standard deviation.

Pseudomonas spp. have been shown to play a beneficial role in plant health. Mohamed et al. (2020), Fouda et al. (2021), and Sallam et al. (2023) suggested they can promote plant growth alongside disease resistance. Specifically, research by Sallam et al. (2023) identified bacterial isolates B4, B7, and B17 as particularly effective in inhibiting the growth of fungal pathogens A. solani, A. alternata, and Colletotrichum lunata compared to a control group. This implies these isolates may suppress these harmful fungi through mechanisms like producing compounds that hinder fungal development or competing with them for essential nutrients, as evidenced by Trung et al.'s work in (2022). In a study by Jayaraj et al. (2007), eight fluorescent Pseudomonas strains isolated from tomato rhizosphere demonstrated antifungal activity against Pythium aphanidermatum, exhibiting the highest growth inhibition zone of 15.5 mm and effectively controlling tomato damping-off by 68.5%. Pseudomonas spp. exhibit antifungal activity against various plant pathogens, potentially through a combination of mechanisms as explored by Sallam et al. (2023). These bacteria may produce lytic enzymes and/or secondary metabolites that degrade fungal cell walls, as suggested by Grata (2016). Sallam et al. (2023) investigated the inhibitory effects of different bacterial filtrate concentrations (20%, 40%, and 60%) against Alternaria spp. in vitro, finding that the highest concentration (60%) displayed the strongest growth reduction. This aligns with Sharma et al. (2021) who reported similar inhibition of A. solani mycelial growth using a Pseudomonas sp. culture filtrate at 40% concentration. These findings support the concept that Pseudomonas spp. harbor antifungal phytocompounds with broad-spectrum activity against plant pathogens. The source of this antimicrobial activity is likely antibiotics produced by these microorganisms, as evidenced by research from Kim et al. (2010) and Wang et al. (2010). Pseudomonas spp. have emerged as a promising source of novel and potent bioactive compounds with significant agricultural applications, particularly in plant disease management (Senthilkumar et al., 2007; Shahul Hameed et al., 2018; Sharifi et al., 2021). Studies by Soliman et al. (2022) identified large peaks in cell-free extracts of Pseudomonas strains, suggesting these bioactive compounds significantly contribute to their antifungal and antibacterial activities. Sallam et al. (2023) further pinpointed phenolic compounds as the most prominent secondary metabolites in endophytic bacterial cultures, potentially explaining their broad spectrum of bioactivities including antifungal properties, as phenolics are known for their diverse functions like antioxidants and antimicrobials (Negreiros de Carvalho et al., 2016). This aligns with Yu et al. (2002) who reported a wide range of antibiotics produced by Pseudomonas spp. and Bacillus spp., including oligomycin, phenazine, and pyoluteorin, which are known to possess antifungal properties. Additionally, Shahul Hameed et al. (2018) identified 24 bioactive compounds from P. fluorescens using GC-MS analysis, highlighting the diverse array of these molecules with potential roles in inhibiting fungal pathogens, as further supported by Montealegre et al. (2003). Overall, this body of research underscores the remarkable potential of Pseudomonas spp. to produce a wealth of bioactive compounds with significant antifungal activity, paving the way for their development as eco-friendly biocontrol agents in agriculture. Pseudomonas sp. combined with salicylic acid treatment demonstrated efficacy as a biological control agent against A. solani in fruits, reducing disease severity. This aligns with findings from Colla et al. (2015), Ruiz-Cisneros et al. (2018), and Hajji-Hedfi et al. (2023), who reported the effectiveness of Pseudomonas spp. against various tomato fruit pathogens (A. solani, Botrytis cinerea, Fusarium oxysporum, and Phytophthora infestans) as measured by multiple parameters including decay, firmness, and biochemical composition. Notably, Seo et al. (2014), Konappa et al. (2020), and Oszust et al. (2020) observed a synergistic effect when combining Pseudomonas spp. with Trichoderma spp. for enhanced postharvest disease control in tomatoes. The underlying mechanism for this resistance may involve the activation of the fruit's innate immune system by Pseudomonas spp., as evidenced by Konappa et al. (2020) and Oszust et al. (2020) who documented increased expression of defense-related enzymes (polyphenol oxidase, phenylalanine ammonia-lyase, and peroxidase) upon treatment. The activity of these enzymes potentially signifies the fruit's resistance to A. solani, with the level of increase depending on treatment type and fruit physiology. This aligns with the established concept that morphological and biochemical changes triggered by plant-pathogen interactions lead to the synthesis of defense chemicals that suppress or retard pathogen development (Gholamnezhad et al., 2016; Konappa et al., 2020).

Conclusions

Pseudomonas sp. holds promise for biocontrol and biostimulant applications in agriculture, potentially promoting crop growth and yield. Studies have shown that culture filtrates from *Pseudomonas* spp. exhibit antifungal activity against *A. solani*, a significant tomato pathogen. This inhibitory effect is likely attributed to the antibiotic properties of secondary metabolites produced by the microbes. This research contributes to a deeper understanding of the underlying mechanisms employed by microbial biocontrol agents and biostimulants in plant disease management.

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A REVIEW OF UTILIZING ENTOMOPATHOGENIC NEMATODES FOR CONTROLLING STORAGE PESTS

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Abstract

In agricultural fields, various factors contribute to crop yield losses. One of the most significant factors is the presence of agricultural pests. Pests can directly or indirectly damage crops leading to reduced productivity. This poses a significant concern for the sustainability of agriculture and the quality of agricultural products. Throughout many years, pesticides have been employed in the control of agricultural pests before and after harvest. However, recent concerns have focused on the toxic effects of pesticides on non-target organisms, particularly as pesticides are increasingly used to control pests in post-harvest storage, potentially exposing humans to pesticide residues. Consequently, restrictions have been implemented on the use of pesticides in the chemical control of pests on agricultural products both pre-harvest and post-harvest. This has highlighted the need for alternative methods, with biological control emerging as a prominent option. Among these methods, Entomopathogenic Nematodes (EPNs) are widely used for managing agricultural pests. However, EPNs are generally more effective under field conditions than in controlling pests in post-harvest storage conditions. Despite efforts to explore the potential of EPNs in controlling pests in storage facilities, progress has mainly been confined to laboratory studies. Therefore, the development of specialized EPN traps is considered crucial for effectively using EPNs in pest management in storage conditions, offering significant potential. Additionally, the authors involved in this study are actively engaged in developing a specific EPN trap.

Keywords: *Biological control, Entomopathogenic nematodes, Storage pests.*

Introduction

In agricultural fields, various factors contribute to yield losses in crops. One of the most significant factors is the presence of agricultural pests. In recent years, the European Union (EU) has imposed restrictions on the use of pesticides, which are primarily employed for controlling agricultural pests, due to their documented toxic effects on non-target organisms (Jayaraj *et al*., 2016; Robin and Marchand, 2019; Cestonaro *et al*., 2022; Dede *et al*., 2022). Particularly concerning are the residual problems these chemical control practices create on products, especially those directly entering the food consumption chain, notably through the potential control of warehouse pests under storage conditions (Carvalho, 2017; Schleiffer and Speiser, 2022). Consequently, these issues have become increasingly hazardous. Therefore, alternative methods to chemical control are supported by the EU as viable alternatives to control the pests (Robin and Marchand, 2019; Dede *et al*., 2022).
Entomopathogenic nematodes (EPNs) are widely used in biological control for the potential management of pests. These organisms belong to the Nematoda phylum, Rhabditida order, and Steinernematidae and Heterorhabditidae families, and they are obligate endoparasites (Dix *et al*., 1992; Glazer, 1996; Ehlers, 2001). Their life cycle consists of an egg stage, four juvenile stages, and an adult stage. These organisms can only infect their hosts during the third juvenile stage (Infective Juvenile or IJ) (Lewis *et al*., 2006; Koppenhöfer *et al*., 2007).

Spending nearly their entire lifecycle underground, they locate their hosts through volatile compounds released by the hosts, entering through natural openings like the mouth or anus, or through wounds on the host's body (Stock *et al*., 2002; Stuart *et al*., 2006; Susurluk and Ehlers, 2008). Once inside, they release gram-negative bacteria belonging to the Enterobacteriaceae family into the host tissues in a symbiotic relationship. These gram-negative bacteria vary at the family level for EPNs (Ehlers, 2001; Susurluk, 2008; Ulu and Susurluk, 2024). Species of the Heterorhabditidae family are symbiotic with *Photorhabdus* spp., while species of the Steinernematidae family are symbiotic with *Xenorhabdus* spp. After being released into the host tissues, these bacteria rapidly proliferate, causing septicemia (blood poisoning) in the host tissue, leading to its death (Park and Kim, 2000; Stuart *et al*., 2006; Ulu and Susurluk, 2014; Bütüner *et al*., 2024). As the host tissue dies and the gram-negative bacteria multiply, it becomes a suitable environment for EPNs to feed and complete their life cycle. Following the depletion of nutrients in the host tissue after 3-4 life cycles, the infective juveniles (IJ) of EPNs exit the host body to search for new hosts (Glazer, 1996; Ehlers, 2001; Lewis *et al*., 2006). Additionally, species of the Heterorhabditidae family cannot kill their hosts without the symbiotic bacteria, whereas species of the Steinernematidae family can kill their hosts without the symbiotic bacteria but cannot reproduce within the host tissue (Stuart *et al*., 2006; Ulu and Susurluk, 2014; Bütüner and Susurluk, 2023a).
Entomopathogenic nematodes are utilized for the potential control of numerous pests in agricultural fields (Dede et al., 2022; Gümüşsoy *et al*., 2022; Bütüner and Susurluk, 2023b; Bütüner *et al*., 2024; Elqdhy *et al*., 2024). However, the ability of EPNs to spend any stage of their life cycle in the soil facilitates easier encounters with their hosts in agricultural settings. In contrast, controlling pests in storage facilities presents greater challenges. Despite efforts to control storage pests using EPNs, these endeavors have largely been limited to laboratory conditions (Bütüner and Susurluk, 2023b; Yağcı *et al*., 2023; Bütüner *et al*., 2024; Yaraşır *et al*., 2024). Developing and utilizing machinery, equipment, or EPN traps that facilitate the application of EPNs in storage conditions is believed to be crucial for achieving significant results in the potential control of storage pests.

Recent Studies on the Efficacy of Entomopathogenic Nematodes for Controlling *Rhizopertha dominica* (Coleoptera: Bostrychidae)

Entomopathogenic nematodes have gained significant attention as a means of controlling storage pests in recent years. *Rhyzopertha dominica* Fab. (Coleoptera: Bostrychidae) is a significant pest in the context of grain storage, posing a substantial threat to various types of stored grains such as wheat, sorghum, rice, and corn across different regions worldwide. Both the adult beetles and their larvae play a crucial role in the degradation of stored grain quality due to their feeding activities. For instance, in a study conducted by Bütüner *et al*. (2024), varying concentrations of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) HBH hybrid strain, *Steinernema carpocapsae* (Rhabditida: Steinernematidae) TUR-S4 isolate, *Steinernema feltiae* (Rhabditida: Steinernematidae) TUR-S3, and S-Bilecik isolates were administered to adult *R. dominica*, resulting in mortality rates reaching as high as 93.33%. These underscores promising prospects for effectively managing *R. dominica* populations. In their study on the potential control of *R. dominica* larvae, Nawab and Javed (2023) utilized four different EPN isolates. Their findings revealed that certain isolates induced mortality rates approaching 100% in the larvae. These studies have carried out significant findings regarding the potential control of *R. dominica*.

Recent Studies on the Efficacy of Entomopathogenic Nematodes for Controlling *Sitophilus oryzae* (Coleoptera: Curculionidae)

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), is recognized as one of the most significant storage pests worldwide, commonly found in grain storage facilities. This pest is known to cause substantial yield losses in commodities such as rice, wheat, barley, and maize. The weevil particularly thrives in storage conditions with humidity levels ranging from 15% to 65%. In recent years, there have been a few studies focusing on the potential control of *S. oryzae*. Bütüner and Susurluk (2023b) evaluated the efficacy of *S. feltiae* TUR-S3 and *S. carpocapsae* TUR-S4 isolates for controlling adult *S. oryzae*. Their findings revealed mortality rates of up to 96.67% for *S. oryzae* across both species. Important results have been obtained in the potential control of *S. oryzae* through these studies. However, studies conducted recent years have been quite limited.

Recent Studies on the Efficacy of Entomopathogenic Nematodes for Controlling *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)

Oryzaephilus surinamensis L. (Coleoptera: Silvanidae) is a polyphagous pest worldwide that causes significant yield losses in stored products under storage conditions. This pest primarily infests and feeds on grains, dried fruits, and spices, leading to reductions in yield and quality. High humidity and warm environments positively influence its development. In recent years, there have been a few studies focusing on the potential control of *O. surinamensis*. In the study conducted by Yaraşır *et al*. (2024), an isolate of *S. feltiae* was evaluated for its effectiveness against adults of *O. surinamensis*. The results revealed mortality rates of up to 83.33% among the adults. This demonstrates promising results for the potential control of *O. surinamensis*.

Recent Studies on the Efficacy of Entomopathogenic Nematodes for Controlling *Tribolium confusum* (Coleoptera: Tenebrionidae)

Tribolium confusum du Val. (Coleoptera: Tenebrionidae) is a storage pest that feeds on a variety of products such as flour, grain, and dried fruits under storage conditions. Adults are brown in color and approximately 4-6 mm in length. Like many other storage pests, both larvae and adults of this species feed on stored products. Yağcı *et al*. (2023) evaluated different isolates of *S. feltiae*, *S. carpocapsae,* and *H. bacteriophora* at varying doses and temperatures against adult *T. confusum* in their study. The results showed mortality rates of up to 90% in treated adults, highlighting the efficacy of certain isolates. Similarly, the study conducted by Yaraşır *et al*. (2024) employed an isolate of *S. feltiae* to assess its efficacy against adult *T. confusum*. Upon analysis of the results, a mortality rate of 83.33% was determined among the adults. These studies have found important results in controlling *T. confusum*. However, there have been relatively few studies conducted in recent years.

Recent Studies on the Efficacy of Entomopathogenic Nematodes for Controlling *Trogoderma granarium* (Coleoptera: Dermestidae)

Trogoderma granarium Everts. (Coleoptera: Dermestidae) is a pest commonly found in storage conditions causing significant yield losses in stored products. This pest poses a serious threat in the storage of dry foods, damaging various products such as wheat, rice, barley, and dried fruits. Its larval stage is particularly threatening to stored commodities. In recent years, there have been a few studies focusing on the potential control of *T. granarium.* In the study conducted by Nawad and Javed (2023), isolates of *Steinernema pakistanense*, *S. bifurcatum*, *S. saimkayai*, and *S. abbasi* were evaluated for their efficacy against adults and larvae of *T. granarium*. The results indicated isolates that caused 100% mortality in larvae and isolates that caused 92% mortality in adults. These studies have found important results in controlling *T. granarium.*

Conclusion

The restriction on pesticide use has been implemented due to the determination of their toxic effects on non-target organisms, particularly those causing losses in stored products directly involved in the consumption chain (Robin and Marchand, 2019; Cestonaro *et al*., 2022; Dede *et al*., 2022). This has led to an emphasis on alternative methods to chemical control, highlighting biological control methods such as EPNs. However, the requirement for a host to pass any stage of its life cycle in the soil facilitates easier encounters between EPNs and hosts, typically feasible in agricultural production areas. In contrast, controlling storage pests poses greater challenges. While effective results have been achieved from studies aiming to control storage pests biologically, these studies have primarily been limited to laboratory settings. Developing and utilizing machinery, equipment, or EPN traps capable of facilitating the applicability of EPNs in storage conditions is considered crucial for achieving significant outcomes in controlling storage pests. Currently, the authors are working on developing an EPN trap specifically designed for potential control with pests under storage conditions.

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OLFACTORY RESPONSE OF AN IMPORTANT TURF PEST, DORCADION PSEUDOPREISSI BREUNING (COL.: CERAMBYCIDAE) TO DIFFERENT ESSENTIAL OILS

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Abstract

The longicorn beetle, *Dorcadion pseudopreissi* Breuning has become a major pest in turf areas in Turkey since 1996. Larvae of the pest feed on turf roots and cause economic damage in turf areas. Environmental hazards, human health and the resistance problems lead researchers to develop alternative methods to pesticides for pest control. In this study, we aimed to investigate olfactory response of *D. pseudopreissi* adults to different commercially available essential oils: lavender, lemon, clove leaf and grass. This study was conducted under laboratory conditions in 2024 at Bursa Uludag University, Turkey. The essential oil solutions were applied at 0.01, 0.1 and 1 µl /1ml doses. Essential oils were diluted with technical acetone. The trial was repeated three times for each doses of essential oils. 36 adult beetles were used for one essential oil test. Experiments were carried out by using Y-tube olfactometer to test whether *D. pseudopreissi* could discriminate plant oils volatiles versus clean air (control). The results showed that *D. pseudopreissi* adults did not show preference to clove leaf oil in low dose (25%), although, low dose of grass oil and lemon oil were attractive (75%) to adults. Also the adults were attracted to lemon oil (medium dose 63%) and grass oil (medium and high dose 64%). Lavender oil showed a 40 % preference for eachdoses. These oils could be potentialy used as attractants and repellents in the trapping systems in integrated pest management programs against *D. pseudopreissi*.

Keywords:, *repellent, Dorcadion pseudopreissi, Y- tube olfactometer.*

Introduction

Increasing urbanization causes negative effects on human life and can increase environmental pollution. The most important effect of rapid urban expansion is the decrease in green areas and the emergence of many environmental problems (Lai *et al*., 2016). In order to reduce the problems caused by this, programs towards healthy urbanization are required. Today, grassed areas constitute a significant part of green areas in many cities of the world (Stewart *et al*., 2009). According to the EU's latest study "Green Surge - A typology of urban green spaces, ecosystem supply services and demands" and green spaces are defined as "Vegetated areas found in the urban environment, including parks, forests, open spaces, lawns, residential gardens or street trees” (Maria *et al*. 2017). The functional benefits of grasses control soil erosion and improve the quality structure of the soil. The type of control is of crucial importance in eliminating dust and mud problems in living spaces (houses, factories, etc.) (Beard and Green, 1994).

Grasses play an important role in controlling climate (Johns and Beard, 1985) and have a superior capacity to capture and retain runoff, resulting in increased infiltration of water throughout the soil–grass ecosystem (Gross *et al*., 1990). Water and sediments from impervious surfaces in urban areas carry many pollutants, including metals such as Pb, Cd, Cu, and Zn, hydrocarbon compounds, and domestic and industrial hazardous wastes (Zhao *et al*., 2006). Turf serves as a low-cost cushioning surface for athletes in football, cricket,

hockey, rugby and many other games (Sithin *et al*., 2021). It provides surface for exercise and meditation in urban areas, which is very necessary to cope with stressful life. It also provides space for families to spend their free time.
Due to concerns over potential toxicity to non-target organisms and potential adverse environmental impacts to soil and water quality, turf research has focused on the fate of agri-chemicals applied to turf for controlling pests. Traditional Integrated Pest Management Bio-control and Best Management Practices have been in place in turf systems to help reduce pesticide application and improve efficacy with tangible impact.
Phytochemicals are the evolutionary-driven products of plant interactions with their environment and among those interactions, ecological relationships with insects are particularly influential on phytochemical diversity and the accumulation of specialized plant metabolites (Kerchev *et al*., 2012; Defossez *et al*., 2021). The nature of such ecological associations allows plants to biosynthesize sophisticated and frequently highly specific metabolites that are toxic and/or can significantly alter the behavior of different species of insects (Wink, 2018). Based on its influence on insects, botanical insecticides can be classified into six groups: repellents, feeding deterrents/antifeedants, toxicants, growth retardants, chemosterilants and attractants (Hikal *et al*., 2017).
It should be our goal to eliminate the negative effects on the environment by using sustainable methods in the management of weeds, diseases and pests. There are many insect pest on the grass and under the grass(white grabs, billbugs, crane flies, mole crickets, armyworms, cutworms, aphids, crickets, grasshopper, etc.) (Barret *et al*., 2014). The longicorn beetle, *Dorcadion pseudopreissi* Breuning, 1962 (Coleoptera: Cerambycidae) has become a major pest in turf areas in Turkey (Özdikmen, 2008). Whitish and legless larvae of the pest feed on turf roots and cause economic damage in turf areas (Kumral *et al*., 2012). It is reported that *Dorcadion* species reproduce every two years (Baur *et al*., 1997). Females lay eggs on the stems of narrow-leaved plants at the end of March (Fabbri and Hernandez, 1996). Adults emerging to the soil surface reach sexual maturity after 1 month (Baur *et al*., 2002). Pathogenicity, reproduction and foraging behaviours of some entomopathogenic nematodes and control potential of *Heterorhabditis bacteriophora* against a new turf pest, *D. pseudopreissi* were studied (Susurluk *et al*., 2009, 2010).
The aim of this study is to investigate the effects of some essential oils, which are environmentally friendly products, against *D.pseudopreissi*, an important grass pest.

Materials and Methods

Dorcadion pseudopreissi adults were collected in early April- early May 2024 from turf grass of Agriculture Faculty of Bursa Uludag University, Bursa, Turkey.
The common oils (clove leaf oil, lemon oil, lavender oil and grass oil obtained in pure form were purchased from the markets (Turkey). In the experiment, three different doses of each essentialoil were determined: 0.01, 0.1 and 1 µl /ml. Oils were prepared by dissolving in technical acetone (70%) (Demeter *et al*., 2021).
The responses of *D.pseudopreissi* to the essential oils were tested using two-choice tests with a closed system Y-tube olfactometer which was a slight modification of Takabayashi and Dicke (1992).
The adults of *D.pseuopreissi* were kept in a jar (14 x 10 cm) with grass for 2-4 day prior to the test. The experiments were carried out in laboratory conditions. First of all, 1ml of the prepared essential oil solution was dropped on the blotting paper and placed in the application compartment of the Y-tube olfactometer, and after waiting for 1 minute, *D.pseudopreissi* adult were released into the tube and monitored until it had walked at least 7 cm up one of the arms. The behavior of each individual was observed for a maximum of 5 min. For three

replicate for each essential oil dose, a total of 12 adult beetles were tested. Clean air on the one side arm was used as a control.

Differences in the proportions of *D.pseudopreissi* adults attraction by moving toward one of the essential oils or toward the control (clean air) were analyzed using the LS Means Student's t test.

Results and Discussion

The response of *D. pseudopreissi* to different essential oils and their different doses are shown in Figure 1. *D. pseudopreissi* adults showed significant preference for grass and lemon oils at low dose compared to control in three out of three replicates. Results showed significant preference for grass and lemon oils at low dose. *D. pseudopreissi* also show a preference for turf grass oil at medium and high dose and medium dose of lemon oil ($F(11,24)=76.39$, $p< 0.0001$) (Figure 1). In this experiment all essential oils were studied for the first time against *D. pseudopreissi* When grasses are cut or damaged, they rapidly release a volatile chemical cocktail called green leaf volatiles (GLV) (Watkins *et al*., 2006). Mayland *et al*. (1997) analyzed the headspace volatiles from freshly harvested samples of *Festuca arundinacea* (tall fescue) and found that, on average, 81.5% of the BVOCs consisted of (Z)-3-hexenyl acetate and 8.9% was (Z)-3-hexen-1-ol. For this reason, it is thought that the reason why *D. pseudopreissi* is attracted to the grass oil is its reaction to some volatiles of grass.

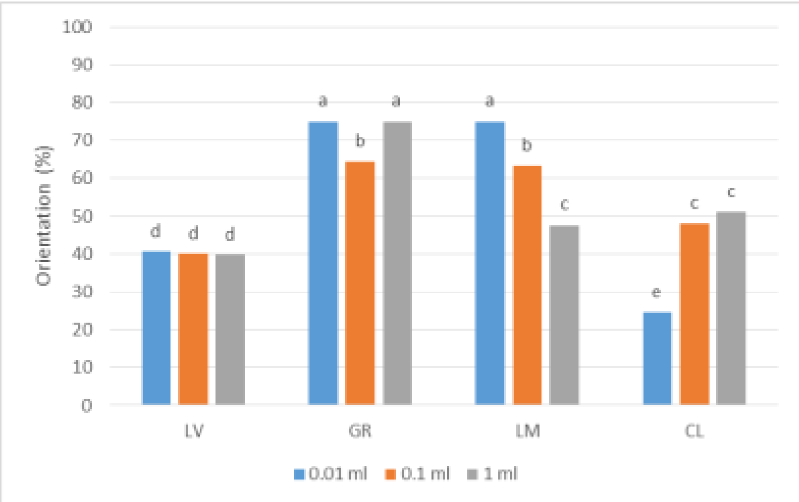

Figure 1. The response of *D. pseudopreissi* when offered 0.01,0.1,1 µl /ml doses of different essential oils (LV: Lavender oil, GR: Grass oil, LM: Lemon oil, CL: Clove Leaf oil) solutions

In this study lemon oil also show attractant effect at low and medium dose on *D. pseudopreissi*. Lemon is a citrus and its essential oil is a pale yellow, oily substance extracted from lemon peels. It contains various volatile components, such as terpenes, aldehydes, and lipids (Kaade *et al*., 2020).

Among the commercial essential oils evaluated in this study, grass and lemon essential oils attract *D. pseudopreissi* in Y-tube olfactometer. Also clove leaf oil repel *D. pseudopreissi*. Lemon and clove essential oils have previously shown insecticidal effects against different insects when applied via fumigation in petri dishes (Babarinde *et al*., 2020), but their attractant and repellent effects against *D.pseudopreissi* is shown here for the first time.

D.pseudopreissi didn't show preference to clove leaf oil at low dose (25%) in three replicates. Medium and high doses attract 48, 51% of the beetle. The main active ingredient in clove leaf oil is eugenol (80–88%) that was toxic effect against various insect pests such as mealworm beetle (*Tenebrio molitor*) (Martínez *et al*., 2018), and cowpea weevil (*Callosobruchus* *maculatus*) (Armijos *et al*., 2019). The other components are eugenol acetate and caryophylene (Nurdjannah and Bermawie, 2012). The contact repellent activity of clove part essential oils was tested and clove parts could be potential biopesticides, and clove stem essential oil could be the most effective alternative for rice weevil management (Bandara and Senevirathne, 2022). Clove essential oil exhibit repellence and toxicity effects against *Anopheles gambiae* and may be considered as a potential tool for controlling mosquito-human contact (Sanga *et al*., 2023).

D.pseudopreissi adults show the same preference at all doses of lavender oil (40%). The aromatic and medicinal properties of *Lavandula dentata* L. is considered as related to the presence of monoterpenes, both hydrocarbon and oxygenated. It is believed that these terpenes assign to *L. dentata* its antispasmodic, antimicrobial, and antioxidant activities (Martins *et al*., 2019). Mambri *et al*. (2018) stated that the essential oil of this species is mainly composed of monoterpenes, such as 1,8-cineole, camphor, borneol, fenchol, α-pinene, β-pinene, trans-pinocarveol, and linalool, among others.

In the present study, we conducted preliminary tests to show the potential attractant and repellent effects of different essentialoils against *D. pseudopreissi* in laboratory conditions, but the safety and effectivity of such products still need to be further studied in additional screenings for them to be considered for attractant and repellent formulations. However, our results offer interesting insights into the potential functions that determined metabolites in plant biotic interactions and identify promising candidate repellents and attractants for future studies focused on the development of plant-based chemicals.

Conclusions

The findings of this study showed that essential oils show both have attractants and repellence effects on *D. pseudopreissi*. Grass oil and lemon oil demonstrated the highest attractant effect while clove leaf oil showed repellent effect. The use of chemicals such as environmentally friendly essential oils in the fight against pests in lawn areas could have great benefits for human and environmental health. Therefore, more laboratory and field studies should be conducted to examine the full potential of essential oils in the control of *D. pseudopreissi*.

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ASSESSMENT OF NON-INTENTIONALLY ADDED SUBSTANCES MIGRATION FROM PLASTIC PACKAGING TO FOOD

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Abstract

Plastic packaging materials(PPMs) are emerging as the primary material used in food packaging due to their functional properties, convenience, and low costs. However, PPMs have a risk known as migration due to the diffusion of low molecular weight substances such as monomers and oligomers. These substances may include lubricants, plasticizers, stabilizers, UV absorbers, antioxidants, antistatic agents, colorants, optical brighteners, etc. These materials are referred to as intentionally added substances. However, the migration risk of PPMs may also include an impurity in the substances used, a reaction intermediate formed during the production process, or a decomposition or reaction product, which are known as non-intentionally added substances (NIAS). Many studies have confirmed that identifying NIAS is a challenging process. One of the main challenges is the complex structure of the material and the need for advanced analytical methods to identify NIAS. The difficulties encountered in determining NIAS have also led to various challenges in classifying NIAS. It has been observed that NIAS originates from degradation processes, by-products, impurities, and oligomers. For the risk assessment of NIAS, the most commonly used risk assessment tool is the threshold of toxicological concern (TTC) concept. Specific regulations are required for the identification and prevention of NIAS, and based on this information, the effects of plastic packaging use on human health worldwide should be reviewed. This review aims to identify potential sources of NIAS, classify NIAS, present the NIAS migration results especially in last years.

Keywords: *NIAS, plastic food packaging, food contact materials (FCMs).*

Introduction

Plastics are in high demand for food packaging due to their high processability, varied permeability, durability, flexibility, lightweight, and low costs. However, it is known that when plastics come into contact with food, they often undergo a chemical interaction, known as migration. Migration generally refers to the transfer of compounds from Food Contact Materials (FCM) to food through a mass transfer mechanism. The presence of monomers and oligomers within the material, due to incomplete polymerization processes, along with low molecular weight compounds and polymer degradation products, occur through molecular diffusion from the high-density region to the low-density region (Ibarra et al., 2019). The components that migrate from plastic to food may include monomers that remain in the structure of plastics, especially when they are not processed with appropriate conditions, and the additives added to facilitate production and to improve the properties of the product such as antioxidants, plasticizers, UV absorbers. Therefore, adding these substances has an intention and these substances are called "Intentionally Added Substances (IAS)". However, substances used in the manufacture of plastic materials or articles may contain impurities originating from their manufacturing or extraction process. These impurities are non-intentionally added together with the substance in the manufacture of the plastic material and these substances are called "Non-intentionally Added Substance (NIAS)".

Challenges About Identification of NIAS

The phrase NIAS is defined as "an impurity in the substances used, a reaction intermediate formed during the production process, or a decomposition or reaction product" under Article 3 of Regulation EU 10/2011 (EU, 2011). The NIAS present in packaging must be controlled according to regulations; however, these NIAS may include unauthorized substances under EU legislation. In this case, the manufacturer should evaluate potential health risks from NIAS in the final product according to internationally recognized scientific risk assessment guidelines. However, identifying NIAS is quite challenging, and several explanations can help to clarify these challenges (Nerin et al.,2013).

Firstly, their complex structures often involve multiple layers with undisclosed compositions, including adhesives and polymers. Secondly, custom-made additives like stabilizers and antioxidants further complicate identification, lacking specific EU regulations. Lastly, detecting NIAS in migration tests requires advanced analytical methods like Mass Spectrometry, yet identifying these substances remains time-consuming and dependent on spectral libraries (Nerin et al., 2013; Ibarra et al., 2019).

The challenges in identifying NIAS have caused the development of different classification categories. Advanced analysis methods have identified many NIAS, but not all have undergone risk assessment. Some NIAS detected through chemical analysis remain unidentified and, thus not assessed for risk. Predictable NIAS, based on chemical processes and manufacturer experience, can be targeted for detection using known compound lists. However, many NIAS are unpredictable and may require untargeted chemical analyses or remain undetected (Kato and Conte-Junior, 2021; Geueke, 2018). Non-target screening is valuable for understanding FCMs completely but is challenging without knowledge of the origins of unknown substances (Su et al., 2019).

Origin of NIAS

Understanding the sources of NIAS is crucial for controlling their formation in FCMs. NIAS can originate from various sources as given below.

By-Product Compounds: These compounds, also known as "neo formed" compounds, can arise during the polymerization reaction of starting substances but also during production stages of the plastic materials. Nevertheless, it is currently difficult to predict each potential side product that could remain in the finished material due to the large variety of starting materials needed to manufacture FCMs and the complexity of production procedures (Kato and Conte-Junior, 2021; Nerin et al., 2013).

One of the most important compounds of this category are oligomers. Although oligomers can also intentionally be used as “prepolymers”, when polymerization is not completed thoroughly or polymer chains break down thermally or hydrolytically during (re)processing of the polymeric material and under use conditions, non-intentional oligomers are chemically formed especially those with molecular weights below 1000 Da. In both cases proper risk assessment is necessary for both intentionally and non-intentionally produced oligomers below 1000 Da in order to make certain the safe usage of packaging materials (Koster et al.,2015)**.** The oligomer pattern that results can be extremely complex, with species that are linear, branching, and cyclic, each of which may have unique toxicological profiles and migrating characteristics (Hoppe et al.,2016). Although their presence is usually known to the manufacturer, the risk assessment of oligomeric mixtures is challenging, because of their complex composition.

Other reaction products that adversely affect consumer health are the primary aromatic amines (PAAs) known as suspected to be carcinogenic compounds. PAAs are formed by polyols and diisocyanate monomers polymerization during the production of polyurethane adhesives commonly used in food contact multilayer laminates. Adhesives are generally not directly exposed to food, but it has been revealed that their constituent compounds can diffuse through the laminated films and potentially migrate into the packaged food (Yan et al., 2020). Therefore, the primary source of PAAs is the residual (unreacted) isocyanic monomers that migrate through the inner layer of laminates, mostly composed of low-density polyethylene (LDPE) or cast polypropylene (C-PP), from the PU adhesive to the surface of the laminate. If the adhesive hasn't fully cured or the ingredients aren't properly mixed, the polymerization reaction will be inefficient, leaving unreacted aromatic isocyanates that can form primary aromatic amines upon contact with water (Kato and Conte-Junior, 2021; Campanella et al., 2015; Nerin et al., 2013).

Degradation compounds: Degradation processes are one of the main reasons for the formation of NIAS. These processes can occur in the polymer as well as in the additives used to enhance packaging properties. The degradation products can result from high temperatures used in the polymerization process as well as from the use conditions of the plastic materials such as microwave applications and radiation process (Kato and Conte-Junior, 2021; Geueke, 2018; Nerin et al.,2013). In addition, other factors such as light exposure, oxidative damage, or chemicals also contribute to the degradation of polymers. These processes can lead to changes in the physicochemical properties of the polymer and cause the oxidation of additives such as antioxidants and light stabilizers (Kato and Conte-Junior,2021). The changes that occur during degradation processes lead to the formation of new molecules with lower molecular weight. These newly formed molecules have higher diffusion coefficients and, consequently, a higher potential for migration, resulting in the transfer from the packaging to the food (Geueke, 2018).
Many studies have highlighted NIAS resulting from polymer degradation processes. Most of these are related to carbonyl compounds such as formaldehyde and acetaldehyde which is generated from the thermal degradation of polyethylene terephthalate (PET) (Kızılırmak Esmer, 2009; Kızılırmak Esmer, 2004). Irganox 1010 and Irgafos 168 are commonly used antioxidants and have been the most widely studied for NIAS as a result of degradation (Kato and Conte-Junior, 2021; Nerin et al.,2013).

Impurities: Impurities coming from the raw materials or additives used in the polymer synthesis process is another frequent cause of NIAS development (Nerin et al., 2013). Although some of these impurities are known by manufacturers, minor impurities are not described in the information datasheets of the starting substances and can remain in the final FCMs, having the potential to migrate into the foodstuffs. Polymer starting materials, additives, and other materials necessary to produce FCMs such as solvents, and ink may include impurities because they are typically of technical grade. According to European regulations, FCMs must be produced with good manufacturing practices, and starting materials that satisfy pre-established requirements (Kato and Conte-Junior, 2021; Geueke, 2018)

Contaminants: The potential migration of NIAS resulting from contamination of food packaging is particularly relevant when recycled materials are used (Nerin et al.,2013). FCMs manufactured from recycled materials are critical to the circular economy, but they raise unique concerns about possible NIAS contamination from a variety of sources. First, recycling streams may contain non-food grade materials, adding undesired contaminants to the recovered product. Second, incompatible materials such as adhesives, printing inks, and coatings might impair recycling operations. Third, plastics can change their physicochemical qualities and produce degradation products when used and recycled. Fourth, food components absorbed by the FCM, as well as chemical residues of process and consumer abuse, can all contribute to NIAS **(**Geueke, 2018).

Migration of NIAS

The presence of NIAS has been determined mostly in multilayer packaging materials, PU adhesives, materials such as PP, PET, LDPE, HDPE, or recycled materials. Degradation products are the main source of NIAS in plastic FCMs, primarily originating from antioxidants like Irganox 1010 and Irgafos 168, followed by oligomers and side reaction products.

For the safety assessment of plastic food packaging materials, the relevance of specific migration limits set by EU 10/2011 should be determined. Then the threshold of toxicological concern (TTC) should be evaluated. However, the challenge is that most NIAS compounds are not explicitly listed in EU regulations. Therefore, the TTC based on a decision-tree procedure with recently available toxicological data regarding the compound's estimated daily intake (EDI) rather than SML should be alternatively applied. The Cramer decision tree classifies organic chemicals into one of three classes (I – low, II – intermediate, and III – high) reflecting the probability of low, moderate, and high toxicity explicitly. The TTC values for Cramer Classes I, II, and III are 30 µg/kg bw per day, 9 µg/kg bw per day, and 1.5 µg/kg bw per day, respectively (EFSA, 2019). In recent years, studies on NIAS in FCMs have significantly increased. Research on the migration of NIAS into food and their potential health effects has garnered substantial interest in food safety. Notably, some studies have shown that the migration of NIAS into food can yield unexpected and striking results, as seen in Table 1.

Table 1. Studies on NIAS migration from food packaging materials into food/food simulants

Material	**Food simulants**	**Evaluation of NIAS**	**Reference**
PE (Polyethylene) breast milk bags	Milk and 50% (v/v) ethanol	The primary migrant compounds have been identified as the degradation products of the antioxidant Irgafos 168. Among these, 1,3-di-tert-butylbenzene exceeded the maximum recommended migration values in two out of the eight bags studied.	Aznar et al., 2023
Three-layered PET/Al/PE packaging material for baby food	3% (w/v) acetic acid and 20% (v/v) ethanol	Thirty-nine NIAS identified in migration analysis including eight NIAS detected for the first time. These NIAS mainly originated from polyurethane layer. Twenty-nine of the identified NIAS were cyclic oligomers and classified as high toxicity according to the Cramer rules. The chemicals detected were similar in both food simulants and baby food.	Bauer et al., 2019
Polyurethane adhesives used in champagne cork stoppers	Champagne, 3% (w/v) acetic acid and 20% (v/v) ethanol	Twelve neo-formed compounds, including amines, amides, and urethanes were identified both in simulants and champagne, resulting from the reaction between acetic acid or ethanol with residual monomers from PU adhesives (diisocyanates) in simulants and between organic acids in champagne and these residual monomers. Migration levels ranged from 70 to 721 µg/kg, and most of them exceeding the specific migration limit of 90 µg/kg	Canellas et al., 2021

		established for Cramer class III compounds.	
Different ratios of virgin (vPET) and recycled PET (rPET), varying from 0% to 100% w/w content using single and twin-screw extruder	50% (v/v) ethanol	PET cyclic oligomers [TPA-EG]2, [TPA-EG]3, and [TPA-EG]2-EG were found in the highest concentrations. It was found that, in a worst-case scenario (40°C/10 days), only [TPA-EG]3 and [TPA-EG]2-EG might pose a potential risk, even with an over-conservative consumption scenario. Differences in cyclic oligomer content were observed between vPET and rPET, with single-screw extrusion showing higher oligomer levels as more rPET was incorporated into the plastic compared to twin-screw extrusion..	Colombo et al., 2024
Vacuum bags (PP/PA and HDPE/PA) for sous vide cooking	10% (v/v) ethanol, , 3% (w/v) acetic acid, ethanol 95% and isooctane	A wide variety of NIAS, originating from degradation processes and contaminants, have been detected, many of which were generated during cooking. In total, twenty-four volatile compounds and four non-volatile compounds were found.	Estremera et al., 2024
Plastic corks, made of ethylene–vinyl acetate (EVA), used to seal wine bottles	3% (w/v) acetic acid and 20% (v/v) ethanol	Fifty compounds were observed to migrate from the corks, including additives like antioxidants and lubricants. A significant proportion (84%) of the detected compounds were NIAS, featuring several cyclic oligomers with various chain sequences. Potential chemical impurities, such as a pair of ethylene-bonded amides, were also identified. One cork was deemed unsuitable for use as a food contact material.	Vera et al., 2022
Microwavable plastic food containers (MPFCs) made of PP, SAN (styrene-acrylonitrile copolyester) or Tritan	Distilled water, 3% acetic acid, 50% ethanol and 95% ethanol.	Over 100 NIAS were identified, with 95% ethanol resulting in higher amounts of NIAS compared to other simulants. NIAS migration gradually decreased with repeated use. Some Cramer class III toxic compounds, including PEG oligomers of N,N-bis(2-hydroxyethyl) alkyl (C8–C18)amines, hexadecanamide and oleamide isomers, and Irgafos 168 OXO, were detected and exceeded the recommended limits in some MPFCs.	He et al., 2021

Conclusion

Apart from IAS, it is evident that NIAS originating from various sources can inevitably be found in food packaging materials. While known and listed NIAS can be detected through targeted chemical analysis, many NIAS remain unpredictable. These substances may be detectable through untargeted chemical analyses or may not be detected at all, especially without knowing the origin of the unknowns. Moreover, studies have shown that it is crucial to not only identify the presence of NIAS in packaging materials but also to investigate their migration into food and to evaluate these compounds toxicologically for potential adverse health effects on humans. Thus, for good manufacturing and safety of plastic food packaging materials, further studies are needed.

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EXTENDING THE POSTHARVEST QUALITY OF MINIMALLY PROCESSED (SLICED) PEACHES BY POSTHARVEST ASCORBIC ACID IMMERSION AND CHITOSAN COATING TREATMENTS

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Abstract

The effect of postharvest 1% ascorbic acid (AA) dipping, 1% chitosan (Ch) coating and their combined application on physiological and biochemical features of minimally processed fresh-cut) peach fruits was investigated during 16 d storage at 4.0±0.5 °C and 90% relative humidity. Remarkable loses in weight of peach fruits occurred from the beginning of the storage duration as expected due to its necessarily perishable fruit tissue. However, both AA and Ch treatments significantly delayed the weight loss incidence although their combined use had the greatest effect. Similar effects of the treatments on fruit firmness were also investigated. Visual quality of peach fruits were superior when they were coated with Ch after AA dipping. Peach fruits showed gradual increase in soluble solid content (SSC) because of their postharvest climacteric physiology. However, both AA and Ch were significantly effective on delaying in increase of SSC with the greatest effect of their combined use. AA remarkably delayed the fruit browning while Ch effectively restricted the weight loss in peaches. Changes in fruit acidity was also restricted by the treatments. Results revealed that combined use of AA and Ch could be recommended for a successful cold storage of minimally processed (sliced) fresh peach fruits.

Keywords: *Peach, fresh-cut, cold storage, ascorbic acid, edible coating, quality extension.*

Introduction

Interest in fresh cut fruits and vegetables have increased due to ready to eat and maintain to fresh like quality. However this produces are more perishable than intact raw materials due to preparation process such as slicing, peeling or shredding results in increased respiration ratio and ethylene production (Velderrain-Rodríguez et al., 2019). This may cause cut surface browning, tissue softening, decreased nutritional value, off-flavor development and microbiological spoilage during storage (Gunes and Lee, 1997; Ma et al., 2017).
Enzymatic browning (surface browning) is one of the factors limiting the shelf life and quality of fresh-cut fruits and vegetables. Operations such as peeling and slicing induce discoloration of surface due to the action of a group of enzymes called polyphenol oxidases (PPO) and enzymatic browning caused by oxidation of phenolic compounds by polyphenol oxidases in the presence of oxygen (Gunes and Lee, 1997; Toivonen and Brummell, 2008). Alone or combine use of certain postharvest physical and chemical methods significantly prevent enzymatic browning. Physical methods are including reduction of temperature or oxygen amount, modified atmosphere packaging or edible film coating and gamma irrigation.
Edible coatings are defined as a thin layer of edible material applied directly on the surface of fresh-cut fruit that can reduce moisture loss, solute migration, respiration and oxidative reactions and retard the natural physiological ripening process (Chiabrando and Giacalone, 2016). The characteristics of the material strongly influence the effectiveness of the coating; thus, biopolymer component should be selected according to physical and chemical properties in consideration (Sharma et al., 2018). Chitosan obtained by the deacetylation of chitin [poly-

b-(1e4)-N-acetyl-D-glucosamine], as a high-molecular polymer, nontoxic, bioactive agent, has become an appreciated compound due to its fungicidal effects and elicitation of defence mechanisms in plant tissues (Sabir et al., 2019).
Although sulfites have been commonly used as chemical application in controlling browning, this chemical was prohibited by FDA in 1986 due to potential health risks posed to sensitive consumers (Garcia and Barrett, 2002). To prevent surface browning, ascorbic acid, citric acid, L-cysteine, 4-hexylresorcinol (4-HR) or N-acetylcysteine are used as alternatives for sulfate (Gunes and Lee, 1997). These substances for browning preventing are used in not harmful limits for human health (Sabır et al., 2011). Ascorbic acid and its derivatives are used effectively in delaying enzymatic browning in many fresh cut fruits and vegetables at concentrations ranging from 0.5% to 4.0% (Sabir, 2017).
The aim of the present study was to evaluate the effectiveness of chitosan and ascorbic acid treatments alone or in combination to on preventing browning and maintaining slice quality in peach *cv* J.H. Hale.

Material and Method

In the study, peach (*Prunus persica*) fruits *cv*. ‘J. H. Hale’ were harvested at commercial mature stage and selected for their health. Then they were immediately transported to Postharvest Laboratory of Horticulture Department, Selcuk University and sorted to obtain homogeneous batches based on fruit color and size. Peaches were washed in tap water then were manually cored and then sliced longitudinally into 8 equal pieces using a stainless steel handheld slicer. Sliced fruits were randomly distributed into four groups and immersed in the treatment solutions for 5 minutes. Treatment solutions were control (distilled water), 1% ascorbic acid (AA), 1% chitosan (Ch) and 1% chitosan + 1% ascorbic acid (Ch+AA). Slices were placed in polystyrene trays and were hand-wrapped with a 20 µm stretch film. Packaged samples were stored at 4.0±0.5 °C and 90% relative humidity for 16 days and sampling was carried on 0, 4,8, 12 and 16 th days of storage.
Weight loss (WL) was determined by weighing the packages at the beginning of the experiment (M_i) and at days 4, 8,12 and 16 (M_s) during storage. WL was calculated by the fromula WL (%) = ([Mi–Ms]/Mi)×100 and values are reported as a percent of weight. Slice color was measured by reading L* a* and b* values on the opposite surfaces of 6 slices from each plate during storage using a colorimeter (Minolta CR 400). Hue angle (h°) values were calculated to determine color changes (McGuire, 1992). The browning index (BI), which is used as an indicator of browning in sugar containing products, was calculated using the following equation. BI= [100 x (X-0.31) / 017]; X= [(a*+ 1.75 x L*) / (5.645x L* + a*- 3.012 x b*)] (Perez-Gago et al., 2006; Putnik et al., 2017). Firmness of slices was measured using a digital penetrometer in opposed side of slices midpoint and results expressed as Newton (N). Visiual quality was evaluated on a scale of 1-9 by panelists at analysis periods (9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1, poor,inedible) (Koukounaras et al., 2008).
Soluble solid content (SSC) was determined by extracting the juice from the slices in each treatment and measuring it with a hand refractometer. Titratable acidity (TA) was determined by titration of fruit juice with 0.1 N NaOH to reach a pH of 8.1 and expressed as % malic acid. Fruit extracts for total phenol content (TPC) and total antioxidant activity (TAA) were prepared using method described by Thaipong et al. (2006) with some modifications. Five grams of peach tissue was homogenized in 25 mL methanol using the Ultra-Turrax homogenizer (IKA, T18 digital, Staufen, Germany) for 5 min. The homogenates were kept at 4 °C for 14–16 h and then centrifuged at 8000 x g for 15 min at 5 °C. The supernatants were recovered and stored at -20 °C in dark color bottles until analysis. TPC were determined

according to the method of Singleton et al. (1999) with slight modifica-tions. The 0.1 mL extract, 6.0 ml distilled water and 0.5 ml Folin-Ciocalteu reagent were mixed and vortexed. The mixture were incubated for 3 min and then 20% sodium carbonate solution was added and volume was made up to 10 ml by adding distilled water. The solution was incubated at room temperature for 2 h and the absorbance was measured at 760 nm. TPC was calculated on the basis of the calibration curve of gallic acid and was expressed as mg/100g. TAA was determined by the ferric reducing ability antioxidant power (FRAP) according to the procedure described by Benzie and Strain (1996). For this, 150 µL of extract and 2.85 mL of the FRAP reagent was incubated at 30 °C for 30 min. After incubation, reaction mixture was measured at 593 nm on a UV-vis spectrophotometer. Standard curve was prepared using different concentrations of 1 mM trolox and expressed as µmol/kg.

The experiment was a completely design with three replication and each replication contained 10 slices. Data from analyzed parameters were subject to variance analysis. Source of variations were treatments, storage days and their interaction. Means were compared by Student’s t-tests at $p \leq 0.05$, using JMP statistical software, version 5.1 (SAS Institute Inc, Cary, NC, USA).

Results and Discussion

The effects of ascorbic acid (AA)and chitosan (Ch) treatments on weight loss of fresh cut peach slices during storage is presented in Figure 1A. The weight loss of slices increased during prolonged storage and treated slices indicated lower weight loss compared with control. In control slices, weight loss sharply increased at 8 days of storage and this increase reached 1.95% at the end of storage. Treated slices indicated lower weight loss with the valus of 1.24% (Ch+AA), 1.38% (Ch) and 1.50% (AA) at 16 days. This finding was in agreement with a report of Kumar et al. (2018) and Özdemir and Gökmen (2017) who reported that the incorporation of anti-browning agents as edible coatings had the lowest weight loss in minimally processed apples and pomegranate. They further suggested that commercial weight loss limit is 4-6% for minimally processed fruits. The weight loss values recorded in this study were under this commercial limits during storage time.

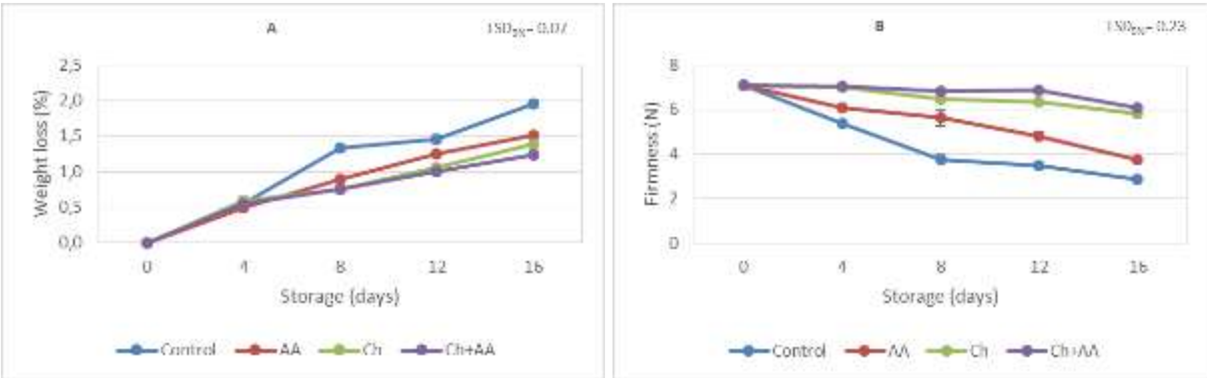

Figure 1. The effects of ascorbic acid and chitosan treatments on weight loss of fresh cut peach slices during storage.

Loss in firmness of both intact or fresh-cut peach fruits affect shelf life and visual or eating quality. The effects of treatments on fruit firmness was statistically significant during storage as illustrated in Figure 1B. Firmness of the slices gradually decreased during the prolonged storage. However chitosan treatments, when used alone or combination with AA, significantly maintained the slice firmness in comparison to control. Initial firmness value of slices were 7.08 N. At the end of the storage period, the highest firmness value was obtained from

Ch+AA treatment (6.09 N), followed by Ch and AA treatments (5.80 and 3.74 N, respectively). On the other hand, slices of control showed the lowest firmness value (2.86 N). Therefore, chitosan treatment might have created an effective thin film on the surface of the slice and slowed down the loss of water. Similar positive effects of chitosan treatment on firmness maintenance during storage was previously reported for fresh-cut banana (Mirshekari et al., 2017).
As presented in Table 1, L* and hue angle values gradually decreased regardless of the storage duration and treatments. The greatest decrease in L* and hue angle occured in control slices along with the storage duration. At the end of the storage, all of the treatments significantly delayed the changes in L* and hue values in comparison to the control. At 16th day, hue angle values ranged from 66.92° (control) to 72.70° (Ch+AA) and combine treatment was the most effective treatment during cold storage.
Browning index (BI) values of slices increased in all treatments during storage and this increases occurred dramatically fort the first four days in especially control slices (Table 1). BI value calculated at 0 day was 100.22. During storage, the highest increase occurred in control slices. All over the storage period, ascorbic acid, chitosan and their combinations effectively prevented the browning in slices compared with control. At the end of storage, the lowest BI value was obtained from Ch+AA (114.66), followed by AA (116.59) and Ch (117.67) treatments. The highest browning was occurred in control slices (124.25). Enzymatic broning is a prime quality parameter indicating the success of postharvest treatment. Positive effects of AA and Ch indicated that these substances could be recommended as antibrowning agent for fresh cut sector (Sabir, 2017).

Table 1. Changes in L*, hue angle and BI values of slices throughout the storage period.

	Treatments	Storage time (days)				
		0	4	8	12	16
L*	Control	67.51 a	62.77 def	57.81 i	53.89 j	52.22 j
	AA		64.64 bcd	61.36 fgh	61.61 e-h	60.11 h
	Ch		65.43 bc	62.95 def	62.19 efg	60.74 gh
	Ch+AA		66.13 ab	65.76 ab	63.52 cde	62.55 efg
Hue angle	Control	74.13 bc	73.87 bcd	72.80 cde	69.25 f	66.92 g
	AA		75.83 a	73.69 bcd	72.92 cde	71.67 e
	Ch		74.45 ab	74.12 bc	72.51 de	71.79 e
	Ch+AA		74.68 ab	74.06 bc	73.38 bcd	72.70 cde
BI	Control	100.22 j	110.16 e-i	116.61 cd	123.65 ab	124.25 a
	AA		106.81 hi	110.87 d-i	113.32 c-g	116.59 cd
	Ch		108.34 ghi	112.96 c-h	116.38 cde	117.67 bc
	Ch+AA		106.47 i	109.81 f-i	111.05 d-i	114.66 c-f

LSD for L^*: 1.96; Hue angle: 1.52; BI:6.25. Means of triplicate measurements are presented and data with different letters are significantly different $P \leq 0.05$.

Changes in soluble solid content (SSC), titratable acidity (TA), total phenolic content (TPC) and total antioxidant activity (TAA) values of peach slices in response to the different treatments during the prolonged cold storage are shown in Table 2. Generally, SSC underwent a slight but insignificant increase through the storage. For TA, which is one of the most effective biochemical components in determining the storage success of horticultural commodities, the espicially coating treatment provided more stable changes, delaying the senescence of slices. The TA content of the must gradually decreased during the cold storage. The greatest and the lowest TA values were observed in Ch+AA (0.78%) treatment and in control (0.56%) at day 16 after storage, respectively.
Changes in TPC of slices in response to the different treatments during the prolonged cold storage was shown in Table 2. TPC at harvest was 93.58 mg/100g, while it ranged from 91.08 mg/100g (Ch) to 120.25 mg/100g (control) at 16th day of storage. In general, a slight increase

occurred in TPC during the storage, but the effect of treatments on this decrease was insignificant. TAA was significantly affected by the treatments. Loss in TAA was easily noticeable in control fruits throughout the 16 day storage period, while all treatments significantly restricted the decrease in TAA during the storage. At the end of the 16 day cold storage, the highest TAA was determined in fruits treated with Ch+AA (3.88 µmol/kg), followed by those treated with Ch (3.34 µmol/kg) and AA (3.29 µmol/kg), respectively. These findings can be explained by the influence of chitosan treatment in slowing the ripening and effectively restricting the metabolic activity.

Table 2. Changes in SSC, TA, TPC and TAA of slices throughout the storage period.

	Treatments	Storage time (days)				
		0	4	8	12	16
SSC	Control	12.07	12.4	12.67	13.00	13.13
	AA		12.67	12.33	12.67	12.93
	Ch		12.33	12.07	12.67	12.93
	Ch+AA		12.13	12.4	12.33	12.73
TA	Control	0.96 a	0.81 e	0.69 hi	0.67 i	0.56 k
	AA		0.85 cd	0.83 de	0.73 g	0.61 j
	Ch		0.89 b	0.85 cd	0.72 gh	0.69 hi
	Ch+AA		0.96 a	0.86 c	0.81 e	0.78 f
TPC	Control	93.58	98.58	102.75	116.92	120.25
	AA		97.75	100.25	98.58	94.42
	Ch		95.25	96.92	98.58	91.08
	Ch+AA		96.08	91.08	100.25	96.08
TAA	Control	5.58 a	3.28 gh	3.71 ef	2.95 h	2.22 i
	AA		5.31 ab	4.99 bc	4.11 d	3.29 gh
	Ch		4.80 c	4.19 d	3.29 gh	3.34 fg
	Ch+AA		5.58 a	5.00 bc	3.91 de	3.88 de

LSD for SSC: N.S.; TA: 0.03; TPC:N.S.; TAC: 0.38. Means of triplicate measurements are presented and data with different letters are significantly different $P \leq 0.05$.

Treatments significantly affected the visual quality performed by the panelists focusing on quality feature such as color, browning and firmness during cold storage (Figure 2). A significant decrease was determened in the control slices from the 8th day while Ch+AA treatment, in particular, significantly protected the quality of slices during the storage period. At the end of the storage, Ch+AA (5.0) treatment alone weas able to yield a score above the marketable limit value. The lowest score (1.0) was determined in the control slices.

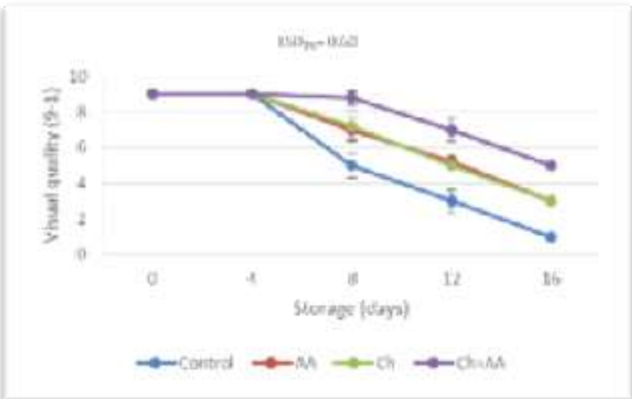

Figure 2. The effects of ascorbic acid and chitosan treatments on visual quality of fresh cut peach slices during storage.

Conclusions

In the present study, the effect of antibrowning (ascorbic acid), edible coating (chitosan) and their combination on slice quality in fresh cut peach was investigated. Combination treatment significantly reduced weight loss and maintained slice L* and hue angle, firmness, antioxidant activity, titratable acidity and delayed broning and visual changes. Overall, the use of chitosan-ascorbic acid coating could be applied on fresh cut peaches to extend shelf-life by maintaining nutritional and sensory quality.

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METHYL JASMONATE MAINTAINS THE POSTHARVEST PHYSICAL AND BIOCHEMICAL QUALITY PROPERTIES OF 'ALPHONSE LAVELLÉE' (*Vitis vinifera* L.) TABLE GRAPES DURING COLD STORAGE

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Abstract

Market demand for table grapes has been increasing due to its premium functional properties and beneficial effects on human health with its rich fenolic and antioxidant contents. However, fresh table grapes are prone to various postharvest disorders such as weight loss, softening, decay and browning. Therefore, this study was performed to reveal the effect of different doses (0, 0.1, 1.0 and 10 mM) of postharvest methyl jasmonate (MeJa) immersion on quality maintenance of 'Alphonse Lavellée' table grapes during 80 d storage at 1.0±0.5 °C and 90% relative humidity. Lower doses of MeJa (0.1 and 1.0 mM) effectively delayed the physiological disorders such as weight loss, skin rupture force and berry detachment force with similar effects. Loses in the antioxidant activity of grapes were significantly delayed by all doses of MeJa treatments with the greatest effect of 0.1 doses during the storage. MeJa treatments in all doses also significantly delayed the changes in total phenols up to the 60th d of storage. Immersing the grape clusters into 0.1 mM MeJa has the greatest effects on maintaining the biochemical features such as total soluble solid, titratable acidity, pH and maturity index of berry juice. The overall investigations indicated that 0.1 mM MeJa treatment could be recommended for restricting the weight loss, maintaining the bioactive and biochemical qualities, delaying the physical changes of 'Alphonse Lavellée' table grapes during 80 days of cold storage.

Keywords: *Table grapes, cold storage, methyl jasmonate, quality extension.*

Introduction

Table grapes, as non-climacteric horticultural crops with a relatively low postharvest physiological activity (Crisosto et al., 2001), are subject to quality losses during storage, resulting in rachis (cluster stem) drying and browning, berry shatter, and even shriveling of berries (Sabir and Sabir, 2013). Fumigation with SO_2 is still the primary means for controlling postharvest disorders due to its excellent effect on diseases (Lee et al., 2015), although SO_2 fumigation is becoming very restrictive due to its dangerous residues. Growing awareness of consumer on the potential harm of chemical treatments for the control of diseases and physiological disorders, has led to the improvement of non-damaging physical treatments for this purpose in horticultural produce. Therefore, harmless or safer compounds should be developed as alternative methods for postharvest diseases (Sabir et al., 2011). Methyl jasmonate (MeJA) is an important phytohormone that regulates the physiology of grape, quality formation, and the biosynthesis of certain biochemicals (Wang et al., 2022). Studies have found that MeJA could regulate the development of postharvest fruit quality, playing an important role in enhancing antioxidant activity, regulating postharvest senescence, and resisting pathogen infections (Garcia-Pastor et al., 2019). MeJA improved the postharvest quality and stress resistance of perishable fruits such as strawberries (Chen et al., 2022), blueberries (Huang et al., 2015) and kiwifruit (Pan et al., 2020). MeJA spraying promoted the synthesis of secondary metabolites during the dehydration and shrinkage process of posthharvest grapes (Modesti et al., 2018). However, experimental and applied data is still a lack of research on the modulatory mechanism of MeJA on the quality maintenance of postharvest table grape. Therfore, this study was performed to reveal the effect of different doses (0, 0.1, 1.0 and 10 mM) of postharvest methyl jasmonate (MeJa) immersion on quality maintenance of ‘Alphonse Lavellée’ table grapes during 80 d storage at 1.0±0.5 °C and 90% relative humidity.

Material and Method

Grapes clusters of ‘Alphonse Lavallée’ harvested at commercial maturity stage (SSC: 16.0) were sorted into four equal groups for treatments (1) immersion in aqueous solutions of 0.1 mM MeJa, (2) 1 mM MeJa, (3) 10 mM MeJa, or (4) conrol. After drying excessive water from grapes, 500 g of grapes per replicate was placed in 30 cm × 40 cm polyamide/polyethylene plastic bags. Samples were stored for up to 80 d in a cold room at 1 °C (90% R.H.). Samples were taken at harvest, 20th, 40th, 60th and 80th days of storage to perform ceratain measurements and analyses. The weight loss was determined by periodical weighing, and calculated by dividing the mass change (Weight loss (%) = [(Mi−Ms)/Mi]×100, where Mi = initial mass and Ms = mass at examined time). Visual quality was scored on a 9 to 1 scale, where 9=excellent, fresh appearance, 7=good, 5=fair (limit of marketability), 3=fair (usable but not salable), 1=unusable. Rachis (stalk) quality was scored on a 1 (fresh appearance) to 5 (fully dessicated brown stalk) scale. A force gauge (DPS-11; Imada, Northbrook, IL) was employed to determine the berry skin rupture force (SRF) and the berry detachment force (BDF) as described by Fidelibus et al. (2007). For determining the SRF, the berries berry from each equatorial section was cradled in a jig attached to a force gauge and the gauge was gently pulled away from the berry until the skin puncture. For BDF, the hook apparatus of the force gauge was fixated around the berry pedicel and the gauge was gently pulled away from the cluster until the berry detached. Total phenol content (TPC) was quantified by the Folin-Ciocalteu method as described by (Singleton et al. 1999). For this aim, 1 mL of the grapes must was added 0.30 mL of the methanol and 1.2 mL of the 7% solution of sodium carbonate and 1.5 mL of diluted Folin–Ciocalteu (Merck, Germany). The mixture was then homogenized and incubated at room temperature for 90 min. The sample absorbance value was read at 750 nm with a spectrophotometer. Gallic acid was utilized as a standard for determining the calibration curve. The phenolic content in the grape must was expressed as mg gallic acid equivalents per 100 g of grape fresh weight according to the calibration curve obtained with the same standard. All samples and standards were diluted with distilled water in a ratio of 1:3 before being read due to the high concentration of samples. The antioxidant capacity of the grape must sample was found with a ferric reducing antioxidant potential (FRAP) assay (Benzie and Strain 1996). The FRAP reagent was a mixture of 2.5 mL of 10 mM 2,4,6-trioyridyl-1,3,5-triazine, 25 mL of acetate buffer pH 3.0 and 2.5 mL of 20 mM ferric chloride hexahydrate. The mixture reaction was performed when 0.5 mL of the supernatant was supplemented into 5 mL of FRAP solution. The reaction solution was incubated at ambient temperature for 30 min and then the absorbance was read at 630 nm. Total soluble solid content (SSC) was measured with a portable refractometer (Atago, Tokyo, Japan) in grape must obtained by whisking the berries from each replication in a blender and then filtering the must. Titratable acidity (TA) was determined by titrating 10 mL of juice using NaOH 0.1 mol L^{-1} to pH 8.1 and expressed as g tartaric acid per 100 g FW. Maturity index (MI) was calculated as soluble solid content/acid content. The pH was measured using a pH meter (Crison, Barcelona, Spain). Berry color of thirty berries per treatment was analyzed using a colorimeter (Minolta® CR-400) to obtain the following variables from two equatorial points of berries: L* (lightness), C* (chroma) and h° (hue). Lightness values may range from

0 (black) 100 (white). Chroma indicates the purity or intensity of color, the distance from gray (achromatic) toward a pure chromatic color and is calculated from the a* and b* values of the CIELab scale system, starts from zero for a completely neutral color, and does not have an arbitrary end, but intensity increases with magnitude. Hue refers to the color wheel and is measured in angles; green, yellow and red correspond to 180, 90 and 0°, respectively (McGuire 1992). Numerical data were submitted to one-way analysis of variance and Student’s t-test ($P<0.05$) using the software SPSS 13.0 for windows.

Results and Discussion

Weight loss of fresh ‘Alphonse Lavellée’ grapes constantly increased during the prolonged cold storage duration in all treatments (Figure 1). After 80 d of storage, the lowest change in weight loss was determined grapes trated with 0.1 mM MeJa treatment (2.92%) which was followed by 1 mM MeJa treatment (3.05%) with a similar effect. On the other hand, the gratest losses in weight were obtained from control (4.61%) and 10 mM MeJa treatment (4.24%). Studies showed that a maximum 6% weight loss in fresh fruits is proven as commercially acceptable (Paniagua et al., 2013). General losses in overall grapes are among the acceptable threshold although control and 10 mM MeJa treated grapes approach the limit. Lower doses of MeJa slowed the moisture loss of fresh grapes.

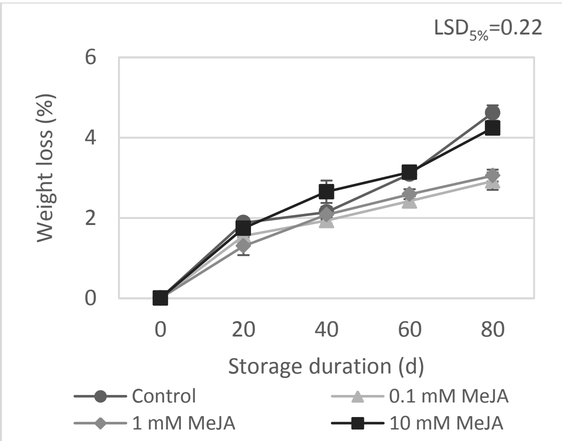

Figure 1. Weight loss changes of berries during the prolonged storage as influenced by MeJA treatments. Error bar stands for the standard deviation of the mean of triplicate determinations.

Visual quality attribute of fresh grapes did not show detectable changes up to the 20th d of storage as illustrated in Figure 2, A. Then, a significant decrease ocurred in visual quality with the highest decrease always determined in control blackberries. In contrast, the lowest loss in fruit visual quality was found in 10 mM MeJa treated or control grapes. At the end of the 80 d storage duration, 10 mM MeJa treated or control grapes were almost under the marketability level with a very low visual quality scores (3.3 and 4.0, respectively) while the grapes treated with 0.1 mM MeJa had a very high score (7.0). Postharvest water loss, enzyme activities and respiration triger the physiological senescence in fresh berries after harvest (Meneghel et al., 2008). Increases in physiological reaction result in rapid development to physiological disorders that cause decreased visual quality and shortened storage life. Cluster stalk quality is one of prime features determining the market quality of table grapes. As depicted in Figure 2, B, stalk quality decreased along with the storage duration. At the end of the storage, the greatest change in stalk quality occurred in control grapes while 0.1 mM MeJa had the highest effect on maintaining the stalk quality.

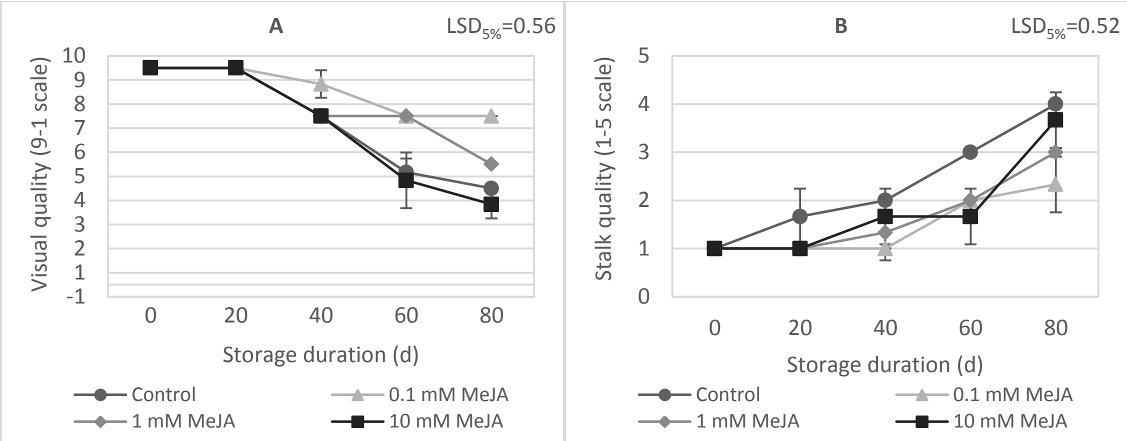

Figure 2. Visual quality (A) and stalk quality (B) changes of berries during the prolonged storage as influenced by MeJA treatments. Error bar stands for the standard deviation of the mean of triplicate determinations.

Changes in skin rupture force (SRF) and berry detachment force (BDF) features have been illustrated in Figure 3 A and B. SRF was almost constant up to the 20th d of storage although there were remarkable decreases in SRF after the 20th d. At the end of the storage, the greatest SRF was obtained from 1 mM MeJa (2.16 N), followed by 0.1 mM MeJa (1.97 N). Similar changes in BDF were also determined during the storage. The lowest and highest changes in BDF were recorded in 0.1 mM MeJa (5.05 N) and control grapes (3.93 N), respectively.

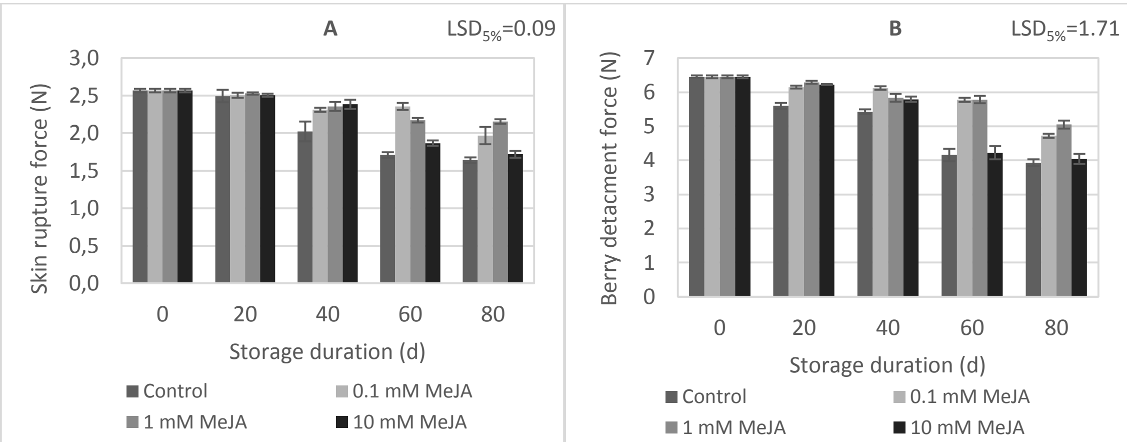

Figure 3. Skin rupture (A) and berry detachment (B) changes of berries during the prolonged storage as influenced by MeJA treatments. Error bar stands for the standard deviation of the mean of triplicate determinations.

The variation in the amount of total phenolic content and antioxidant activity in response to different MeJa concentrations during storage was illustrated in Figure 4A and B. As the cold storage prolonged, the amount of phenolic matter increased across the grapes up to the 60th d. At the 60th d of the storage, the highest phenol increase was detected in control grapes (57.5 mg GAE 100 g^{-1}), although all doses of MeJa provided an effective maintenance in total phenol. There were no significant differences in total phenol content of the grapes. In contrast to total phenol, antioxidant activity displayed gradual decrease during the storage. The greatest decrease was always determined in control grapes, while MeJa doses retarded the loss in antioxidant activity. At the end of the storage, control grapes had significantly lower antioxidant activity than those of MeJa doses.

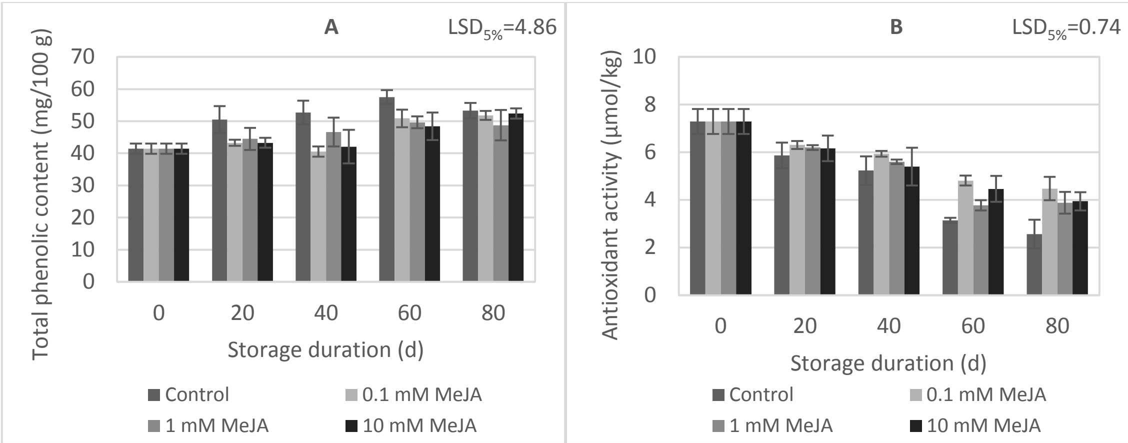

Figure 4. Total phenolic content (A) and antioxidant activity (B) changes of berries during the prolonged storage as influenced by MeJA treatments. Error bar stands for the standard deviation of the mean of triplicate determinations.

As presented in Table 1, soluble solid content (SSC) and maturity index (MI) of fresh grapes significantly increased during cold storage, whereas titratable acidity (TA) and pH gradually increased over the extended storage period. The increase trend of SSC in postharvest period was reported for different grape cultivars (Ayala-Zavala et al., 2004). The highest changes in both SSC and MI were determined in control fruits, while the lowest changes in these biochemical parameters were found in grapes subjected to 1 mM MeJa treatment (except for pH). Accelerated respiration rate is a well knownwell-known postharvest physiology occurred in vegetables and fruits (Jiang and Li, 2001; Ayala-Zavala et al., 2004) causing to reduction in SSC as the organic sugars and acids are the pioneering phytochemical matters for respiration process (Meighani et al., 2015). However, MeJa, 1 mM doses in particular, was effective on delaying the biochemical changes.

Table 1. Changes in SSC (°Brix), TA (%), MI and pH of berries throughout the storage period.

	Treatments	Storage time (days)				
		0	20	40	60	80
SSC	Control	16.00	16.40	16.80	16.86	17.00
	0.1mM MeJA		16.13	16.43	16.66	16.60
	1 mM MeJA		16.33	16.46	16.46	16.93
	10 mM MeJA		16.40	16.46	16.60	16.86
TA	Control	0.762 a	0.698 bc	0.622 de	0.598 e	0.465 g
	0.1mM MeJA		0.752 a	0.699 bc	0.675 c	0.517 f
	1 mM MeJA		0.737 a	0.681 bc	0.635 d	0.489 g
	10 mM MeJA		0.736 a	0.706 b	0.615 de	0.463 g
MI	Control	21.00 k	23.47 gh	27.00 de	28.17 d	36.50 a
	0.1mM MeJA		21.44 jk	23.51 fg	24.69 f	32.07 c
	1 mM MeJA		22.15 ıjk	24.17 fg	25.90 e	34.56 b
	10 mM MeJA		22.27 hıj	23.34 ghı	26.96 e	36.40 a
pH	Control	3.97 a	3.81 b-e	3.85 abc	3.74 cde	3.68 e
	0.1mM MeJA		3.76 b-e	3.88 ab	3.83 a-d	3.79 b-e
	1 mM MeJA		3.67 e	3.79 b-e	3.75 b-e	3.69 de
	10 mM MeJA		3.78 b-e	3.83 abc	3.47 f	3.41 f

LSD for SSC: N.S.; TA: 0.03; MI: 1.20; pH: 0.14. Means of triplicate measurements are presented and data with different letters are significantly different $P \leq 0.05$.

All the grape berries displayed a gradual treatment dependent decrease in L* and C* values while hue angle did not significantly change during the storage period (Table 2). Studies indicated that decreases in color coordinates of the fresh commodities s in the cold storage could be probably due to the oxidation of phenolic matters as well as other physicochemical phenomena (Pathare et al., 2012) and the weight loss that take place during storage (Cortés Rodríguez et al., 2020). At the end of the storage time, the highest changes in L* and C* values were determined in control grapes, while the lowest decreases occurred in the fruits subjected to 0.1 mM MeJa which were followed by 1 mM MeJa. These findings implied the protective effect of lower doses of MeJa on berry skin.

Table 2. Changes in L*, C and Hue angle values of berries throughout the storage period.

	Treatments	Storage time (days)				
		0	20	40	60	80
L*	Control	30.67 a	29.78 bc	28.51 def	28.30 ef	27.86 fg
	0.1 mM MeJA		30.01 ab	29.74 bc	28.58 def	28.31 ef
	1 mM MeJA		30.45 ab	30.30 ab	29.02 cde	28.17 f
	10 mM MeJA		30.38 ab	29.12 cd	27.89 fg	27.16 g
C*	Control	2.67 ab	2.34 def	2.43 cde	2.33 def	2.18 fg
	0.1 mM MeJA		2.65 ab	2.56 abc	2.31 efg	2.28 efg
	1 mM MeJA		2.76 a	2.62 abc	2.24 efg	2.12 g
	10 mM MeJA		2.69 ab	2.53 bcd	2.30 efg	1.54 h
Hue angle	Control	288.80	297.70	287.99	311.09	316.43
	0.1 mM MeJA		292.68	291.77	308.38	305.93
	1 mM MeJA		295.40	297.02	308.02	307.71
	10 mM MeJA		297.01	297.88	309.98	312.53

LSD for L*: 0.76; C*: 0.21; Hue: N.S. Means of triplicate measurements are presented and data with different letters are significantly different $P \leq 0.05$.

Conclusions

MeJa in 0.1 and 1.0 mM doses maintained the postharvest quality of 'Alphonse Lavellée' grapes by delaying weight loss, extending skin rupture force and berry detachment force with similar effects. Total phenol and antioxidant activity of grapes were significantly extended all doses of MeJa treatments. Immersing the grape clusters into 0.1 mM MeJa has the greatest effects on maintaining the biochemical features such as total soluble solid, titratable acidity, pH and maturity index. The results indicated that 0.1 mM MeJa treatment could be recommended for maintaining the bioactive and biochemical qualities 'Alphonse Lavellée' table grapes during 80 days of cold storage.

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DETERMINATION METHOD OF HEXAKANAZOL AND TEBUCUNAZOL RESIDUES BY CAPILAR ELECTROPHORESIS

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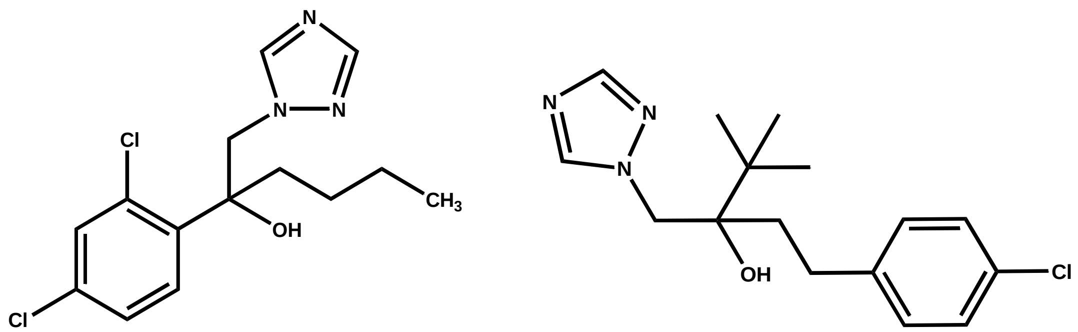
Abstract

A method was developed to determine the triazole derivative fungicides hexakanozole and tebucanazole residues by capillary electrophoresis. The total amount of these two fungicides was determined. With this method, the lowest detection limit for total amount was calculated as 0.0303 μg mL^{-1} and the limit of quantification was calculated as 0.101 μg mL^{-1}. The capillary electrophoresis system was equipped with a 50 cm × 50 μm column. The electrophoretic conditions were as follows: 7.5 mM NH_4HCO_2 with 10% methanol at pH 2.1. The column temperature is 30°C and the detection wavelength is 200 nm.

Keywords: *Hexaconazole, tebuconazole, fungicid, capillary electrophoresis.*

Introduction

Fungicides are used on a large scale for agricultural purposes to control plant diseases caused by fungal attack. The negative effects of fungicides on both human and environmental health are a matter of public concern. So, both the actual amount and migration of fungicide residues in various matrices such as water, soil and agricultural products should be comprehensively monitored (Sakamoto *et al.*, 2004). Fungicides pollute crops and natural waters because of their widespread use. It is thought that triazole derivatives represent the most important fungicide category to date, thanks to their excellent protective, therapeutic and destructive powers against a wide range of plant diseases (Wu *et al.*, 2001). Hexaconazole (HEX) is a member of the triazole family. Triazoles are known to inhibit cytochrome P-450-mediated oxidative demethylation reactions required for ergosterol synthesis and the conversion of kaurene to kaurenoic acid in the gibberellin biosynthetic pathway. Hexaconazole [(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol] is a potent triazole fungicide consisting of a pair of enantiomers in which the (-)-enantiomer is more fungicidally active than the (+)-enantiomer (Figure 1) (Wang *et al*., 2005). This drug, released in 1986; is registered as a foliar applied fungicide for cereals, vegetables, farm plants and fruits (Paredes and Munos, 2002; Navarro *et al.*, 2000).
Tebuconazole (TEB)- (RS)-1-p-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol- is a broad-spectrum triazole fungicide used to control soil-borne and foliar diseases in peanuts and other crops. Tebuconazole has one chiral carbon atom and a pair of enantiomers as shown in Figure 1. Tebuconazole is often used as a seed coating agent applied to agricultural soil. So, earthworms, which are beneficial organisms in terms of bioavailability, can be directly exposed to high doses of tebuconazole . (Dingyi *et al*., 2012,).



a b

Figure 1. Chemical structures of a) HEX and b) TEB

There are several methods aimed at the determination of these fungicides and many other pesticides. These are HPLC (Carmona *et al*., 2020; Ekiert *et al.*, 2010; Huang *et al.*, 2012 and GC methods (Ekiert *et al.*, 2010; Cserháti and Szőgyi, 2012). Capillary electrophoresis (CE) is a promising alternative method to these analytical techniques. The versatility of CE separation modes (zone electrophoresis, isotachophoresis, isoelectric focusing, electrokinetic chromatography) enables the determination of a wide variety of compounds with different sizes and characters (Voeten *et al*., 2012).
An overview of the validation of CE methods for the separation and determination of pesticide residues is summarized in the literature (Pico *et al*., 2003; Bol'shakova and Amelin, 2016). On the other hand, the disadvantage of the CE method are relatively low detection limit and a limited number of selective detectors (Pico *et al*., 2003). Moreover, the disadvantage of the lower sensitivity of CE can be enhanced by offline or online sample preconcentration.
A validated capillary electrophoresis (CE) method with on-line preconcentration technique was developed for the determination of the sum of hexaconazole and tebuconazole fungicides in trace amounts in this study.

Material and Methods

The capillary cartridge temperature was fixed at 25 °C and capillary was conditioned for 15 minutes in succession with 1.0 mol L^{-1} NaOH, 0.1 mol L^{-1} NaOH, H_2O and running electrolyte, respectively. Between two successive injections, the capillary was flushed with deionize water for 2 minutes and then with a running electrolyte for 5 minutes. Membrane filters (0.45 µm porous RC) were used before all injections to prevent contamination. All the samples and solutions were sonicated for 5 minutes before the injection step. The recorded migration times at 200 nm were 9.42 and 2.21 minutes for HEX + TEB and internal standard (IS), respectively.

In the experiments, peak normalization area ratios (rPN) were evaluated by dividing the peak normalization value of the analytes (PNanalyte or PNstd) by the peak normalization of the IS (PNIS).

$$rPN = PN_{Std} / PN_{IS}$$

Best peak normalization area ratio values for Standard and IS were calculated by dividing the areas of each by the Standard and IS retention times.

$$PN_{Std} = \text{Peak Area}_{Std} / \text{Retention Time}_{Std}$$
$$PN_{IS} = \text{Peak Area}_{IS} / \text{Retention Time}_{IS}$$

According to these calculation results, optimum conditions were determined by taking into account the electropherograms that gave the largest rPN value and the best peak morphology.

Results and Discussion

Method Optimization for Studied Fungicides

First of all, experiments were conducted with acidic solutions to determine the type of working electrolyte to be used in the study. Experiments were carried out using KCl-HCl buffer, sodium dihydrogen phosphate phosphoric acid buffer, acetic acid acetate buffer and formic acid-ammonium formate electrolyte. The best results were observed when working with formic acid-ammonium formate electrolyte. After determining the type of working electrolyte, its pH was optimized. The solution consisting of a mixture of fungicides studied at a final concentration of 8.0 x 10^{-5} M and 1.0 x 10^{-5} M IS was injected at the optimum pH values specified in Table 1. As can be seen from the table, the rPN values of these fungicides that are most suitable for the study are almost close to each other, but the value that looks relatively better in terms of peak morphology and has the highest value is pH 2.14.

Table 1. rPN values of 8.0 × 10^{-5} M standard solutions of the studied fungicides depending on the pH of the working electrolyte (at 200 nm)

pH of the working electrolyte	**rPN values of the standards**
1.80	0.245
2.05	0.252
2.14	0.273
2.26	0.232

Injection of the solution consisting of a mixture of the studied fungicides at a final concentration of 8.0 x 10^{-5} M and 1.0 x 10^{-5} M IS, with NH_4HCO_2 buffer solutions prepared at different concentrations (0.5-15 mM) to pH 2.14. has been made. However, at concentrations higher than 7.5 mM of buffer solution, the column was constantly blocked. For this reason, 7.5 mM ammonium formate-NH_4HCO_2 – concentration was chosen as an optimum. In order to obtain better peak resolution in the electropherogram, organic solvents are added to the buffer solution to increase the viscosity of the buffer solution. The most commonly used solvents for this purpose are acetonitrile (ACN) and methanol (MeOH). For this reason, the standard fungicide mixture solution prepared at fixed concentrations (8,0 x 10^{-5} M) has been analyzed. The MeOH percentage with the best rPN values of the fungicides was 10% (v/v), and this concentration was selected in terms of appropriate analysis time. The potential was increased from 10 kV to 30 kV and 25 kV was chosen as operating potential in terms of appropriate analysis time. Standard solutions, prepared by diluting the stock solutions prepared by dissolving with MeOH, were injected in hydrodynamic mode before injection, and then injected in electrokinetic mode. When the standards are injected into the capillary in a large volume in a low-conductivity solution in water, and the concentrated working electrolyte is prepared, when the ions moving rapidly in the sample region with a high electric field reach the buffer region, they will suddenly slow down- as the electric field will decrease- and become compressed and concentrated at the buffer boundary. Thus, the applied voltage and time for the electrokinetic injection of the standards, the pressure and time of the water in the hydrodynamic mode were optimized separately. 8.0 × 10^{-5} M standard solutions of the

fungicides were prepared at pH 2.14 and 7.5 mM of NH_4HCO_2 concentration in 10% (v/v) MeOH buffer at 25 kV applied voltage, 10 kV electrokinetic injection voltage. It was observed that the best rPN values of the peaks obtained at 200 nm due to the water injection of 50 mbar hydrodynamic pressure for 3 seconds during the application period of 25 seconds were observed. The column temperature of the standard solutions of fungicides prepared at a final concentration of 8.0 x 10^{-5} M was optimized under the conditions recommended above. 30 °C was observed as the temperature that gave the best rPN value and peak morphology.
The electropherogram obtained under optimum conditions is given in Figure 1.

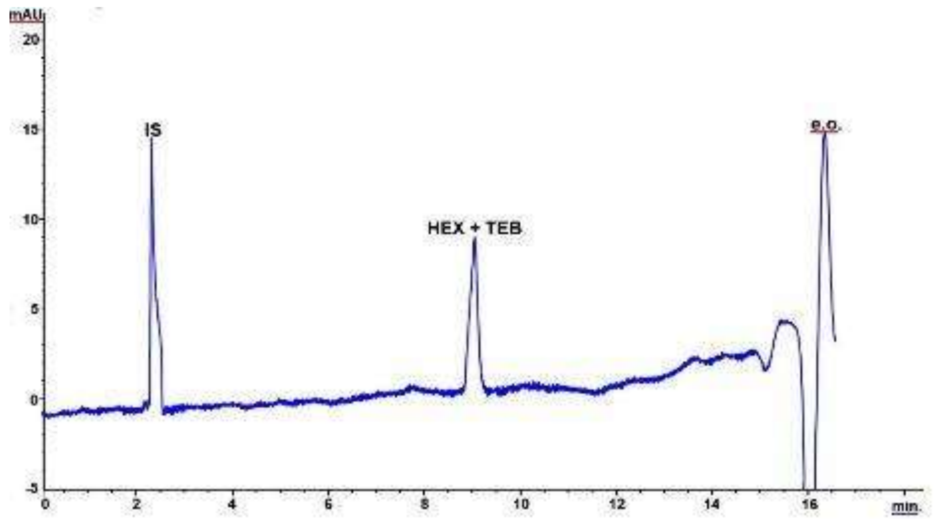


Figure 1. Electropherogram of HEX+TEB at a final concentration of 8.0 x 10^{-5} M and IS at 1.0 x 10^{-5} M under optimum conditions (at pH=2.1, 7.5 mM NH_4HCO_2 buffer, 10% methanol, 50 mbar, 3 sec water and 10 kV, 25 sec standard mixture injection time, 200 nm wavelength, 30 ^{o}C column temperature and 25 kV)

Method Validation of The Fungicide

The developed capillary electrophoretic method was validated by investigating the linearity, limit of detection (LOD), limit of detection (LOQ), precision and accuracy parameters of the fungicides studied according to the International Conference on Harmonization (ICH).
The linearity of this method was examined with a six-point calibration curve using a final concentration of 1.0 x 10^{-6} mol L^{-1} IS and standard fungicide solution each. For the fungicides, the solutions were in the concentration range of 3.00×10^{-6} mol L^{-1} and 4.00×10^{-5} mol L^{-1} at a wavelength of 200 nm and six repeated injections were made on three separate days. In experiments, peak normalization ratios (rPN) were evaluated by dividing the peak normalization value of the analyte by the IS peak normalization [rPN = PNStd / PNIS]. The limit of detection (LOD) and limit of detection (LOQ) of the method were calculated from the calibration curve with the equation given below:
LOD = 3,3 × s/m
LOQ = 10 × s/m

In these formulas, s = standard deviation value of the lowest concentration of the analyte solution and m = slope value of the calibration equation.
Determination range results and LOD and LOQ values for the fungicides are given in Table 2.

Table 2. Calibration data for the fungicide

	I. Day, n=6	II. Day, n=6	III. Day, n=6	Inter-day, n=18
m^a	0.0618	0.0618	0.0618	0.0618
n^b	-0.0378	-0.0367	-0.0379	-0.0379
R^{2c}	0.9990	0.9991	0.9990	0.9990
s_m^d	0.0011	0.0011	0.0011	0.0011
LOD[e] μg mL^{-1}	0.0406	0.0354	0.0521	0.0303
LOQ[f] μg mL^{-1}	0.1354	0.1180	0.1735	0.1010

[a] m is slope
[b] n is intercept
[c] R is correlation coefficient
[d] s_m is the standard deviation of calibration curve. ($s_m = \sqrt{\frac{s_r^2}{s_{xx}}}$), s_r is standard deviation of regression
[e] LOD is limit of detection
[f] LOQ is limit of quantification

The precision of the method was evaluated through intra-day and inter-day repeatability studies. Three sets of standard solutions were prepared by adding 1,00 x 10^{-6} mol L^{-1} M IS. Injections were done 6 times (n = 6) over 3 successive days. Precision results are shown in Table 3.

Table 3. Inter-day and Intra-day results of 9.00 × 10^{-6} mol L^{-1} the fungicide (2.83 μg mL^{-1} HEK, 2.77 μg mL^{-1} TEB)

	I. Day (n=6)[a]	II. Day (n=6)[a]	III. Day (n=6)[a]	Inter-day (n=18)[a]
$\overline{X}^b$	0.321	0.322	0.323	0.322
s^c	0.006	0.005	0.006	0.005
RSD%[d]	1.749	1.509	1.757	1.624
CL[e]	0.006	0.006	0.007	0.005
CL[e]	0.001	0.001	0.001	0.001

[a] n is the number of experiments.
[b] is the mean ratio of peak normalization.
[c] s is the SD of the mean response.
[d] RSD% is relative standard deviation, %
[e] CL confidence limit, (ts/√n)

Conclusion

The CE method proposed in this study, which aims at the combined determination of HEX + TEB; has important advantages such as cheapness, accuracy, selectivity, reliability and environmental friendliness. To the best of our knowledge, this validated method is the first example based on HEX+TEB determination using online preconcentration technique by capillary electrophoresis. The developed method has been verified according to the ICH guideline. All the ICH parameters give good results.

Acknowlegdement

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DETERMINATION METHOD OF HEXACONAZOLE AND TEBUCUNAZOL RESIDUES BY CAPILLARY ELECTROPHORESIS

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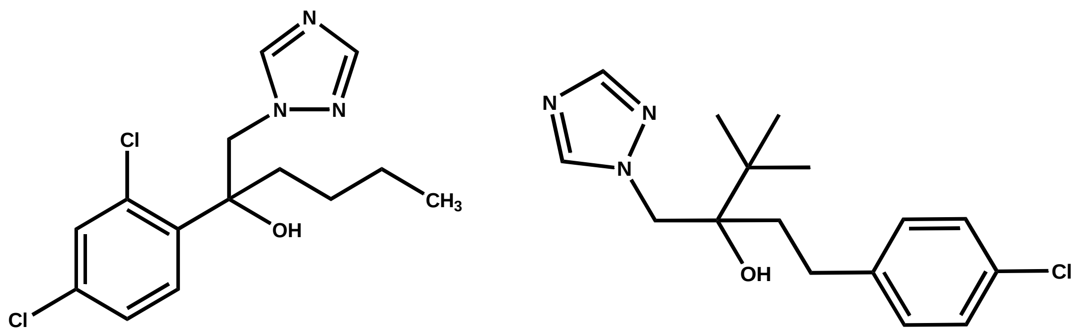
Abstract

A method was developed to determine the triazole derivative fungicides hexaconazole and tebucanazole residues by capillary electrophoresis. The total amount of these two fungicides was determined. On an uncoated fused silica capillary column (containing 50 cm effective length, 50 μm inner diameter) consisting of background electrolyte of 7.5 mM NH_4HCO_2 solution, 10% (v/v) methanol (pH=2.1), capillary electrophoretic separation was obtained by applying a potential of 25 kV and measuring at a wavelength of 200 nm and a temperature of 30 °C. Field-amplified sample injection (FASI) was utilized with the parameters of 3 s hydrodynamic injection of a water plug and 25 s electrokinetic injection of the sample in order to increase sensitivity. Additionally, a 1:1 volume ratio of methanol to water was used as the sample solvent. With this method, the lowest detection limit for total amount was calculated as 0.0303 μg mL^{-1} and the limit of quantification was calculated as 0.101 μg mL^{-1}. The established method has been verified by according to the guidelines set out by the ICH. The validated method is the first that we are aware of for simultaneous measuring hexaconazole and tebuconazole combining online preconcentration techniques and capillary electrophoresis.

Keywords: *Hexaconazole, tebuconazole, fungicide, capillary electrophoresis.*

Introduction

In agriculture, fungicides are widely employed to manage plant diseases brought on by fungal invasions. Public concern is raised by fungicides' detrimental impacts on environmental and human health. Therefore, it is important to carefully monitor the amount and migration of fungicide residues in many matrices, including soil, water, and agricultural products (Sakamoto and Tsutsumi, 2004). Fungicides pollute crops and natural waters because of their widespread use. Triazole derivatives are considered to be the most significant class of fungicides available today because of their superior abilities to prevent, treat, and eradicate a variety of plant diseases (Wu *et al.*, 2001). One member of the triazole family is hexaconazole (HEX). Triazoles are known to impede the oxidative demethylation processes performed by cytochrome P-450, which are necessary for the synthesis of ergosterol and the gibberellin biosynthesis pathway's conversion of kaurene to kaurenoic acid. Hexaconazole, also known as (RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexane-2-ol, is a powerful triazole fungicide that consists of two enantiomers, the (-)-enantiomer being more fungicidally active than the (+)-enantiomer (Figure 1) (Wang *et al.*, 2005). Released in 1986, this medication is approved for use as a foliar-applied fungicide on fruits, vegetables, cereals, and farm plants (Paredes and Munos, 2002; Navarro et al., 2000). Tebuconazole (TEB)- (RS)-1-p-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol- is a triazole fungicide with a broad range of application that is used to manage foliar and soil-borne diseases in peanuts and other crops. Tebuconazole has one chiral carbon atom and a pair of enantiomers as shown in Figure 1. Tebuconazole is often used as a seed coating agent applied to agricultural soil. So, earthworms, which are beneficial organisms in terms of bioavailability, can be directly exposed to high doses of tebuconazole (Yu *et al*., 2012).



a b

Figure 1. Chemical structures of a) HEX and b) TEB

There are several methods aimed at the determination of these fungicides and many other pesticides. These are HPLC (Carmona et al., 2020; Ekiert et al., 2010; Huang et al., 2012 and GC methods (Ekiert et al., 2010; Cserháti and Szőgyi, 2012). As an alternative to traditional analytical methods, capillary electrophoresis (CE) shows great promise. According to Voeten et al. (2012), the many different compounds with varying sizes and characteristics can be determined thanks to the adaptability of CE separation modes, which include zone electrophoresis, isotachophoresis, isoelectric focusing, and electrokinetic chromatography.

The literature provides an overview of the validity of CE methods for pesticide residue separation and determination (Pico et al., 2003; Bol'shakova and Amelin, 2016). However, the CE method's drawbacks include a small number of selective detectors and a comparatively low detection limit (Pico et al., 2003). Moreover, offline or online sample preconcentration may increase the drawback of CE's reduced sensitivity (Quirino et al., 2002; Sentellas et al., 2003; Sentellas et al., 2004; Shihabi, 2000).

This work used a validated capillary electrophoresis (CE) method with an online preconcentration methodology to determine the total amount of fungicides, tebuconazole and hexaconazole, in trace levels.

Material and Methods

The temperature of the capillary cartridge was set at 25 °C, and the capillary was conditioned for 15 minutes continuously using 1.0 mol L^{-1} NaOH, 0.1 mol L^{-1} NaOH, H_2O, and flowing electrolyte, in that order. The capillary was washed with deionized water for two minutes in between each of the two injections, and then with a running electrolyte for five minutes. To avoid contamination, membrane filters (0.45 μm porous RC) were utilized prior to each injection. Prior to the injection procedure, all of the samples and solutions were sonicated for five minutes. For HEX + TEB and internal standard (IS), the migration times at 200 nm were found to be 9.42 and 2.21 minutes, respectively.

By dividing the peak normalization value of the analytes ($PN_{analyte}$ or PN_{Std}) by the peak normalization of the IS (PN_{IS}), peak normalization area ratios (rPN) were calculated for each experiment.

$rPN = PN_{Std} / PN_{IS}$

By dividing each area by the Standard and IS retention times, the best peak normalization area ratio values for Standard and IS were determined

$$PN_{Std} = \text{Peak Area}_{Std} / \text{Retention Time}_{Std}$$
$$PN_{IS} = \text{Peak Area}_{IS} / \text{Retention Time}_{IS}$$

According to these calculation results, optimum conditions were determined by taking into account the electropherograms that gave the largest rPN value and the best peak morphology. Within the scope of this study, the injection type, injection time and voltage were optimised as in the study by Fei et al. (Fei, et al., 2007) to increase the sensitivity of the method using the online enrichment technique and to achieve low LOD values.

Results and Discussion

Method Optimization for Studied Fungicides

First of all, experiments were conducted with acidic solutions to determine the type of working electrolyte to be used in the study. Experiments were carried out using KCl-HCl buffer, sodium dihydrogen phosphate phosphoric acid buffer, acetic acid acetate buffer and formic acid-ammonium formate electrolyte. The best results were observed when working with formic acid-ammonium formate electrolyte. After determining the type of working electrolyte, its pH was optimized. The solution consisting of a mixture of fungicides studied at a final concentration of 8.0×10^{-5} M and 1.0×10^{-5} M IS was injected at the optimum pH values specified in Table 1. The table indicates that these fungicides with the highest rPN values for the study are almost equal, however pH 2.14 is the value with the highest morphology and appears to be substantially better.

Table 1. rPN values of 8.0×10^{-5} M standard solutions of the studied fungicides depending on the pH of the working electrolyte (at 200 nm)

pH of the working electrolyte	rPN values of the standards
1.80	0.245
2.05	0.252
2.14	0.273
2.26	0.232

Injection of the solution consisting of a mixture of the studied fungicides at a final concentration of 8.0×10^{-5} M and 1.0×10^{-5} M IS, with NH_4HCO_2 buffer solutions prepared at different concentrations (0.5-15 mM) to pH 2.14. has been made. However, at concentrations higher than 7.5 mM of buffer solution, the column was constantly blocked. For this reason, 7.5 mM ammonium formate concentration was chosen as an optimum. In order to obtain better peak resolution in the electropherogram, organic solvents are added to the buffer solution to increase the viscosity of the buffer solution. The most commonly used solvents for this purpose are acetonitrile (ACN) and methanol (MeOH). For this reason, the standard fungicide mixture solution prepared at fixed concentrations (8.0×10^{-5} M) has been analyzed. The MeOH percentage with the best rPN values of the fungicides was 10% (v/v), and this concentration was selected in terms of appropriate analysis time. The potential was increased from 10 kV to 30 kV and 25 kV was chosen as operating potential in terms of appropriate analysis time. Standard solutions, prepared by diluting the stock solutions prepared by dissolving with MeOH, were injected in hydrodynamic mode before injection, and then injected in electrokinetic mode. When the standards are injected into the capillary in a large volume in a low-conductivity solution in water, and the concentrated working electrolyte is prepared, when the ions moving rapidly in the sample region with a high electric field reach the buffer region, they will suddenly slow down- as the electric field will decrease- and

become compressed and concentrated at the buffer boundary. Thus, the applied voltage and time for the electrokinetic injection of the standards, the pressure and time of the water in the hydrodynamic mode were optimized separately. 8.0 × 10^{-5} M standard solutions of the fungicides were prepared at pH 2.14 and 7.5 mM of NH_4HCO_2 concentration in 10% (v/v) MeOH buffer at 25 kV applied voltage, 10 kV electrokinetic injection voltage. It was observed that the best rPN values of the peaks obtained at 200 nm due to the water injection of 50 mbar hydrodynamic pressure for 3 seconds during the application period of 25 seconds were observed. The column temperature of the standard solutions of fungicides prepared at a final concentration of 8.0 x 10^{-5} M was optimized under the conditions recommended above. 30 °C was observed as the temperature that gave the best rPN value and peak morphology.
The electropherogram obtained under optimum conditions is given in Figure 2.

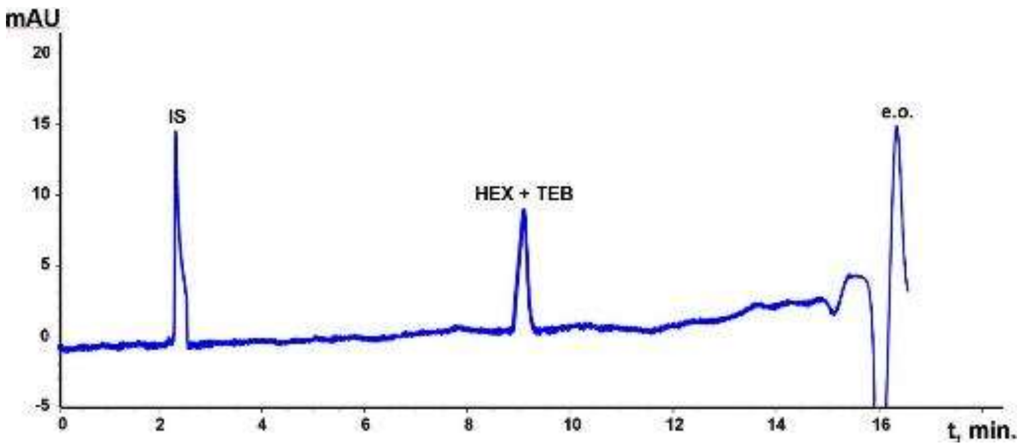


Figure 2. Electropherogram of HEX+TEB at a final concentration of 8.0 x 10^{-5} M and IS at 1.0 x 10^{-5} M under optimum conditions (at pH=2.1, 7.5 mM NH_4HCO_2 buffer, 10% methanol, 50 mbar, 3 s water and 10 kV, 25 s standard mixture injection time, 200 nm wavelength, 30 ^{o}C column temperature and 25 kV)

Method Validation of The Fungicide

By examining the linearity, limit of detection (LOD), limit of detection (LOQ), precision, and accuracy criteria of the fungic(ides under study in accordance with the International Conference on Harmonization (ICH) (Guideline, Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1), the devised capillary electrophoretic method was verified.
Using a six-point calibration curve and a final concentration of 1.0 x 10^{-6} mol L^{-1} IS and standard fungicide solution each, the linearity of that method was investigated.
For the fungicides, the solutions were in the concentration range of 3.00×10^{-6} mol L^{-1} and 4.00×10^{-5} mol L^{-1} at a wavelength of 200 nm and six repeated injections were made on three separate days. Peak normalization ratios (rPN) were calculated in experiments by dividing the analyte's peak normalization value by the IS peak normalization [$rPN = PN_{Std} / PN_{IS}$]. Using the following equation, the method's limit of detection (LOD) and limit of quantification (LOQ) were determined from the calibration curve:

$$LOD = 3.3 \times s/m$$
$$LOQ = 10 \times s/m$$

These calculations use **s** as the lowest concentration of the analyte solution's standard deviation value and **m** as the calibration equation's slope value.

Determination range results and LOD and LOQ values for the fungicides are given in Table 2.

Table 2. Calibration data for the fungicide

	I. Day, n=6	II. Day, n=6	III. Day, n=6	Inter-day, n=18
m^a	0.0618	0.0618	0.0618	0.0618
n^b	-0.0378	-0.0367	-0.0379	-0.0379
R^{2c}	0.9990	0.9991	0.9990	0.9990
$s_m{}^d$	0.0011	0.0011	0.0011	0.0011
LOD[e] μg mL^{-1}	0.0406	0.0354	0.0521	0.0303
LOQ[f] μg mL^{-1}	0.1354	0.1180	0.1735	0.1010

[a] m is slope
[b] n is intercept
[c] R is correlation coefficient
[d] s_m is the standard deviation of calibration curve. ($s_m = \sqrt{\frac{s_r{}^2}{s_{xx}}}$), s_r is standard deviation of regression
[e] LOD is limit of detection
[f] LOQ is limit of quantification

Through repeatability experiments conducted both within and between days, the method's precision was assessed.
In order to prepare three sets of standard solutions, 1.00 x 10^{-6} mol L^{-1} M IS was added. Injections were done 6 times (n = 6) over 3 successive days. Precision results are shown in Table 3.

Table 3. Inter-day and intra-day results of 9.00×10^{-6} mol L^{-1} the fungicide (2.83 μg mL^{-1} HEX, 2.77 μg mL^{-1} TEB)

	I. Day (n=6)[a]	II. Day (n=6)[a]	III. Day (n=6)[a]	Inter-day (n=18)[a]
$\overline{X}^b$	0.321	0.322	0.323	0.322
s^c	0.006	0.005	0.006	0.005
RSD%[d]	1.749	1.509	1.757	1.624
CL[e]	0.006	0.006	0.007	0.005
CL[e]	0.001	0.001	0.001	0.001

[a] n is the number of experiments.
[b] is the mean ratio of peak normalization.
[c] s is the SD of the mean response.
[d] RSD% is relative standard deviation, %
[e] CL confidence limit, (ts/√n)

Conclusion

The CE method proposed in this study, which aims at the combined determination of HEX + TEB; has important advantages such as cheapness, accuracy, selectivity, reliability and environmental friendliness. As far as we are aware, this verified approach is the first instance of HEX+TEB measurement via capillary electrophoresis and online preconcentration methods. The ICH guideline has been followed in the verification of the established method. Every ICH parameter produces positive outcomes.

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SUPPRESSION OF OSTRINIA NUBILALIS IN CORN WITH UAV – SPRAY APPLICATION

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Abstract

The corn borer (*Ostrinia nubilalis*), along with the cotton bollworm (*Helicoverpa armigera*) and the western corn rootworm (*Diabrotica virgifera*), is one of the most important corn pests. Over 600,000 hectares planted in corn in Serbia are threatened by this pest. Crop height and a lack of adequate machinery limit the effective control of this pest. In order to explore the possibility of suppressing corn borer by using unmanned aerial vehicles (UAVs) for crop protection, an experiment was conducted at the location of Sremska Mitrovica (Glac) close to the Sava River (44.953980, 19.664681). The date of the treatment was determined taking into account the intensity of the moth flight and the threshold. Treatments with the DJI AGRAS T30 drone were performed on 17 June 2022 and 20 July 2022 with 15 l/ha of working liquid with insecticide based on chlorantraniliprole (150 ml/ha), with the addition of adjuvant agent. In addition to the yield, the researchers also analyzed the place where the larvae of the corn borer penetrated into the stalk and the cob, the position of the hole in the trunk, cob damage, cob disease, and the number of pupae in the stalk. Within the trial variants, three randomized repetitions were designed in the form of microplots. The laboratory analysis of the sampled corn plants showed that the average yield in the control was 5.18 t/ha, while in the treatment it was 6.94 t/ha, which was a difference of 34% due to cob damages and diseases, stalk breakage and all other symptoms found only in the untreated variant. The difference in yield shows that the drone treatment is effective and economically justified.

Keywords: *UAV-spray application, insecticide, crop treatment, suppression, Ostrinia nubilalis.*

Introduction

Corn as a crop in Serbia is produced every year on about one million hectares. In production conditions in Serbia, besides cotton bollworm (*Helicoverpa armigera*) and the western corn rootworm (*Diabrotica virgifera*) most important pest is the european corn borer *(Ostrinia nubilalis)*. Every year, to a greater or lesser extent, about 600,000 hectares are threatened by corn borer. This pest significantly affects the yield volume as well as quality in wide production areas. The corn borer is a polyphagous insect from the family *Crambidae*, which belongs to the order of *Lepidoptera*. It feeds with wide variety of wild and cultivated plants, but in its diet it prefers to choose corn (Bourguet et al., 2000). The caterpillars of the corn borer feed on the leaves and the inner parts of the corn stem (Umeozor, O.C. et all, 1985), causing indirect damage, reducing the plant's physiological activity and thereby weakening its potential for yield (Patch, L.H. et all, 1951). Caterpillars can also cause direct damage, feeding on tassel and cobs, damaging grains, and often causing plant breaks and cobs falling off. Direct damage to the yield can be from 15 to 25%, and in certain years with favorable climate conditions for insects, these damages can exceed 50%. In addition to mechanical damage, the action of corn borer also creates conditions that favor the development of infections caused by various pathogens (*Fusarium spp. or Aspergilus spp.* infections), which produce mycotoxins, that can be very toxic if they enter the food chain of animals and humans (Franeta, F., 2019). In production conditions in Serbia, the corn borer usually has two generations (Čamprag, D., 1994, Almaši et al., 2002), but in some warm years and in conditions of warm and humid autumn, even third generation occurs. The flight of the first generation usually occurs from mid-May to the end of June. The flight of the second generation usually can start already at the beginning of July and last until the middle of August.

During the flight period of the corn borer moth, the corn is usually in advanced stages of development, in the tassel stage, when its height is a limiting factor for the use of conventional sprayers, so treatments can only be carried out with sprayers with high clearance. In recent years, the application of drones for crop treatments has been imposed as a new technique for treatments in protection against corn borer. Drones perform the treatments in a low flight above the crop (2-2.5 m), so the possibility for the treatment itself is not affected by the height of the crop, crop density or the condition of the soil moisture.

During the 2022 production season, an trial was conducted with the aim to evaluate the possibility of suppressing corn borer by drone DJI Agras T30 treatment, as well as evaluating the difference in yield achieved with this treatment.

Material and method

Experimental field was set up on the experimental field of the agricultural advisory service of Sremska Mitrovica at the Glac location (44.953980, 19.664681). Total area of the trial was 0,4 ha. The trial was carried out in two variants, a drone-treated variant and untreated variant. Within the trial variants, randomized repetitions of the size defined by the EPPO standard (10 x 4.2 m) were formed.

Image 1. –Trial location Glac, Sremska Mitrovica (44.953980, 19.664681), variants (treated and untreated) and micro plots positions (R1-R3)

Drone DJI AGRAS T30 was used for the treatment (https://www.dji.com/global/t30), which used XR11001VS nozzles. The treatment was performed in low flight from a height of 2 m. The swath width was 6 m. Speed of the drone during the treatment was 4 m/s.

Table 1. Environmental conditions during first treatment with drone

Date of treatment	6.17.2022.	Location	Glac, Sremska Mitrovica
Terain type	Flat, close to river Sava	Air temperature (℃)	22
Field area	0,4 ha	Air humidity (%)	52
Wind speed	1,5 (m/s)	Wind direction	NW

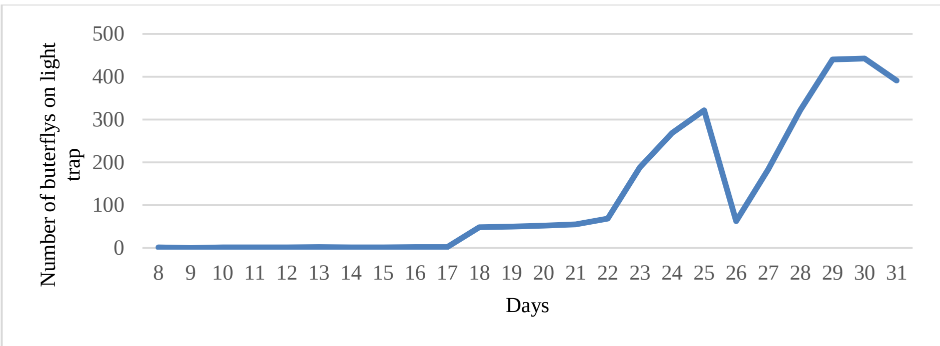
Table 2. Environmental conditions during second treatmant with drone

Date of treatment	7.20.2022.	Location	Glac, Sremska Mitrovica
Terain type	Flat, close to river Sava	Air temperature (℃)	24
Field area	0,4 ha	Air humidity (%)	40
Wind speed	1,5 (m/s)	Wind direction	NW

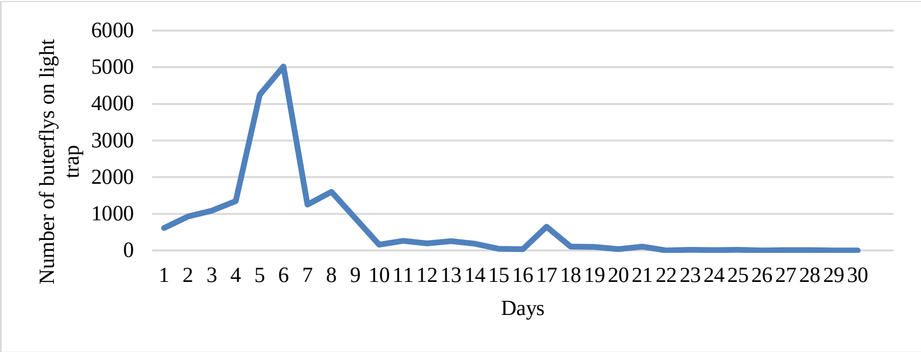
During the treatment, an insecticide based on chlorantraniliprole was used in the amount of 150 ml/ha, with the addition of a wetting agent based on fatty alcohol ethoxylate 20.3% and polydemethylsiloxane 1.0%. For the treatment, 15 l/ha of working liquid was used.

Image 2. –Maize insecticide treatmment with drone DJI AGRAS T30

In the treated variant, two treatments were carried out, the moment of which was determined by counting of moths on the nearby light traps and temperature thresholds for sawing the larvae of the corn borer.



Graph 1. Number of corn borer moths in May 2022. (locaton Laćarak)



Graph 2. Number of corn borer moths in June 2022. (locaton Laćarak)

The evaluation of the effects of the treatment was done 10 days before the harvest, by taking samples from all repetitions - micro plots within the variants of the trial (treated and non-treated). Three random samples (complete plants with roots) were taken from each micro plot - repetition, and in laboratory conditions an evaluation was carried out, which included: counting the punctures on the trunk of corn plants, locating the puncture site on the trunk, counting the punctures on the cobs, the number of pupae in the plants , occurrence of cob disease (*Fusarium spp. and Aspergillus spp.*), stem diameter, number of grain rows in the cob and weight of corn cobs by variant and repetition. Laboratory analysis of the sampled plants was done by longitudinal cross-section of the trunks and detailed examination of the cobs condition and cob measurement.

Results and Discussion

Stem and cobs damage analysis on the sampled plants showed that damage from the corn borer in the untreated variant of the experiment was present on all plants. On average, there were 6 punctures in the stem (min 2 max 16) and 6 punctures, in the cob (min 1 max 27). In 66% of the plant samples, a trunk breakage appeared under the tassel, on 22% plants under the cob, while in 12% of the plants no breakage occurred. The presence of pupae in the xylem on cross section of the trunks was found on 66% of the plants. The presence of fungal diseases was found on 45% of the cobs of the plant samples taken in untreated variant. On all cobs of plant samples taken in the untreated variant of the experiment, cob deformation was noted.

Image 3. - Damages caused by corn borer, larvae of the corn borer found in the corn trunk

Table 3. Laboratory evaluation of corn drone insecticide treatment samples

Varriant	Sample	No of holes in trunk	No of holes in cob	Stem brakage possition	No of pupae	Cob damage	Desease on cob	Stem diametar (mm)
Not treated rep 1	1	2	2	Below tassel	1	Yes	-	18
	2	6	1	Below cob	0	Yes	-	18
	3	16	12	Below cob	1	Yes	Fus. Asp.	17
Not treated rep 2	1	9	27	Below tassel	0	Yes	Fus. Asp.	17
	2	4	2	Below tassel	1	Yes	Fus. Asp.	17
	3	3	3	Below tassel	2	Yes	Fus. Asp.	17
Not treated rep 3	1	4	1	-	1	Yes	-	18
	2	3	3	Below tassel	0	Yes	-	19
	3	8	2	Below tassel	2	Yes	-	18
Treated 1	1	0	-	-	-	-	-	21
	2	0	-	-	-	-	-	24
	3	0	-	-	-	-	-	24
Treated 2	1	0	-	-	-	-	-	24
	2	0	-	-	-	-	-	23
	3	0	-	-	-	-	-	26
Treated 3	1	1	-	Below tassel	-	-	-	25
	2	0	-	-	-	-	-	24
	3	0	-	-	-	-	-	22

In the treated variant of the experiment only one sampled plant, in the cross-section of the trunk, had the presence of in the position below the tassel and above the cob. In the treated trial variant no cob deformations were detected, as well as no fungal diseases of any kind. In the untreated variant, the average thickness of the tree at the first internode was 17.67 mm, in the treated trial variant it was 23.67 mm. The grain rows number in the cob in the untreated variant was 14 (on 33% of cobs) and 16 (on 67% of cobs) while in the treated variant it was 16 (on 45% of cobs) and 18 (on 55% of cobs). Average weight of the cobs in treated variant was 162.6 g and in treated variant was 246.3 g with the difference in yield of 51.5%.

Table 4. Laboratory yield measurement

Varriant	Sample	Cob weight (g)	Average in repetition (g)	Average in variant (g)	Number of rows in cob
Not treated rep 1	1	98,7	165,3	162,6	16
	2	243			16
	3	154,3			16
Not treated rep 2	1	129,1	151,0		16
	2	139,7			16
	3	184,2			14
Not treated rep 3	1	212,2	171,4		14
	2	125,5			14
	3	176,6			16
Treated 1	1	297,2	255,5	246,3	18
	2	303,1			18
	3	166,1			16
Treated 2	1	210,2	212,6		16
	2	197,1			16
	3	230,4			18
Treated 3	1	345,6	270,8		16
	2	275,8			18
	3	190,9			18
Yield difference				**51,5%**	

After the harvest, the yield was measured with field weight. Results showed that the yield in the variant that was not treated was 5.18 t/ha, while in the treated variant yield was 6.94 t/ha, with the difference of 34% between variant.

Image 4. – Visual difference between cobs – treated and untreated trial variants

The difference in yield shows that the investment in crop treatment by drones is fully justified. The calculation of gross margin difference (based on the average yields in Serbia) shows that the investment in the drone for crop treatment pays off by treating corn on an area of 103 hectares in one season.

Conclusion

The results of the experiment show that drones for crop treatments during treatment with small amounts of treatment solution with properly designed and profiled air support can effectively and efficiently control the corn blight in the corn crop. The difference in yield achieved with this treatment justifies the investment not only in the treatment itself but also in the purchase of equipment. Drones for crop treatments can significantly contribute to pest protection in maize crops and reduce the risk of not only yield losses, but also cob and grain diseases, thus ensuring that the products of these pathogens do not enter the human and animal food chain.

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APPLICATION OF BACILLUS SPP. IN PLANT PROTECTION AND GROWTH PROMOTION OF CEREALS

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Abstract

The research objective is the application of new bacterial isolates as an alternative to chemical pesticides for controlling plant diseases caused by *Fusarium* spp., aiming to reduce reliance on conventional interventions, minimize environmental impacts, thus fostering a shift towards environmentally friendly plant disease management strategies. In this study, the effects of four *Bacillus* spp. (BHC 2.3, BHC 2.4, BHC 4.5, BHC 4.7) isolated from two soil samples on suppressing the *Fusarium* spp. plant pathogens (*F. poae*, *F. proliferatum*, *F. oxysporum* and *F. graminearum*), as well as their effect on seed germination (wheat, oat and barley) during the fungal infection were determined. Basic chemical analyses of soil samples used for bacterial isolation and plant growth promoting experiments were performed. Antifungal activity of isolates was tested *in vitro* on PDA medium, seed germination test was performed in Petri dishes on filter paper, while the ability of isolates to promote plant growth under semi-controlled conditions was also tested. Inhibition percentage of mycelial growth of *F. poae* was up to 20% (for isolate BHC 4.5) and up to 15% (for isolate BHC 4.7). Seed germination test indicated the effectiveness of seed inoculation by selected bacterial isolates. In the case of infected seeds, germination percentage was up to 80% (wheat) and up to 90% (barley and oat), without bacterial inoculation. On the other hand, germination percentage for infected seeds inoculated by all four applied isolates was increased. Results of the experiment performed under semi-controlled conditions showed that bacterial isolate BHC 4.5 had the best overall effect on the shoot length of all used cereals. The results of this study indicate the potential of different *Bacillus* spp. soil isolates for formulating new biofertilizers to be used in plant protection and plant growth promotion of cereals.

Keywords: *Bacillus* spp., PGP bacteria, seed germination, oat, barley and wheat seedling shoots.

Introduction

The effects of climate change and global warming has a strong impact on the agricultural sector. Higher air temperatures, relatively less rainfall, the rarity of continuous rainfall, the frequency of torrential rains can promote more frequent plant infections caused by pathogens (Modrzewska et al., 2022). A warmer climate and frequent extreme phenomena increase the incidence of pathogens, mostly fungi of the *Fusarium* genus, which are responsible for causing fusariosis in cereals (Siebold and von Tiedemann, 2012, West et al., 2012). The most commonly grown cereal plants include maize, wheat and rice (FAO, 2021) and diseases of these cereals result in a loss of nearly 1/6 of crops before harvest (Popp and Hantos, 2011). The major *Fusarium* species that infect many crops and represent some of the most critical soil-borne plant pathogen and are responsible for massive crop losses include *F. oxysporum*, *F. proliferatum* and *F. poae* (Baard et al., 2023).

Synthetic pesticides have a constant importance for protection of cereal crops, and chemical control is one of the most commonly used methods for preventing crops diseases. However, conventional plant protection strategies are costly and have been the subject of debate due to the possible soil pollution and the presence of residues in food (Braun et al., 2018; Ntalli & Menkissoglu-Spiroudi, 2011). Biological control has been considered as an effective alternative method to chemical control, as it poses no health risks and has an insignificant impact on the environment (Xu et al., 2020).
Rhizosphere soils from healthy plants that survived in a plot infested with phytopathogens are a good source for the isolation of microorganisms with biostimulator and biopesticides potential (Modrzewska et al., 2022, Huang at al., 2013). Using plant growth-promoting rhizobacteria (PGPR) in biological control of phytopathogens represent an eco-friendly strategy which is reported in variety of research papers (Mulk et al., 2022, Sharma et al., 2020). Microorganisms offer many advantages, as they can improve the overall fitness of plants by promoting their growth and resistance to abiotic stress and promote the suppression of pathogens (Modrzewska et al., 2022). The commercial application of bioinoculants is still limited, due to a lack of consistent field efficacy and poor product shelf life (Mulk et al., 2022, Valente et al., 2020).
The variety of microorganisms that might potentially be used as biological agents and promotors of cereal crop growth include bacteria and fungi, and they have proven to be effective in reducing fungal infection in the greenhouse and field trials (Modrzewska et al., 2022, Huang at al., 2013). Various PGPRs have been successfully isolated from the rhizosphere soils of healthy plants and the most studied ones belong to *Bacillus*, *Pseudomonas* and *Streptomyces* genus (Huang at al., 2013).
The objective of this paper is to test and compare biocontrol and plant growth promoting potential of bacteria isolated from two rhizosphere soil samples with different acidity. Biocontrol potential experiments against four *Fusarium* species is conducted, while the plant growth promoting potential of bacterial isolates through the seed germination test and seedling parameters of tree cereal plants (oat, wheat and barley) is also examined.

Material and Methods

The isolation of bacterial isolates was conducted from soil sampled in the territory of Lazarevac (soil sample BHC 2) and Smederevska Palanka (soil sample BHC 4). The basic soil chemical parameters are shown in Table 1:

Table 1. Basic soil chemical parameters

Soil sample	pH		$CaCO_3$	P_2O_5	K_2O	Humus content
	KCl	H_2O	%	mg·100 g^{-1}		%
BHC 2	5.49	7.33	< LOD*	2.94	25.9	2.59
BHC 4	7.80	8.37	13.2	28.4	39.3	3.79

*The limit of detection (LOD) 0,04%

For the isolation of bacteria from soil samples, a series of decimal dilutions of the soil suspension from 10^{-2} to 10^{-6} was prepared. To ensure the isolation of bacteria from the genus *Bacillus*, tubes with dilutions from 10^{-4} to 10^{-6} were incubated for 15 min at 80 °C in a water bath. One ml of these dilutions was then inoculated onto Petri dishes with Nutrient agar. Petri dishes were incubated at 28 °C for 72 h, and pure cultures of bacterial isolates were obtained by several rounds streaking on Nutrient agar. The obtained pure cultures were stored on slanted Nutrient agar at 4 °C.

The antagonistic effect of bacterial isolates against different *Fusarium* species (*F. poae*, *F. proliferatum, F. oxysporum* and *F. graminearum)* was evaluated on potato dextrose agar (PDA). Fungi used for testing the antagonistic activity of bacterial isolates were cultured on PDA medium for 72 h at 28 °C, from which sterile plugs of fungal mycelium (approximately 2 mm in diameter) were excised for further use. A plug of fungal mycelium was placed in the centre of a Petri dish, and 20 µL of overnight bacterial cultures was inoculated approximately 1 cm from the edges of the Petri dish (on two sides of the Petri dish). Petri dishes were then incubated at 28 °C, and the results were obtained after 7 days of incubation. After incubation, the diameter of fungal colony growth was measured in millimetres and compared with the bacteria-free control. Tests were conducted in triplicate, and the antagonistic activity of bacterial isolates was determined based on the following equation (Ogbebor & Adekunle, 2005): Inhibition (%) = [(Fungal control (mm) - Treatment (mm))/Fungal control (mm)] x 100.
The ability of bacterial isolates to induce germination of wheat (*Triticum aestivum*), oat (*Avena sativa*) and barley (*Hordeum vulgare*) seeds (infected and non-infected by *F. poae*, *F. proliferatum, F. oxysporum* and *F. graminearum*) was accessed *in vitro* on Petri dishes by using filter paper method. Ten seeds of each plant species were soaked in over-night bacterial culture for each isolate (BHC 2.3, BHC 2.4, BHC 4.5 and 4.7), while non-inoculated seeds were used as the control sample. Further, seeds infected with each fungal pathogen were used as fungal infection control, while the seeds infected by fungi and inoculated by each bacterial isolate were also used. Petri dishes were kept for two weeks in a transparent sealed box. Relative seed germination index (RSGI%) was estimated as described by Knezevic et al. (2021). All tests were done in three independent replications.
For PGP test, seeds of wheat, oat and barley were placed in seedling trays (three seeds per one hole) in triplicates. The following treatments were applied: control (Ø, no fungal or bacterial inoculation), fungal control (FØ, fungal infection), bacterial inoculation by four isolates (BHC 2.3, BHC 2.4, BHC 4.5 and BHC 4.7), as well as mixed inoculation by *F. poae* and bacterial isolates (F-BHC 2.3, F-BHC 2.4, F-BHC 4.5 and F-BHC 4.7) for each cereal. The experiment was kept under semi-controlled conditions for 21 days, after which the shoots of all seedling were measured.

Results and Discussion

The soils used in this research (BHC 2 and BHC 4) belong to a group between acid and moderately acidic (pH value in KCl 5.49) and alkaline (pH value in KCl 7.80) soils, respectively (Manojlović et al., 1969). A total of four *Bacillus* spp. isolates (BHC 2.3, BHC 2.4, BHC 4.5 and BHC 4.7) isolated from soil samples were used in this research. In literature, effectiveness of different *Bacillus* species has been previously demonstrated against *F. graminearum, F. poae* and other fusarium head blight (FHB) causative agents in cereals, such as barley and wheat (Mischler et al., 2024; Zanon et al., 2024). Among tested fungal pathogens, used bacterial isolates showed the strongest antagonistic effect against *F. poae.* The percentage of *F. poae* inhibition was 20%, 15% 12% and 7% by BHC 4.5, BHC 4.7, BHC 2.4 and BHC 2.3, respectively. In addition, BHC 4.7 and BHC 4.5 showed weak antagonistic effect against *F. graminearum* (Figure 1). Similarly, Bjelic et al. (2017) reported effectiveness of *Bacillus* spp. isolated from the soils of Province of Vojvodina against *F. proliferatum* and *F. oxysporum*.

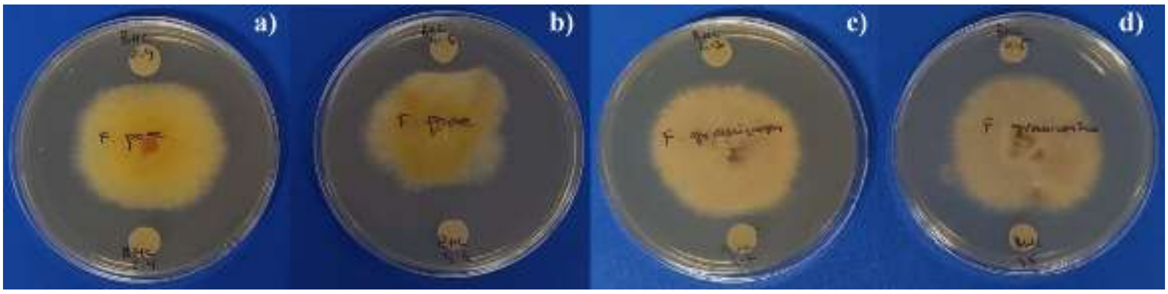

Figure 1. Antifungal potential of *Bacillus* spp. isolates against *F. poae* and *F. graminearum. a)* BHC 2.4 + *F. poae;* b) BHC 4.5 + *F. poae*; c) BHC 4.7 + *F. graminearum;* d) BHC 4.5 + *F. graminearum*

Relative seed germination index percentage (RSGI, %) of seeds infected by *F. poae* was 70% (barley), 75% (oat) and 37.5% (wheat). Application of all four bacterial treatments increased RSGI of all cereals. BHC 2.4 treatment increased seed germination for infected barely and oat seeds, with the strongest effect on wheat seeds, in comparison to the uninfected control. Furthermore, application of all bacterial treatments (except F-BHC 2.3 in oat) increased the root length of all cereals, in comparison to infected control (Figure 2).

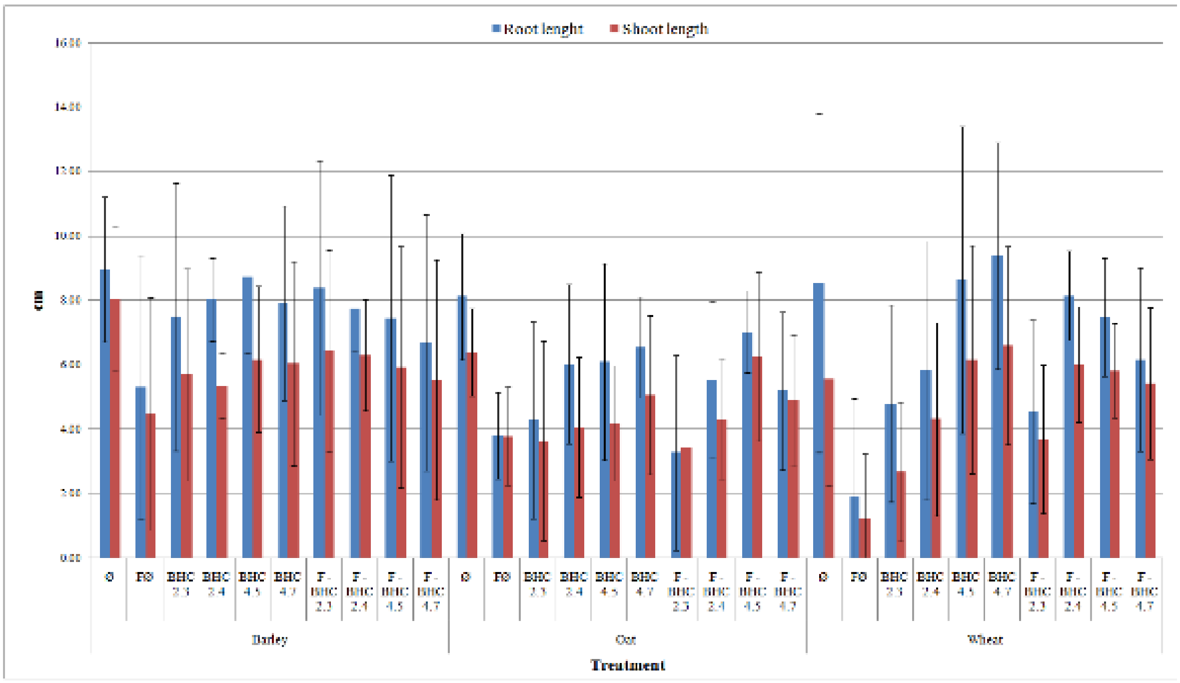

Figure 2. Impact of bacterial treatment/fungal infection on root and shoot length of barley, oat and wheat seedlings.

Similar results were obtained for the shoot length, where only treatments BHC 2.3 and F-BHC 2.3 had values of shoot length (oat) lower than infected control. Previously, it has been shown that the application of *Bacillus* spp. improved soybean seed quality compared to control, as well as the germination of garden pea seeds in the presence of *F. proliferatum* infection (Miljaković et al., 2022, Miljaković et al., 2024). In addition, *B. pseudomycoides* improved seed germination of alfalfa in the presence of *F. oxysporum* (Knezevic et al., 2021).
Further, PGP test showed that applied bacterial treatments increased fresh plant biomass of infected wheat, barley and oat in comparison to the fungal control (FØ). The highest increase of fresh biomass in comparison to the infected control was recorded by BHC 4.5 treatment for oat (18.43%), followed by wheat (14.04%) and barley (2.35%), while the other bacterial

treatments increased fresh biomass in a range from 0.31 (wheat) - 3.70% (barley). Similar, it has been shown that PGP bacteria (most commonly from *Bacillus*, *Brevibacterium*, *Pseudomonas* and *Arthrobacter* genus) can increase biomass, leaf area, plant height, root length, dry weight and fresh weight of different cereals including wheat, barley, maize and rye (Wijekoon and Weerasinghe, 2024). The results of this research confirm positive effects of bacterial inoculation and the possibility of using *Bacillus* spp. for improving seed germination and seedling biomass infected by *Fusarium* species.

Conclusions

In conclusion, this study demonstrates the promising potential of selected *Bacillus* spp. soil isolates as effective alternatives to chemical pesticides for managing plant diseases caused by *Fusarium* spp. The isolates, particularly BHC 4.5, exhibited notable antifungal activity against *Fusarium* pathogens while also promoting seed germination and enhancing plant growth in wheat, oat, and barley under semi-controlled conditions. These findings highlight the feasibility of utilizing these bacterial isolates in formulating biofertilizers, thereby offering sustainable and environmentally friendly strategies for plant disease management and crop enhancement in cereals. By reducing reliance on chemicals, these bio-based approaches hold significant promise for mitigating environmental impacts associated with conventional pesticide use allow a transition towards more eco-friendly agricultural practices.

Acknowledgement

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ORGANIC AGRICULTURE

VARIATION OF ORGANIC CARBON CONCENTRATION IN BUCKWHEAT GROAT WASTE EXTRACTS

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Abstract

It is generally accepted that organic carbon plays a crucial role in soil health and plant growth~~,~~ because it serves as a source of energy and nutrients for soil is microorganisms and is essential for maintaining soil fertility, supporting plant growth, and contributing to overall ecosystem health. Carbon entering with solid materials has a greater impact on the soil, but for a quick effect, it is more convenient for plants to use liquid extracts of organic carbon, which can be sprayed on the leaves of plants or inserted into the soil. Various organic materials (peat, compost) are often used to obtain such liquid extracts. "Ekofrisa", a company which produces organic buckwheat groats in Lithuania, accumulates 600 tons of raw biomass (BM), 700 tons of buckwheat husk (BH) per year. Therefore, how the concentration of water-soluble (dissolved) organic carbon (DOC) from buckwheat biomass and buckwheat husk on such factors as the extraction method (mixing, ultrasound, heating), extraction duration (3, 6, and 9 days at room temperature without mixing), without mixing (at temperature 50, 70, and 90 °C for 3, 6, 9 hours), whit mixing (at temperature 30, 50, 70, and 90 °C for 3, 6, 9 hours), and in an ultrasonic bath (3, 6, and 9 hours at 30, 50, and 60°C temperature without mixing) the research was carried out in this study. The concentration of organic carbon in the extracts was determined using a TOC analyser (TOC-L, Shimadzu, Japan) following the LST EN 1484:2002 standard. The best results were obtained when the biomass was mixed with water for 9 hours at a temperature 90 °C.

Keywords: *Buckwheat waste, Extraction, Organic carbon, Liquid fertilizers.*

Introduction

Soil organic carbon (SOC) plays a pivotal role in soil health and plant productivity. As a fundamental component of organic matter, it influences various soil properties and processes, contributing to the sustainability of agricultural systems and the environment (Blanchart, 2007; Murphy, 2015). Organic carbon is derived from decomposed plant and animal residues, microorganisms, and other organic substances. Its presence in the soil improves structure, water retention, nutrient availability, and microbial activity, all of which are essential for healthy plant growth. SOC increases the soil's water-holding capacity, reducing the need for frequent irrigation and helping plants withstand drought conditions. Furthermore, organic carbon fosters a thriving microbial ecosystem within the soil. Microorganisms, such as bacteria and fungi, decompose organic matter, releasing nutrients and promoting soil fertility. These microorganisms also help suppress soil-borne diseases, contributing to healthier plants and higher crop yields (Ontl, 2012; Tang, 2022; Martínez-García, 2018).
Many soils in Europe – largely those under grassland, forests, and natural vegetation – are accumulating carbon and thus act as carbon stores or sinks. However, despite many soils can sequester C from the atmosphere, the process is slow, easily reversible, and time limited. In addition, soil disturbance during intensive farming increases soil erosion and nutrient leaching. Also, the cultivation and drainage of soil causes significant CO_2 emissions and the

total amounts of organic carbon held in soils across Europe are gradually decreasing (Baritz, 2010; Grilli, 2021; Soil carbon, 2023).
Restoring organic matter levels requires understanding the ecological processes essential for its storage. Effective restoration techniques can help rejuvenate terrestrial ecosystem functions and increasing soil organic C contents through sustainable soil management (SSM) practices can improve soil health, the efficiency of food production.
Soil organic carbon represents the carbon fraction of organic compounds in soil. Since measuring soil organic matter (SOM) directly is challenging, laboratories typically measure and report soil organic carbon SOC instead. Organic matter is crucial for soil's physical, chemical, and biological properties, which are vital for its proper functioning and, by extension, human society. SOM is mostly composed primarily of carbon, hydrogen, and oxygen, but organic residues contain small amounts of other elements such as nitrogen, phosphorus, sulphur, potassium, calcium, and magnesium. There are 4 different fractions that differ greatly in size, circulation time and composition in the soil: dissolved organic matter; particulate organic matter; hummus; resistant organic matter (Soil organic carbon, 2022).
One of the most recent and effective methods is enriching soil and plants with organic carbon is the dissolved organic matter (DOM) or dissolved organic matter (DOC). DOM is widely known to play a dominant role in several soil processes. Furthermore, DOM molecules contain nutrients such as N, P, and S, and DOM dynamics thus affect their mobility and availability.
Large variations in DOC are found among biomes across space and the soil DOC concentration declines exponentially along soil depths. DOC generally accounts for < 1% of total organic carbon in soils, and DOC in 0–30 cm contributes more than half of the total DOC in the 0–100 cm soil profile. Variations in DOC are primarily controlled by soil texture, moisture, and total organic carbon (Guo, 2020).
Since soil properties greatly influence the amount and quality of DOM and it is difficult to predict the dynamics of DOM in the soil in detail, it is reasonable to use pre-prepared organic carbon extracts (Filep, 2011; SOIL, 2024).
After assessing the deteriorating carbon situation in soils and possible ways to improve it, this work aimed to determine the optimal conditions for the extraction of water-soluble (dissolved) organic carbon (DOC), when the carbon source is food (buckwheat groats) industry waste.

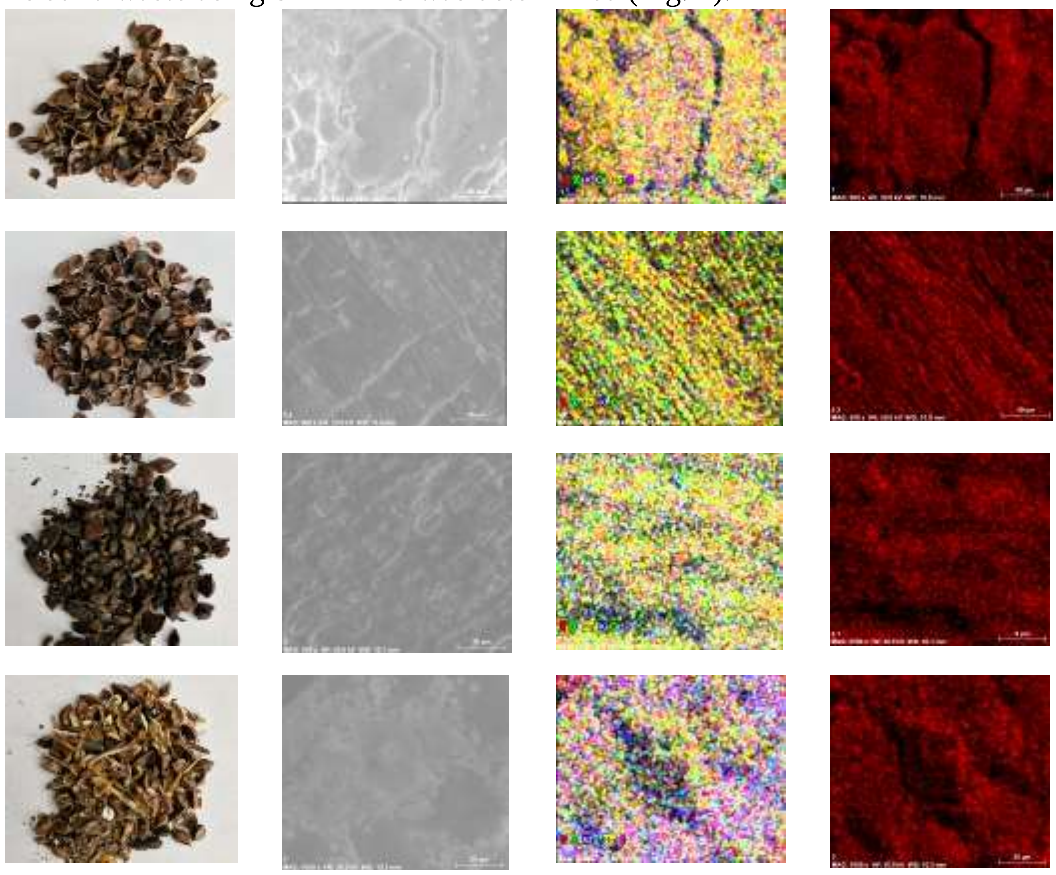
Materials and Methods

Waste from the organic buckwheat groats production company "Ekofrisa" (Lithuania) was used as organic carbon sources. “Ekofrisa” accumulates 600 tons of raw biomass (BM) and 700 tons of buckwheat hulls (BH) per year. To evaluate the raw materials in detail, different types of buckwheat husk were collected and analyzed: partially cleaned buckwheat husk (BH1), cleaned buckwheat husk (BH2), and partially composted buckwheat husk (BH3).
Whole raw materials with a size of 3.15–7.00 mm was used for analysis. This fraction was separated using a RETSCH (Retsch, GmbH, Haan. Germany) woven wire.
The amount (%) of organic matter in this waste was determined according to the LST EN 13039:2012 standard. Samples of the raw materials were weighed with an accuracy of ±0.001 g, and reduced to ash at 900 °C (1 hour) in a muffle furnace. The amount of organic matter in the samples was calculated from the weight loss (UNE EN, 2012).
The morphological structure and chemical composition of solid raw materials was determined using a scanning electron microscope and energy dispersive spectroscopy (SEM-EDS) using the scanning electron microscope model S-3400N by Hitachi company (Tokyo, Japan). It has a built-in Bruker Quad 5040 EDS detector.

Samples for extraction were prepared by ratio waste: water = 1:50. The extracts were prepared: 3, 6, and 9 days at room temperature without mixing; without mixing and heating at 50, 70, and 90 °C for 3, 6, 9 h; mixing for 3, 6, 9 hours and heating at 30, 50, 70 and 90 °C and in an ultrasonic bath for 3, 6 and 9 hours at 30, 50 and 60 °C.
The concentration of dissolved organic carbon in the extracts was determined using a TOC analyzer (TOC-L, Shimadzu, Japan) following the LST EN 1484:2002 (EN 1484:1997) standard.
The presented results were calculated with 95% probability (the significance level was $p \leq 0.05$) and are expressed as the arithmetic mean. One-way analysis of variance (ANOVA) was used to evaluate the differences of the means between groups. Research was carried out in 2023-2024 at the Department of Physical and Inorganic Chemistry of the Kaunas University of Technology.

Results and Discussions

According to Jha R., buckwheat is one example of an underutilized crop species, despite having abutant nutritional and bio-active components (Jha, 2024). The same can be said about the waste produced during the production of buckwheat groats. Currently, they are collected on the territory of the company, part of this waste is composted, and another part of buckwheat husks is used as fuel in the company's bio-boiler. The chemical composition of this solid waste using SEM-EDS was determined (Fig. 1).



The amount of organic matter 98.27%

The amount of organic matter 98.54%

The amount of organic matter 81.51%

The amount of organic matter 96.16%

Fig. 1. Photos, SEM-EDS images and amount of organic matter: a – BH1; b – BH2; c – BH3; d – BM.

In principle, a very similar morphological structure can be seen in BH SEM images. However the structure of the sample BH3 is slightly split, and this corresponds to the state of buckwheat hulls (they are already slightly decomposed, i.e. partially composted). From the

figure, all samples contain C, O, K, Mg, Ca, S, and the BM additionally contains P (Fig. 1 d). Therefore, in agreement with the statements of Wloch, buckwheat waste contains various nutrients necessary for plants (Wloch, 2016). However, since the object of this work is organic carbon, the amount of organic matter was determined (Fig. 1).
The data presented in Fig. 2 show that when the samples are poured with water (ratio waste:water = 1:50), DOC concentration after 9 h is from 70.12±0.61 mg/L to 168.64 ±2.82 mg/L when water is poured on buckwheat husk. However, when producing the extract from BM after 9 h at room temperature, DOC concentration is nearly 600 mg/L. This result prompted the investigation and evaluation of the effects of mixing, temperature, and ultrasound on the concentration of DOC.

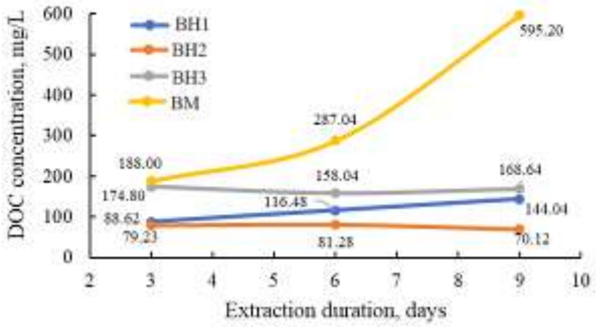


Fig. 2. Dependence of DOC concentration on extraction duration at room temperature (20–21 ºC) without mixing.

Analysis of the effect of temperature (Fig. 3) shows that it has a positive influence on the concentration of DOC. Especially considering the extraction duration of 6 and 9 hours. It can also be seen from the presented data that a high concentration of DOC concentration was found in extracts made from BH3 (783.60±4.25 mg/L) and BM (745.60±2.32 mg/L) samples at 90 ºC temperature after 6 and 9 hours.

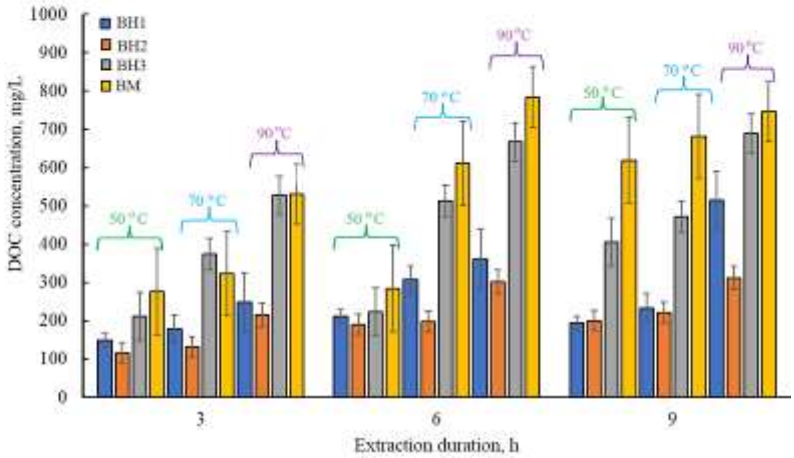


Fig. 3. Dependence of DOC concentration on extraction duration at 50, 70, and 90 ºC temperature without mixing.

A similar dependence of DOC concentration on the extraction time and temperature was also found when the extracts were produced by mixing (Fig 4).

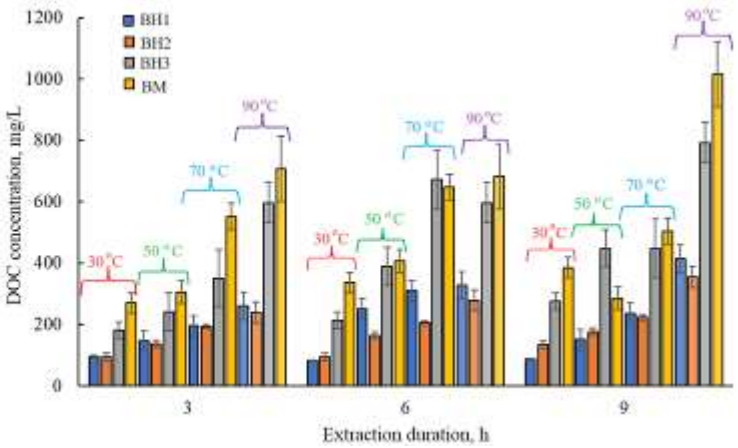


Fig. 4. Dependence of DOC concentration on extraction duration at 30, 50, 70, and 90 ºC temperature with mixing.

High DOC concentration (596.00–793.20 mg/L) were determined in BH3 and MB extracts at 90 °C not only after 6 and 9 h, but also after 3 h. The highest DOC concentration (1015.20±3,92 mg/L) was found in the extract prepared from BM at 90 ºC temperature while mixing for 9 hours.
Despite the good results obtained with mixing and heating, the effect of ultrasound on the concentration of DOC in the extracts was also investigated in this study (Fig. 5).

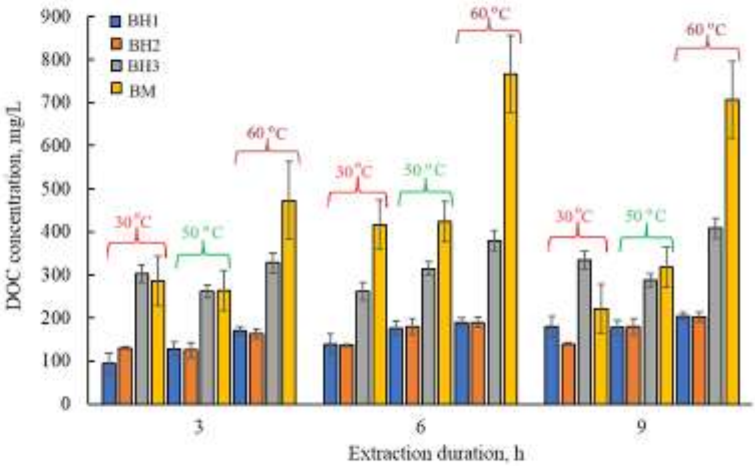


Fig. 5. Dependence of DOC concentration on extraction duration at 30, 50, and 60 ºC temperature in an ultrasonic bath without mixing.

It should be noted that in the investigated temperature range (30–60 ºC), the influence of ultrasound on DOC concentration is not as great as that of mixing. The highest values of DOC concentration during the production of extracts in an ultrasonic bath were determined when MB was extracted with water at a temperature of 60 ºC for 6 and 9 hours (766.60±2.48 mg/L and 706.80±1.98 mg/L respectively). It is likely that by further increasing the temperature, extending the extraction time, or crushing the solid waste, even higher concentrations of DOC in the aqueous extracts could be obtained.

Conclusions

By using buckwheat groats production waste (especially biomass) in a simple way (mixing and heating) it is possible to produce aqueous extracts with a high DOC concentration, which are suitable for direct application to plants and soil. It is also likely that such solutions can be

used as an additive in concentrated liquid fertilizers, but scientific experimental studies are needed to substantiate this hypothesis.
In conclusion, the incorporation of organic carbon into soil management practices offers numerous benefits for both soil health and plant growth. By understanding and leveraging these benefits, farmers and land managers can enhance soil quality, boost plant resilience, and contribute to sustainable agricultural practices. Thus, this work will continue in order to produce a product of maximum quality and efficiency.

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EFFECTS OF HARVESTING TIME ON ESSENTIAL OIL YIELD AND COMPONENTS OF ORGANICALLY GROWN THYME (*Thymus vulgaris* L.)

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Abstract

Timing of essential oil-bearing plant harvest is a key determinant in achieving higher oil content and quality in the essential oil production. Thyme (*Thymus vulgaris* L.), an herbaceous perennial plant from the Lamiaceae family, is widely cultivated for its essential oil. Thyme essential oil has attracted much attention, due to its usage in medicine, pharmacy, perfumery-cosmetic and food industries. The aim of this study was to determine the effects of harvest time on essential oil content and composition of organically grown thyme under continental type of climate. The study was carried out in growing season of 2021 to determine the effects of harvesting hours on essential oil content and composition of thyme using a completely randomized design with four replications. Thyme was harvested at a two-hour intervals starting at 6:00 and ending at 18:00. Essential oils of freshly harvested and dried plants were extracted by hydro-distillation for three hours and essential oils were analyzed by gas chromatography to determine the composition and quality of the essential oil. Essential oil content of thyme varied slightly among the harvest time of the day. The highest essential oil yield was obtained from early morning harvest hours. More than 27 essential oil components were found in the essential oil of each harvest hour. Thymol, γ-terpinen, p-cymene and carvacrol were the main essential oil components of all harvest hours. The major essential oil components did not significantly fluctuate among the harvesting hours. The results of this study showed that the best harvesting hours of thyme for higher essential oil yield were between 6:00 and 10:00 AM in the morning.

Keywords: *Harvesting time, Essential oil, Thyme, Organic farming.*

Introduction

Thymus vulgaris is a perennial plant from the Lamiaceae family native to southern Europe. It is about 15-30 cm tall and 30-40 cm wide. Thymus species are grown in most of the European countries, especially France, Spain, Italy, Bulgaria and the Portuguese Greece, Turkey, Israel, Morocco and North America (Stahl-Biskup and Sáez, 2002; Maghdi and Maki, 2003). Today there are approximately 250 taxa divided into eight divisions (214 species and 36 subspecies) are accepted.

Thyme, used for food and medical purposes, which has great economic importance due to its monoterpene derivatives such as thymol, γ-terpinen, p-cymene and carvacrol (Aeschbach *et al.,* 1994; Grigore *et al*., 2010).. Thyme was known to have hepatoprotective properties and its effectiveness as an expectorant, anti-acne agent, and fungicidal and antiviral effects. Thyme, used for food and medical purposes, has great economic importance due to its monoterpene derivatives such as thymol, γ-terpinen, p-cymene and carvacrol. Thyme was known to have hepatoprotective properties and its effectiveness as an expectorant, anti-acne agent, and fungicidal and antiviral effects (Bukovska et al. 2007). These numerous industrial applications are mainly due to its antioxidant, antimicrobial, anti-inflammatory and anticancer effects (Miura and Nakatani, 1989; (Katsiotis, *et al*., 2009).

Essential oil content and components of thyme vary depending on the development stages in which they are harvested. Depending on the harvest periods, the essential oil content of the thyme varies between 0.4-4% (Ozguven and Tansi, 1998; Carlen *et al*., 2010). The major component of thyme, thymol, is active against enterobacteria and cocci bacteria. Thyme may also improve liver function and act as an appetite stimulant. Thyme used as a gargle is beneficial in the treatment of laryngitis and inflammation.

Most of the essential oil bearing plants is mostly harvested at the beginning of the flowering or during the flowering periods. (Bagdat, 2006; Karik et al., 2007; Hazrati et al. 2022). The content and composition of the essential oil varies with harvest hours of a day. Changes in the content and composition of essential oils at different hours of a day have been less studied (Salehi and Hazrati, 2017; Salehi et al., 2017; Kaya et al., 2013). Secondary metabolite content and composition are under influence of environmental factors such as soil profile, plant nutrition, light, photoperiod, temperature, day/night temperature ratio, precipitation, moisture, irrigation conditions and altitude (Silva et al., 1999; Rao et al., 2001; Figueiredo et al. 2008; Ramezani et al., 2009; Hassiotis et al., 2010; Bufalo et al., 2015). Therefore, to determine the most optimum harvest hour, it is necessary to consider essential oil content and essential oil composition to have maximum economic gain from the cultivated essential oil bearing plants. The purpose of this study was to determine an optimum harvest time (hour) in a day for higher essential oil yield and higher thymol content of thyme.

Material and Methods

The study was carried out at the Experimental Field of the Faculty of Agriculture, Erciyes University in Turkey, in 2021 growing seasons. One-year-old thyme plant was used for determining the best harvest time. At the beginning of the flowering stage, plants were harvested at two-hour intervals starting from 6:00 to 18:00 in 25 June 2021. Freshly harvested plant samples in each harvesting hour were sent for essential oil extraction and shadow drying to determine essential oil contend of both fresh and dry thyme plant. The fresh and shadow dried thyme plants were steam distilled for 3 h using a Clevenger type apparatus. The oil ration was expressed as w/v with respect to the fresh and dry herb of the initial material.

Analysis of the essential oil was carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and Rtx®-5 capillary columns (60 m x 0.32mm, 0.25µm film thickness). Helium (99.9%) was the carrier gas at a flow rate of 1 mL/min. MS transfer line temperature was 250 °C, MS Ionization source temperature was 220oC, the injection port temperature was 220 °C. The samples were injected with 250 split ratio. The injection volume was 1µL. The oven temperature was programmed to 50 °C to 220 °C at 3 °C /min. The structure of each compound was identified by comparison of their mass spectrum (Wiley) using the Xcalibur software, version 2.0.7.

Results and Discussion

The essential oil content and components of fresh and dry herbage of thyme were given in Table 1, Table 2 and Table 3. The essential oil content significantly varied among the harvesting hours (Table 1).

Essential oil contents of fresh herbage varied between 0.75 and 0.98%. The lowest essential oil content was obtained from 12:00 PM harvesting hour, while the highest was obtained from 6:00 o’clock harvesting hour. When dry herbage was in consideration, essential oil content varied between 1.92 and 2.40 %. Like fresh herbage, the highest essential oil content was obtained from early morning harvest (6:00 AM), and the lowest was obtained at 2:00 PM harvesting hour. Our results are in good agreement with the findings of Ozguven and Tansi, (1998); Carlen *et al.*, (2010) that the essential oil content of the thyme within the reported limits.

Table 1. The effect of harvest hours of thyme on essential oil content.

Harvesting hour	**Essential oil content (%)**	
	Fresh herbage	**Dry herbage**
6:00 AM	0.98$_{\pm 0.01}$	2.40$_{\pm 0.02}$
8:00 AM	0.84$_{\pm 0.01}$	1.98$_{\pm 0.02}$
10:00 AM	0.86$_{\pm 0.02}$	2.23$_{\pm 0.02}$
12:00PM	0.75$_{\pm 0.01}$	1.95$_{\pm 0.01}$
2:00 PM	0.77$_{\pm 0.01}$	1.92$_{\pm 0.03}$
4:00 PM	0.79$_{\pm 0.02}$	1.97$_{\pm 0.01}$
6:00 PM	0.96$_{\pm 0.03}$	1.97$_{\pm 0.01}$

Table 2. The effect of harvest hours of fresh thyme on essential oil components

Components	RT	Harvesting hours of fresh herbage						
		6:00 AM	8:00 AM	10:00 AM	12:00 PM	2:00 PM	4:00 PM	6:00 PM
α-Pinene	3.63	0.90	0.74	0.65	0.62	0.85	0.68	0.69
α-Thujene	3.69	1.34	0.96	0.88	0.92	1.17	0.77	0.97
Camphene	4.34	0.67	0.65	0.51	0.42	0.68	0.78	0.46
β-Pinene	5.13	0.25	0.23	0.18	0.20	0.25	0.19	0.22
β-Myrcene	6.48	1.52	1.31	1.08	1.06	1.43	1.09	1.22
δ-4-Carene	6.88	1.20	1.06	0.91	0.86	1.05	0.93	1.20
Bornylene	7.40	0.41	0.41	0.32	0.29	0.41	0.32	0.37
Eucalyptol	7.59	0.83	0.78	0.57	0.90	0.72	0.63	0.72
β-Phellandrene	7.67	0.14	0.11	0.11	0.67	0.07	0.11	0.16
γ-Terpinene	7.96	9.40	10.10	11.72	9.60	7.38	8.85	8.71
p-Cymene	10.28	15.31	18.05	19.12	17.14	16.21	15.13	15.09
1 Octen 3 ol	16.13	0.44	0.42	0.45	0.51	0.42	0.45	0.42
Cis-Sabinene hydrate	16.54	0.86	0.87	1.03	1.04	0.88	0.94	0.80
Camphor	18.31	0.39	0.40	0.44	0.42	0.34	0.36	0.71
Linalool	19.87	1.92	2.12	1.30	1.70	1.19	1.12	1.44
Linalyl acetate	20.24	0.19	0.12	0.17	0.06	0.13	0.21	0.22
Caryophyllene	21.35	1.55	1.32	1.98	1.40	2.47	4.04	2.41
Thymyl methyl ether	21.62	0.63	0.77	0.25	0.20	0.62	0.25	0.22
Terpinene 4-ol	21.74	0.68	0.50	1.44	0.54	1.06	1.75	1.81
Carvacrol methyl ether	22.00	0.23	0.15	0.18	0.21	0.36	0.25	0.17
β-Selinene	24.02	0.09	0.05	0.18	0.08	0.20	0.23	0.15
Borneol	25.33	1.79	1.51	1.00	1.42	1.96	1.36	1.50
Caryophyllene oxide	34.79	0.76	0.74	0.58	0.62	0.60	0.50	0.33
Thymol	41.73	46.30	45.42	44.90	43.19	38.63	35.20	32.74
Carvacrol	32.09	2.95	5.11	5.19	9.43	12.37	15.27	23.22

Twenty-five essential oil components was obtained in the essential oil of fresh herbage (Table 2). Thymol, carvacrol, p-Cymene and γ-terpinene were the major essential oil components. When major essential oil components were compared among the harvesting hours, they

fluctuated among the harvesting hours. Thymol which is the most important essential oil components of thyme oil varied among harvesting hours. The highest thymol rate was obtained early morning harvest hours with 46.30%. On the other hand, carvacrol content was low in the early morning harvest hours. Thymol content negatively correlated with carvacrol content. p-Cymene and γ-Terpinene ratios also fluctuated among harvesting hours.
The analysis of dry herbages resulted in detection of 26 essential oil components (Table 3). Like fresh herbage, the major essential oil components were thymol, carvacrol, p-Cymene and γ-terpinene. Essential oil components varied among harvest hours. The highest thymol rate was obtained for the early morning harvest hours. Thymol contains decreased with the midday and late harvest hours. Senatore (1996) stated that the best time for thyme harvest was during or immediately after the full flowering stages. In the present harvesting study, thyme plant was harvested at the beginning of flowering stage.

Table 3. The effect of harvest hours of dry thyme on essential oil components.

Components	RT	Harvesting hours of dry herbage						
		6:00 AM	8:00 AM	10:00 AM	12:00 PM	2:00 PM	4:00 PM	6:00 PM
α-Pinene	3.63	0.14	0.70	0.61	0.59	0.46	0.67	0.54
α-Thujene	3.69	0.18	0.93	0.96	1.66	0.71	1.18	0.96
Camphene	4.34	0.05	0.56	0.41	0.52	0.27	0.44	0.34
β-Pinene	5.13	0.07	0.22	0.20	0.17	0.15	0.21	0.19
Sabinene	5.43	0.08	0.03	0.47	0.00	0.14	0.30	0.19
β-Myrcene	6.48	0.36	1.23	1.15	1.03	0.94	1.31	1.28
δ-4-Carene	6.88	0.41	1.04	1.26	0.94	1.12	1.92	1.77
Bornylene	7.40	0.11	0.36	0.38	0.29	0.30	0.44	0.44
Eucalyptol	7.59	0.28	0.80	0.68	0.56	0.40	0.47	0.43
β-Phellandrene	7.67	0.00	0.11	0.16	0.11	0.16	0.25	0.25
γ-Terpinene	7.96	11.23	9.45	13.70	8.10	9.87	9.96	10.14
p-Cymene	11.20	16.35	18.20	16.61	15.42	16.01	15.22	14.83
α-Terpinolene	10.02	0.15	0.29	0.06	0.07	0.00	0.05	0.32
1 Octen 3 ol	16.13	0.37	0.43	0.21	0.51	0.47	0.30	0.40
Cis-Sabinene hydrate	16.54	0.85	0.97	0.51	0.89	0.96	0.57	0.96
Trans-sabinene hydrate	19.69	1.95	1.97	0.88	0.60	1.04	1.53	1.67
Linalool	19.87	1.24	1.57	0.46	1.65	1.46	1.12	1.42
Linalyl acetate	20.24	0.61	0.33	0.22	0.12	0.32	0.11	0.31
Caryophyllene	21.35	3.36	2.78	2.69	1.60	2.50	2.72	1.99
Thymyl methyl ether	21.62	0.18	0.07	0.00	0.95	0.14	0.33	0.56
Terpinene 4-ol	21.74	2.81	2.94	1.35	0.89	2.16	2.99	2.89
Borneol	25.33	1.31	1.60	1.06	1.51	1.23	1.30	0.68
Farnesol	26.34	1.83	0.43	0.32	0.16	0.13	0.95	0.31
Caryophyllene oxide	34.79	0.54	0.48	0.88	0.66	0.54	0.69	0.49
Thymol	41.73	33.50	35.42	36.90	34.16	32.21	30.23	32.29
Carvacrol	31.78	23.72	18.60	16.61	20.24	22.80	23.30	24.11

Conclusions

Different harvesting times were evaluated to understand the importent parameters for producing the highest quantity and quality of thyme essential oil. Harvesting time schedules in day for the thyme (6:00 AM, 8:00 AM, 10:00 AM, 12:00PM, 2:00 PM, 4:00 PM and 6:00

PM) greatly affected essential oil content and composition of thyme. Both fresh and dry herbages, the highest essential oil content was obtained from 6:00 AM harvesting hour with 2.40 % and the lowest essential oil content was obtained from 2:00 PM with 1.92 %. The essential oil contents in midday harvests were lower than the early and late harvesting hours due to increased air temperature. The major essential oil component ratios varied among harvesting hours. The high thymol content is in trend to associate with the early morning harvest hours optimum harvesting hours for thyme.

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USE OF ESSENTIAL OIL FOR WEED CONTROL IN ORGANIC FARMING

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Abstract

Consumers demand for organic foods is increasing all over the world due to heavily used synthetic pesticides in conventional agriculture that severally treats human and environment health. Organic farming is the way of producing good quality crops in harmony with biodiversity and ecosystems. Weeds remain one of the most important problems associated with organic crop production since organic farming excludes the use of synthetic herbicides. Essential oils have a great potential to be used as bio-herbicide to control problem weeds in organic farming. A study was conducted to determine the bio-herbicidal potential of organically grown oregano (*Origanum onites*), rosemary (*Rosmarinus officinalis*), thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*) essential oils. The experimental design was a completely randomized design with three replications. Different concertation of essential oil (2, 4, 8, 16 and 32 ml/L) alone and vinegar mixed were applied on the four leaf stage pigweed (*Amaranthus retroflexus*). The major essential oil components of oregano, rosemary, thyme and sage essential oils were carvacrol, thimol, 1,8 cineole and b-thujone, respectively. The highest herbicidal effects on pigweed were obtained from vinegar + 32 ml/l essential oil mixed treatments. Herbicidal effects of essential oil increased with the increasing concentration of essential oils. Among the tested plant species, oregano and rosemary essential oils were found the most effective when used as herbicide. Vinegar alone had low herbicidal effect on pigweed seedlings. However, the vinegar and essential oil mix had the highest herbicidal effects on pigweed seedlings. Essential oil and vinegar mixtures have potential to be used in organic farming to control problem with weeds in organic farming.

Keywords: *Essential oil, Bio-herbicide, Organic farming weed control.*

Introduction

Weed control is one of the most troublesome practices in organic crop production areas, since there are a few organically approved herbicides available for weed control. Organic agriculture is Anon (1997) defined organic farming as a production system that sustains health of soil, eco-system and people, by relying on ecological process, bio diversity and natural cycles and adapted to local conditions than use of inputs with adverse effects. Successful weed management is crucial for a successful organic crop production. In conventional crop production, weed control is an important part of applied practices to increase crop yield and quality. Conventionally chemical pesticides are applied to minimize or completely eliminate the damage of weeds. Today, many conventional producers quickly accepted the use of herbicides due to ease application. Like conventional farming, weeds are one of the worst problems in organic farming. Weed control in organic farming is one of the most important obstacles for traditional farmers to switch to organic farming.

During the production process in the organic farming areas, studies are widely carried out on different methods and machines other than chemical pesticides. Environmentally friendly and new control methods such as allelopathy, organic herbicides, solarization, robotics, freezing, microwave, UHF rays, laser rays, ultraviolet rays, infrared rays, electricity, steam and pressurized water applications are the weed control practices applied in organic crop production areas (Quarles, 2013; Pacanoski, 2015). Many natural herbicides that are not organic licensed yet can be approved to use in organic farming by certification companies. One of the main disadvantages of organically approved herbicides are high costs, higher quantities, non-selectivity, effective only in the early growth stages of the weeds (Young, 2004; Lanini, 2010; Webber *et al.*, 2012; Bari and Kato-Noguchi, 2017).

Essential oils extracted from plants can suppress weeds and they can be used as bio-herbicide for weed control (Campiglia *et al*., 2007; Frabboni *et al*., 2009; Synowiec and Nowicka-Połeć, 2016; Werrie *et al*. 2020). It has already emphasized by many researchers that essential oils can inhibit seed germination of weed species (Dudai *et al.* (1999).

The aim of this study was to determine the contact herbicidal potential of oregano, lavender, thyme and sage essential oil on problem weeds.

Materials and Methods

The dried aerial parts of oregano (*Origanum onites*), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris*) and sage (*Salvia officinalis*) plants were collected in the organically grown medicinal and organic plant production areas of Erciyes University, Kayseri, Turkey. The dry herbage of plant parts were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus, to extract essential oil. The extracted essential oils were dried over anhydrous sodium sulphate and stored in low temperature until use.

The herbicidal effect of the essential oils was tested under field condition. Pigweed seeds were planted in the experimental farm of Erciyes University. To applied as herbicide, 2, 4, 8, 16 and 32 ml/L of oregano, rosemary, thyme and sage essential oil were mixed with 1 ml tween 20 and 1 L distilled water and shaken for 30 minutes in a shaker at 700 rpm. In the case of vinegar and essential oil mixture, 2, 4, 8, 16 and 32 ml/L of essential oil oregano, rosemary, thyme and sage essential oil were mixed with 1 L commercial vinegar and 1 ml tween 20. When the pigweed seedlings were in the four leaf stages the prepared essential oil were sprayed on the weed seedlings. The application doses of essential oil were 2, 4, 8, 16 and 32 ml in the prepared mix of bio-herbicide. One week after application growth injury of treatments were recorded by using 1-5 scale.

GC-MS analysis

Analysis of the tested essential oil were carried out by using Thermo Scientific Focus Gas Choromatograph equipped with MS, auto sampler and Rtx®-5 capillary columns (60 m x 0.32mm, 0.25µm film thickness). Helium (99.9%) was the carrier gas at a flow rate of 1 mL/min. MS transfer line temperature was 250°C, MS Ionization source temperature was 220^{o}C, the injection port temperature was 220°C. The samples were injected with 250 split ratio. The injection volume was 1µL. Oven temperature was programmed to 50°C to 220°C at 3°C /min (Kaya *et al*., 2003). The structure of each compound was identified by comparison of their mass spectrum (Wiley) using the Xcalibur software program.

Results and Discussion

Chemical composition of oregano, rosemary, thyme and sage essential oil were given in Table 1. Only major essential oil components are shown. The major components of oregano oil were carvacrol (60.03%), p-cymene (8.81%) and a-terpinene (2.79%). The 1,8 cineole (22.25%) and thymol (20.21%) were the major components of rosemary oil. Thymole /37.13%), p-cymene 18.46%) and a-terpinene (4.14%) were the major components of thyme oil. The major components of sage oil were b- thujone (20.38) and L-camphor (19.41%).

Table 1. Chemical composition of essential oils used in experiment (major components in %)

Essential oil components	Oregano	Rosemary	Thyme	Sage
1,8 cineole	-	22.25	2.6	24.51
a-terpinene	2.79	1.04	4.14	0.85
p-cymene	8.81	3.04	18.46	-
Thymol	0.95	20.21	37.13	-
Carvacrol	60.03	-	4.12	-
b- thujone	-	-	-	20.38
L-camphor	-	-	-	19.41
b-pinene	0.5	-	0.4	7.62

Herbicidal effects of essential oils alone and their mixture with vinegar was given in Table 2. Essential oils have long been recognized for their anti-microbial activities. The lowest herbicidal rate was obtained from 4 ml essential oil treatments, while the highest was obtained from 32 ml/l essential oil treatments mixed with vinegar for all tested essential oils (Table 2). The herbicidal effects of oregano, rosemary, thyme and sage essential oil increased with the increasing essential oil concentrations. Maximum herbicidal effects were obtained when essential oil was mixed with vinegar. Vinegar alone has equal herbicidal effect on pigweed seedling regardless of the applied dose, but it has not enough potential to kill pigweed seedlings.

Table 2. Bio-herbicidal effect of oregano, rosemary, thyme and sage essential oil on pig weed seedlings

	Dose of applied essential oil (ml/L)/ Bioherbicidal effect				
Treatments	*2*	*4*	*8*	*16*	*32*
Vinegar only*	3	3	3	3	3
Oregano	1	2	2	3	4
Rosemary	1	2	2	3	4
Thyme	1	2	2	3	4
Sage	1	2	2	3	4
Vinegar+Oregano	1	2	3	4	5
Vinegar+Rosemary	1	2	3	4	5
Vinegar+Thyme	1	2	2	3	5
Vinegar+Sage	1	2	2	3	5

1: weeds are not injured, 2: weeds are slightly injured; 3: weeds are injured but not death; 4: most of the weeds are severely injured; 5: All weeds are death.

* Vinegar was not diluted. Pure vinegar was applied and no water or any other ingredients was not added.

The herbicidal effects of tested essential oils can be attributed to their major components (Table 1). The given compounds were found to be the most active components of tested essential oils (Frabboni *et al*., 2009; Synowiec and Nowicka-Połeć, 2016; Werrie *et al*., 2020). In most cases, the inhibitory effect of essential oils has been attributed to its major components (Farag *et al.* 1989). These essential oil components were reported as strong biocidal (phytotoxic, antifungal, and insecticidal) compounds (Arslan *et al*. 2010; Bendre *et al*., 2018; Liu et al., 2019; Muñoz *et al*., 2020).

Conclusion

Oregano, rosemary, thyme and sage essential oils had the similar herbicidal effects on pigweed seedlings. Herbicidal effects of tested essential oil on pigweed seedlings increased with the increasing essential oil concentrations. Vinegar alone had moderate herbicidal effect on pigweed seedlings. The bio-herbicidal effects of tested essential oils were increased when vinegar was used instead of water for delivering the essential oil on pigweed seedlings. This study showed that Oregano, rosemary, thyme and sage essential oils and their vinegar mixtures have potential to be used as bio-herbicide in organic farming.

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ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

EVALUATION OF GROUNDWATER QUALITY OF THE TIRANA (ALBANIA) WASTE TREATMENT AREA

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Abstract

Waste management has a significant impact on groundwater quality. Waste is a source of groundwater pollution through leachate release, in cases that waste is disposed in non-sanitary landfills and dumpsites. Sharra landfill was one of the most polluted areas in the country until 2018. The disposal of Tirana waste in Sharra started at the end of the '80s. Until 2018, the waste was disposed in an uncontrolled manner causing high surface water and groundwater pollution. In 2010, UNDP identified Sharra landfill as an environmental hotspot in Albania. For the rehabilitation of this hotspot, in 2018 the Tirana Waste Treatment Area (TWTA) was created to improve the waste management system in this area. The objective of this study is to evaluate the groundwater quality in TWTA after the change of the waste management system. To achieve the objective, 32 water samples were taken within the TWTA during two years with a frequency of every three months and the physico-chemical parameters such as pH, conductivity, chlorides, fluorides, phenols, ammonia, nitrates, nitrites were analysed. The analysis results demonstrated that the groundwater quality in the TWTA is within the threshold values, meanwhile parameters such as ammonia, nitrates and nitrites were nearly at the threshold values. Through this study, it was indicated that the change in the waste management system in TWTA and discontinuance of the uncontrolled leachate discharge in soil led to the improvement of the groundwater quality in the TWTA.

Key words: *groundwater, waste, ammonia, dissolved oxygen, conductivity*.

Introduction

Sharra landfill, located near Tirana, Albania, used to be recognized as an environmental hotspot due to several critical issues associated with waste management and its environmental impacts (UNDP, 2010). Starting from the 1980s Sharra landfill has been a source of significant pollution, affecting soil, water, and air quality in the surrounding area, since different typologies of waste used to be disposed in an uncontrolled manner. In 2010, UNDP identified Sharra landfill as an environmental hotspot in Albania. Until 2018, Sharra was a non-sanitary dumpsite.

Decomposing waste produced greenhouse gases like methane, which contributed to air pollution and climate change. Additionally, improper waste burning (which was a common practice by the local community) used to release harmful pollutants into the atmosphere (Alcani et al., 2010). In addition, the dumpsite used to emit volatile organic compounds (VOCs) and other harmful substances, such as dioxins and furans since the waste was burnt on a daily basis. Methane contributes to climate change, while VOCs and other pollutants can degrade air quality, leading to respiratory issues and other health problems for nearby residents (Alcani et al., 2010; Vaverková, 2019).

Leachate, the liquid that drains from decomposing waste, contained a mixture of harmful chemicals, heavy metals, and organic pollutants, since the municipal solid waste was mixed with hazardous waste. Leachate used to seep into the soil and contaminated groundwater, posing risks to drinking water sources and aquatic ecosystems nearby, such as the water basins located in Sharra and also Erzeni river (Koto et al., 2023, Keçi et al, 2013). Contaminated runoff used to harm aquatic life and degrade water quality, affecting ecosystems and potentially entering human water supplies (Beqiraj A., Cenameri M., 2010).. Hazardous substances from the landfill used to leach into the soil since they were not adequately contained. Soil contamination was an environmental threat as it could lead to the loss of soil fertility and harm plant life. This level of environmental pollution also poses health risks to humans and animals through direct contact or through the food chain (Psomas et al., 2021; Cantor et al., 2018).

Taking into consideration all of the above-mentioned environmental and public health consequences of the improper management of waste, in 2018, the project of Tirana Waste Treatment Area (TWTA) took off in Sharra.

The Tirana Waste Treatment Area was created to improve the waste management system in this area, by implementing best practices of waste disposal, in compliance with national and applicable international standards. To achieve this, the TWTA project includes the rehabilitation of the old dumpsite, construction of waste categorization facility (material recovery facility, where the recyclables are set aside and later on sold to licensed recycling companies); Municipal Solid Waste (MSW) landfill; 2 inert waste landfills; a leachate treatment plant and a Waste-to-Energy plant, as well as its ashes landfill (European Commission, 1991; European Commission, 2000; European Commission, 2006; INSTAT, 2023; European Environment Agency, 2021).

This study is focused on the quality of the groundwater of the TWTA. Since the old waste management practices have had quite a negative impact on the environment and the waters, being these surface waters or groundwaters, this study aims to evidence if there are any improvements in the quality of the groundwaters of the TWTA.

The main objective of this study is to evaluate the groundwater quality in TWTA after the change of the waste management system.

Material and Methods

To achieve the study’s objective, 40 groundwater samples were taken within the TWTA during two years with a frequency of every three months and the physico-chemical parameters such as pH, conductivity, chlorides, fluorides, phenols, ammonia, nitrates, nitrites were analysed.

The samples were taken in a 3 months frequency, respectively in: February, May, August, November of each year, starting from February 2022, until May 2024.

In order to monitor the level and quality of the groundwaters, there are 4 piezometers installed in the TWTA. Every three months 4 samples were collected, one in each of the piezometers located in TWTA.

The piezometers are located in the coordinates as shown in the table below:

Table 1. The location of the piezometers

Nr.	X	Y
P1	4397102.231	4574217.758
P2	4397248.779	4574118.671
P3	4397150.276	4573984.668
P4	4397039.36	4574080.665

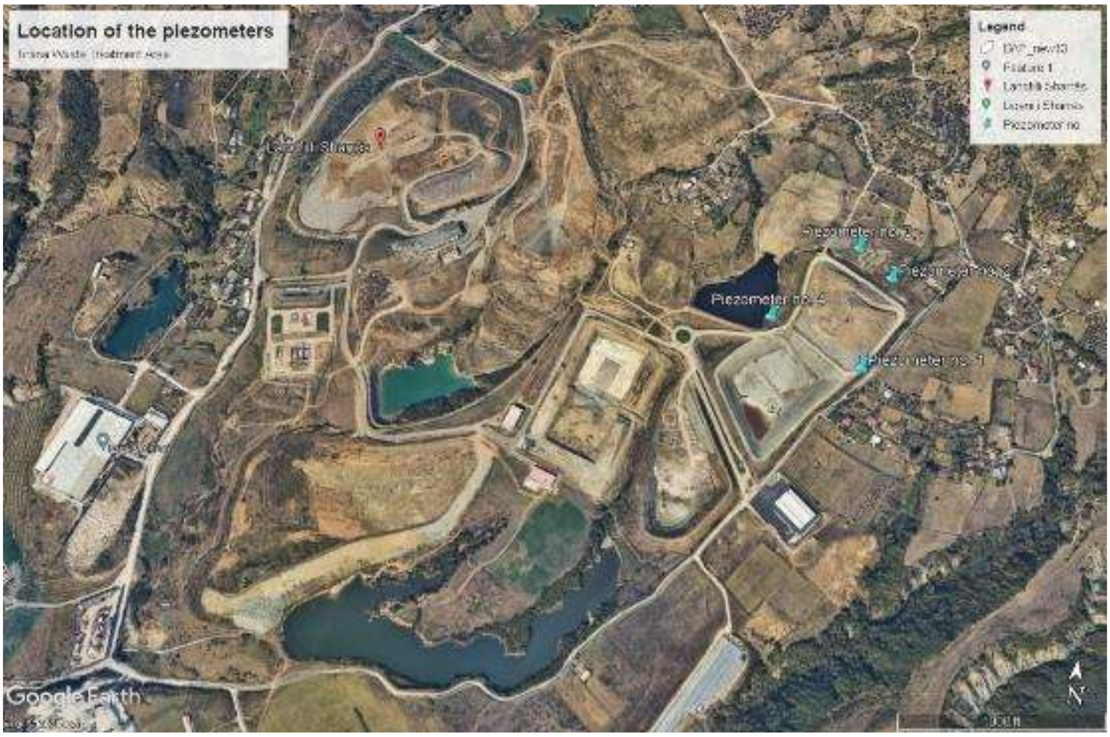

Figure 1. The location of the piezometers (Source: Google Earth)

In the paragraph below, a few pictures of the piezometers are shown:


Figure 2. Photos of the piezometers that are installed in the TWTA

The groundwater quality analyses were conducted by the accredited laboratory: Qendra e Monitorimit të Mjedisit (Environmental Monitoring Center). The methodology of the analysis is described below:
For the analysis of groundwater, water sampling was done using a Ruttner sampler. Groundwater sampling was performed using a special device for this type of sampling, and the collected water was poured into 1.5-liter bottles that have been pre-cleaned. The transportation of samples was carried out in a thermal box at a temperature of 4˚C according to the laboratory's technical requirements. The number of samples and the sampling point were determined according to the monitoring program. The container wass rinsed to normalize it. The bottle was completely filled with the sample, and the cap was placed so that no air remained in the bottle. A standard label was placed on it, and it was put into a black plastic bag to avoid contact with light and was stored in a cool place to prevent nitrification or stratification.

Results and Discussion

Groundwater quality standards for landfills are essential to protect the environment and public health from the potential contamination associated with landfill leachate. The parameters to be analyzed are determined by the EU Landfill Directive (1999/31/EC), as well as by the national legal framework.
The EU Landfill Directive (1999/31/EC) sets strict requirements for the location, design, operation, and monitoring of landfills, including groundwater protection measures. Regulatory frameworks at the national and international levels provide the guidelines and enforcement mechanisms necessary to maintain groundwater quality around landfill sites.
Below are given the results of the analysis that were performed during the period of time from February 2022 until July 2024:

Table 2. Results of the analyses conducted in the year 2022

Parameter	February, 2022				May, 2022				August, 2022				November, 2022			
	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4
pH	7.47	7.42	7.64	7.52	7.37	7.18	7.56	8.02	7.67	7.79	7.81	7.89	7.62	7.41	7.51	7.81
Temperature (°C)	6.1	5.9	6	6.4	15.9	15.9	15.9	16	16.9	16.7	16.6	16.7	13.6	13.8	14	13.9
Conductivity (µS/cm)	281	278	271	281	524	847	1002	1085	1435	1411	1482	1502	285	579	791	701
Dissolved oxygen (DO, mg/L)	5.4	5.2	5.2	5.6	3.7	2.1	3.5	4.8	4.1	3.9	3.9	3.9	4.5	4.4	2.1	5.1
Chlorides (mg/L)	23.2	24.6	20.7	21.5	11.58	11.56	10.88	10.97	12.34	12.78	11.87	12.07	10.92	11.01	10.69	10.72
Sulphates (mg/L)	2.2	2.8	3	3.4	5	6	6	5	6	7	5	6	4	6	4	5
Fluorides (mg/L)	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1
Phenols (mg/L)	<0.1	<0.10	<0.10	<0.10	<0.1	<0.1	0.1	0.11	0.11	0.12	0.16	0.19	<0.1	<0.1	<0.1	<0.1
N-NH4 (mg/L)	<0.05	< 0.05	<0.05	<0.05	1.32	1.1	1.74	0.17	1.32	1.42	1.44	1.62	1.26	1.17	1.56	0.19
N-NO2 (mg/L)	0.049	0.055	0.038	0.067	0.07	0.05	0.03	<0.02	0.05	0.04	0.06	0.04	0.06	0.03	0.03	<0.02
N-NO3 (mg/L)	<1	<1	1	1	3.2	2.9	2.1	1.9	2.9	3.4	2.4	2.8	2.9	3.1	2	1.2

Table 3. Results of the analyses conducted in the year 2023

Parameter	February, 2023				May, 2023				August, 2023				November, 2023			
	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4
pH	7.93	7.78	7.64	7.62	7.33	7.63	7.59	7.68	7.33	7.42	7.64	7.52	7.22	7.18	7.29	7.37
Temperature (°C)	6	6.2	6.5	6.3	5.2	5.1	5.4	5.2	6.1	5.9	6	6.4	5.8	5.7	5.8	6.2
Conductivity (µS/cm)	278	285	354	291	243	230	365	374	281	278	271	281	279	283	276	276
Dissolved oxygen (DO, mg/L)	5.1	4.7	4.2	5.9	5.6	4.9	4.6	5.9	5.4	5.2	5.2	5.6	5.6	5.4	5.1	5.4
Chlorides (mg/L)	25.9	26.8	26.2	26.5	25	25	24.6	25.3	23.2	24.6	20.7	21.5	24.7	24.7	21.3	22.6
Sulphates (mg/L)	3	4	3	4	2.8	3	3.2	3.8	2.2	2.8	3	3.4	2	2.6	2.7	2.23
Fluorides (mg/L)	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Phenols (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	<0.10	<0.10	<0.10	<0.1	<0.1	<0.1	<0.1
N-NH4 (mg/L)	<0.05	0.05	0.1	<0.05	<0.05	<0.05	0.04	<0.05	<0.05	< 0.05	<0.05	<0.05	0.07	0.09	0.84	0.93
N-NO2 (mg/L)	0.02	0.02	<0.02	<0.02	0.047	0.052	0.031	0.073	0.049	0.055	0.038	0.067	0.051	0.052	0.042	0.067
N-NO3 (mg/L)	1.1	1.4	<1	<1	<1	1	1	1.1	<1	<1	1	1	<1	<1	2.5	2.7

Table 4. Results of the analyses conducted in the year 2024

Parameter	February, 2024				May, 2024			
	P no. 1	P no. 2	P no. 3	P no. 4	P no. 1	P no. 2	P no. 3	P no. 4
pH	7.22	7.34	7.32	7.28	7.3	7.39	7.27	7.31
Temperature (°C)	5.8	6.1	6.2	6.4	6.1	6	6	6.2
Conductivity (µS/cm)	279	278	274	282	271	269	282	280
Dissolved oxygen (DO, mg/L)	5.6	6.1	5.7	5.9	6.1	6.1	6.1	6.1
Chlorides (mg/L)	24.7	14.4	25	3	21.4	16.3	21	23
Sulphates (mg/L)	2	2.2	2.5	3	2.2	2.3	2.3	2.8
Fluorides (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Phenols (mg/L)	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
N-NH4 (mg/L)	0.07	1.2	0.97	0.99	0.09	1.02	0.91	0.87
N-NO2 (mg/L)	0.051	0.099	0.087	0.078	0.049	0.057	0.074	0.071
N-NO3 (mg/L)	6.3	4.2	2.3	3.9	4	3.1	1.9	3.1

By analyzing the results of the performed tests, it can be deducted that each of the analyzed parameters represents an average value.

However, during the study, it was taken in consideration that there are no determined groundwater quality standards in a national range.

In the paragraphs below is given the interpretation for each of the analyzed parameters:

- pH levels for TWTA groundwater typically range between 7 and 8.
- Conductivity measures the ability of water to conduct electrical currents, which correlates with the presence of dissolved ions (e.g., salts). High conductivity indicates contamination from industrial or agricultural activities. In the performed analysis the conductivity was in the range of 200 – 1500, with the highest value measured in the Piezometer no 4, on August 2022. This value indicates the low humidity and rainfall, which makes the groundwater more concentrated and therefore increases its conductivity. In the following periods of monitoring the value of conductivity is visibly lower, indicating the improvement of the quality of groundwaters in TWTA.
- DO levels are crucial for aquatic life. Low DO can indicate microbial decomposition of organic matter (e.g., from landfill leachate), leading to oxygen depletion and potential harm to aquatic ecosystems. The lowest DO in the period of study was noticed in Piezometer no. 3 during May 2022, with the value of 2.1.
- Chloride levels indicate contamination from road salts, industrial processes, or seawater intrusion. Chlorides levels in TWTA groundwaters vary from 10 to 27 mg/L, with the highest concentration corresponding to Piezometer no.2 during February 2023.
- Sulphate levels originate from natural sources or industrial activities. High sulphate concentrations indicate contamination from industrial discharges, which in this case is waste management. Sulphates levels in TWTA are from 2-6 mg/L. The highest concentration of the sulphates has been documented during May to November 2022, and then the trend was decreasing.
- Fluoride concentrations in the TWTA groundwaters have maintained a value of <0.1 mg/L throughout the period of the study.
- Phenols are toxic organic compounds often associated with industrial activities. Their presence in groundwater indicates contamination and poses health risks. Phenols as well have kept a steady trend, by <0.1 mg/L in each of the samples that were taken during this study.
- Ammonium levels indicate organic decomposition or agricultural runoff. Elevated levels may indicate contamination and impact aquatic life. The highest ammonium level in TWTA groundwaters during the monitoring period was evidenced on November 2022 at Piezometer no.3, with a concentration of 1.56 mg/L.
- Nitrite levels are indicators of potential pollution sources, such as agricultural runoff or inadequate wastewater treatment. European standards specify limits to prevent nitrite-related health risks and ecological disturbances in groundwater ecosystems. Nitrite levels in the TWTA groundwaters are in <0.02 – 0.073 range.
- Nitrates are common contaminants from agricultural fertilizers, sewage, or waste. High nitrate levels pose health risks, especially for infants (methemoglobinemia or "blue baby syndrome"). The nitrates concentration in the TWTA groundwaters is <1 up to 6 mg/L.

Conclusions

The physical parameters which were analyzed in TWTA over the 25 months (February 2022 – May 2024) demonstrated a positive trend of groundwaters quality improvement, since the level of the pollution was diminished. Especially for the parameters of Dissolved Oxygen (DO) and conductivity, the change was more visible.The water quality in TWTA's groundwater generally remains stable with slightly alkaline characteristics. Consistently low levels of parameters such as fluorides, chlorides, phenols and sulphates suggest minimal risk, while other indicators such as ammonia, nitrates, nitrites hint at issues related to organic material from decomposition of waste which was previously disposed in an uncontrolled manner. There is a positive trend in the environmental condition of the TWTA, nonetheless, taking in consideration that TWTA is still being implemented and the rehabilitation measures will be applied in a longer period of time, the improvement might be more visible after a few years.

By complying with the applicable standards and following up with regular monitoring and reporting of the groundwater quality in TWTA, decreasing and stable trends in pollutant levels were shown over time.

While progress has been made, ongoing efforts are necessary to ensure that the landfill's environmental impact is minimized and that public health is protected.

The progress made in the Tirana Waste Treatment Area, including efforts at the Sharra old dumpsite (which had been operative since the 1980s), has been focused on improving waste management practices and safeguarding groundwater quality. By implementing modern infrastructure, advanced treatment technologies, and rigorous monitoring, significant strides are expected in mitigating environmental impacts and achieving sustainable waste management.

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SOLID-STATE FERMENTATION CELLULASES PRODUCTION ON WHEAT BRAN

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Abstract

The solid-state fermentation (SSF) process is one of the most applied in industry with low investment and operation costs. This has made it an attractive alternative to produce cellulases, the enzymes that break down cellulose, which are widely used in the textile industry, in detergents, pulp and paper industry, improving digestibility of animal feeds, in food industry, and the enzymes account for a significant share of the world enzyme market. However, the most promising use in the current economic climate seems to be the production of alternative fuels. Production of cellulases using agricultural residues or wastes is one of the most efficient biological ways by which these residues can be recycled using a value-added process for conversion of these materials into renewable chemicals and fuels. The purpose of this work is to evaluate cellulases activities of *Bjerkandera adusta* in a solid substrate fermentation process using wheat bran. SSF was carried out in 500 ml flasks containing 15 g of wheat bran moistened with distilled water. After sterilization, the medium was inoculated with 12 ml of homogenized mycelium. Cultures were incubated at 28°C for 25 days. The results obtained show maximum carboxymethyl cellulase (CMCase) (1.31 U/ml), β-glucosidase (BGL) (4.50 U/ml). Wheat bran can be used and valorized for the cellulases production by *Bjerkandera adusta* under SSF.

Keywords: *Basidiomycetes, Cellulases, Solid-State Fermentation, Wheat bran, Valorization.*

Introduction

There are three major macromolecular components of plant cell walls which are found in litter, namely cellulose, hemicellulose and lignin, of which the latter is the most recalcitrant. All three component form a complex referred to as lignocellulose. The degradation of lignocellulose requires a complex set of extracellular enzymes. Extracellular hydrolases and oxidoreductases are involved in the breakdown of lignocellulose and are produced by many known lignolytic fungi. Both hydrolases and oxidoreductases have already been discussed in earlier papers, but there is still an ongoing interest, especially in biotechnological applications (Hofrichter, 2002; Baldrian, 2006). Cellulases, in particular the complex consisting of endoglucanase, cellobiohydrolase and beta-glucosidase, hydrolyze the long chains of cellulose, resulting in the liberation of cellobiose and finally glucose. There is a significant interest in solid-state fermentation (SSF) techniques as an efficient biotechnological process for basidiomycetes cultivation and the production of a wide variety of enzymes.
The purpose of this work is to evaluate the growth and cellulases production (Carboxylmethyl cellulase: CMCase and β-glucosidase ; BGL) of *Bjerkandera adusta* in a solid substrate fermentation process using wheat bran.

Material and methods

Strain

Bjerkandera adusta, has been isolated from the Yakouren forest (Tizi-Ouzou / Algeria). The stock cultures were maintained on Potato Dextrose Agar (PDA) plates at 4°C and transferred every 6 months to PDA plates and kept at 28°C for 10 days before being used for inoculums preparation.

Solid-state fermentation

Inoculums’ preparation

The inoculum was prepared, as described by Elisashvili et *al.* (2008). Fungus was grown in 500 ml flasks containing 200 ml of standard medium with the following composition (in g/L): glucose 10.0, ammonium tartrate 2.0, KH_2PO_4 0.8, K_2HPO_4 0.2, $MgSO_4 .7 H_2O$ 0.5, yeast extract 2.0. The medium was adjusted to pH 6.0 with 2 M NaOH and incubated at 180 rpm and 28 °C. After 5 - 6 days of fungal cultivation mycelia pellets were harvested by sterile distilled water and homogenized with a laboratory blender. The same identical inoculum was used to conduct the SSF.

SSF and cultivation conditions

SSF was carried out in 500 ml flasks containing 15 g of wheat bran substrate moistened with 45 ml of distilled water. About 12 ml of homogenized mycelium was used to inoculate the flasks containing wheat bran. Flasks were incubated at 28 °C. Each sampling day (2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 and 25 days) 10 g of the homogenized cultures were soaked with 50 ml of distilled water at 28 °C, on a shaker (180 rpm) for 1h. The solids were separated by centrifugation at 10,000 x *g* for 30 min at 4 °C. The supernatants collected were further used as enzyme source.

Enzymes assays

Carboxymethyl Cellulase (CMCase) was determined as the amount of total sugars released from the substrates by the crude enzyme preparation and was monitored by the method of Ghosse (1987). The reducing sugars formed were determined using the DNS method of Miller (1959). One unit of CMCase was defined as the amount of the enzyme which released 1µM of reducing sugars per min. β-glucosidase (BGL) activity was determined as the amount of *p*-nitrophenol (*p*NP) released from *p*-nitrophenyl β-D- glucopyranoside (*p*- NPG) by the enzyme preparation. It was measured according to the method of Norkrans (1957). One unit of β- glucosidase was defined as the amount of the enzyme which released 1µM of *p*-NP per min.

Results and Discussion

During the growth of the selected strain on wheat bran under SSF, CMCase and BGL secretion and increase gradually to reach a maximum of 1.31 and 4.50 U/ml respectively, in the end of fermentation (Fig. 1). Several researchers have reported CMCases production in *B. adusta* culture. The maximum CMCase produced varies from 0.02 at 0.33 U/ml (Dinis et *al*., 2009 ; Quiroz-Castan˜eda, 2009). A lower CMC ase activity was detected on SSF wheat bran with *Iprex lacteus* and Euc-strain with values of 0.16 (in 35 days of culture) and 0.07 U/ml (in 10 days of culture) (Dias et *al*.,2010). However, the submerged fermentation (SF) of lignocellulosic residues favored the maximum hydrolases accumulation by white-rot basidiomycetes. For example, high levels of cellulolytic enzyme activities appeared during SF of banana peels by *Pycnoporus coccineus* (111 U/ml) and only 32 U/ml activity was found in SSF (Elisashvili et *al*.,2008). The existence of endoglucanase and exoglucanase (as well glucosidase and xylanase) with elevated action on microcrystalline cellulose has been reported by Quiroz-Castan˜eda et *al*. (2009). Additionally, lignocellulosic substrates induce different quantities and/or types of enzymes (cellulases and xylanases) (Quiroz-Castan˜eda et *al*., 2011). CMCase activity (under SSF) revealed in the selected strain is higher than these reported already for *B. adusta*. In addition to endoglucanase and cellobiohydrolase, an effective hydrolysis of cellulose also requires β-glucosidases, which break down cellobiose releasing two glucose molecules. However, during enzymatic hydrolysis, insufficient BGL activity causes an accumulation of cellobiose with the consequence that both endoglucanase and exoglucanase activities are severely inhibited. BGLs play an important role in efficient hydrolysis of cellulosic biomass, as they relieve the inhibition of the cellobiohydrolases and endoglucanases by reducing cellobiose accumulation. The β-glucosidase was detected in *Bjerkandera* sp. strain BOL13, however, the activity was very low, recorded as 0.017 U/ml, with cane molasses, wood chips and wheat husk substrates in the batch culture medium (Romero et *al*., 2006). The brown-rot basidiomycete *Fomitopsis palustris* has high specificity only for *p*-NPG and cellobiose. This BGL plays an important role in the enzymatic saccharification of lignocellulosic biomass to glucose (Yoon et *al*., 2008). Recently, Chicatto et *al*. (2014) reported a BGL activity of 0.31 U/l in *Lentinula edodes* EF52 strain. Comparatively to these results, strain BN4 showed a high activity toward *p*-NPG.

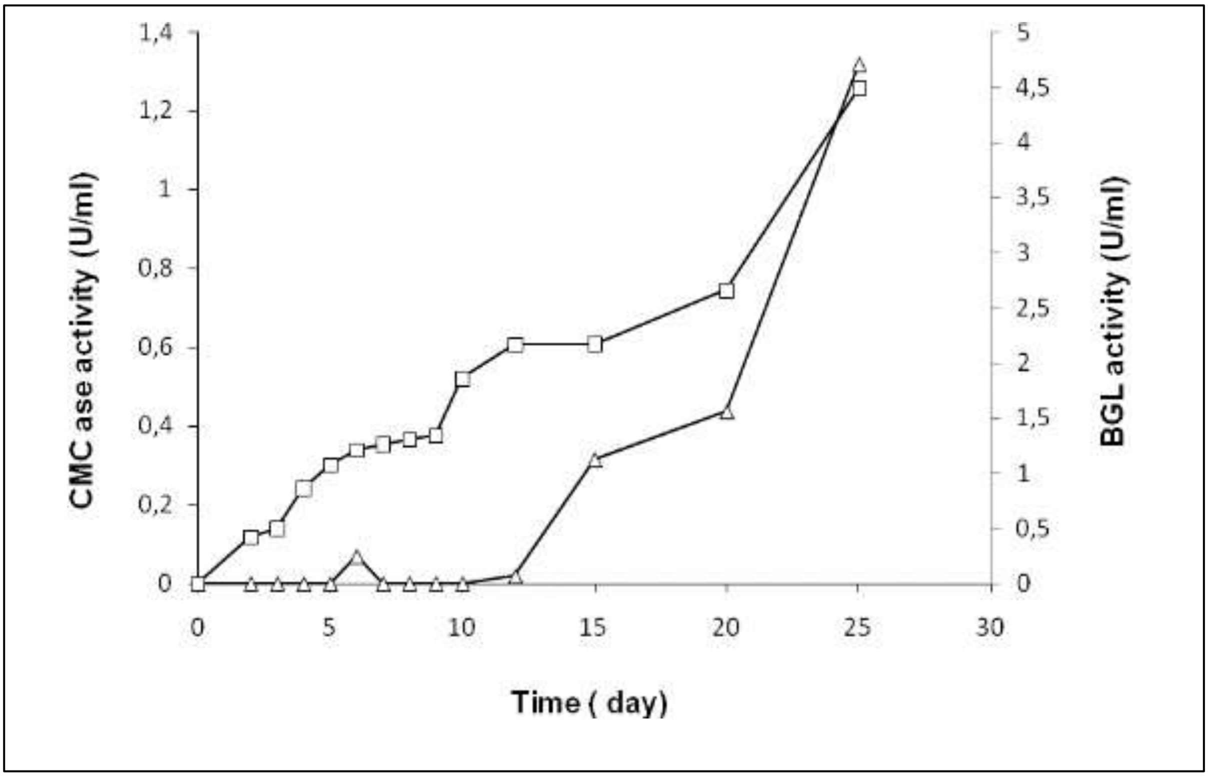

Figure 1. CMCase (Δ) and BGL (□) activities measuring during solid state incubation with wheat bran

Conclusion

A white-rot fungus collected from a forest in Algeria has a potential to produce, on wheat bran (an abundant and economic substrate) under SSF, a good CMCase and BGL activities (1.31 U/ml) and (4.50 U/ml) respectively. Further studies are required to explore this strain for large-scale enzymes production for bioconversion of lignocelluloses.

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INVASIVE SPECIES IN THE VINEYARDS OF BOSNIA AND HERZEGOVINA

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Abstract

Invasive plant species become dominant in the new habitat by suppressing native flora and often have a negative effect not only on biodiversity, disrupting the natural balance in the environment, but also damage the economy and/or human health. The aims of this paper are to identification of invasive plants species in the vineyards in Bosnia and Herzegovina and comparison of their presence in some vineyards in western Balkan countries (Republic of Slovenia, the Republic of Croatia, the Republic of Serbia, and the Republic of Montenegro). The research on the invasive flora of the vineyards of Bosnia and Herzegovina was conducted during three vegetation seasons (2021, 2022, 2023). In this study the 14 invasive plant species were recorded, from which nine species are therophytes, followed by geophytes and hemicryptophytes with two species, and phanerophytes with one species. The analysis of flora elements, that is, the phytogeographical analysis of the invasive flora with 10 species showed the dominance of cultivated and adventitious species and a lower representation of invasive species of wide distribution (4 species). Comparing the status of these 14 invasive species with their status in the countries in the region, In vineyards in Serbia, the 13 of the same invasive plant species have been identified, out of 14 identified invasive plant species in Bosnia and Herzegovina, and only 5 of the same invasive plant species in the Republic of Slovenia.

Keywords: *Invasive plants, Vineyards, Phytogeographical analysis.*

Introduction

Foreign or non-native species are those that have been brought from another area, accidentally or intentionally, and not every non-native species will become an invasive species. Research shows that approximately 10 out of every 100 introduced species are successfully naturalized, while out of every 100 naturalized, ten become invasive (Williamson and Fitter, 1996).

In Europe, parties are usually divided into so-called archaeophytes, species that were introduced to Europe before 1500, and neophytes, species that appeared in Europe after 1500 (Richardson et al., 2006, Lambdon et al., 2008).

Due to their ability to successfully reproduce, spread and conquer new areas, invasive plant species seriously threaten different ecosystems, and are therefore considered the second most significant threat to biological diversity, coming in importance immediately after direct habitat destruction (Randall, 1996). Invasive plant species often have a negative effect not only on biological diversity, disrupting the natural balance in the environment, but also damage the economy and/or human health (Kowarik, 2003). Unlike natural habitats, anthropogenic habitats, such as vineyards, are significantly more susceptible to invasions because they are habitats where disturbances are more pronounced (Vuković 2015). The cultivation of vines in Bosnia and Herzegovina has a tradition longer than 2000 years. The Greeks brought the grapevine from the Adriatic coast to the area of southern Herzegovina, and with the arrival of the Romans, the grapevine spread to the north of the country. Apart

from southern Herzegovina, the vine was most widespread in the area of Rama, Konjic, around Foča, Goražde and Višegrad, in the Tuzla and Zvornik areas and around Derventa and Banja Luka (Beljo et al., 2018).
There are two wine-growing regions in Bosnia and Herzegovina: the region of northern Bosnia and the region of Herzegovina. Today, according to official data, vines occupy an area of about 3,500 ha, some data (Rotim, 2017) state as much as 4,000 ha, which is less than 1% of the arable land of Bosnia and Herzegovina.
The aims of this paper are identification of invasive plants species in the vineyards in Bosnia and Herzegovina and comparison of their presence in some vineyards in western Balkans countries (Republic of Slovenia, the Republic of Croatia, the Republic of Serbia, and the Republic of Montenegro).

Material and methods

The research on the invasive flora of the vineyards of Bosnia and Herzegovina was conducted during three vegetation season (2021, 2022, 2023).
The research covered 73 vineyard locations, larger than 1 ha in the territory of Bosnia and Herzegovina, from Prijedor, the wider area of Banja Luka, Gradiška, Prnjavor, via Žepč, Tomislavgrad, Mostar, Široki Brije, Grud, Ljubuški, Čitluk, Čapljina to Stolac and Trebinje (Figure 1).

Figure 1. Layout of the locations of the investigated vineyards

For each locality, latitude, longitude and altitude were determined using a GPS device. For each locality, the date of sampling and the name of the place were recorded. The size of the area for one phytocenological survey was 100 m^2, and the phytocenological survey was made before the application of agrotechnical measures.
Each invasive taxon is associated with a corresponding flora element and life form. Flora elements were analyzed and determined according to Horvatić (1967) and Pignatti (1982). Life forms were determined according to Horvat (1948) and Pignatti (1982).

For data processing, the first detailed list of invasive species with their distribution in the Federation of Bosnia and Herzegovina, made in 2019 as part of the project "Inventory and geographic interpretation of invasive species in the Federation of Bosnia and Herzegovina", was used.
The report on foreign and invasive species of plants present in the territory of Montenegro was monitored through the project "Determination of the list of the presence and distribution of invasive species of plants in the territory of Montenegro". The invasive plant species of Slovenia were determined according to Zelnik (2012) and Kuzmič (2017), while for the invasive flora of Serbia, Lazarević (2012), Gavrilović (2016) and Petrović (2016) were used. Beršić et al. (2008) were used for the invasive flora of Croatia.

Results and discussion

During the three-year research of the invasive weed flora of the vineyards of Bosnia and Herzegovina, 14 species were recorded (Table 1).

Table 1. Invasive species in the vineyards of Bosnia and Herzegovina

	Invasive plant species	**Life form**	**Flora groups**
1.	*Ailanthus altissima* (Mill) Swingle	F	Cultivated and adventitious plants
2.	*Amaranthus albus* L	T	Cultivated and adventitious plants
3.	*Amaranthus retroflexus* L.	T	Cultivated and adventitious plants
4.	*Ambrosia artemisiifolia* L.	T	Cultivated and adventitious plants
5.	*Asclepias syriaca* L.	G	Cultivated and adventitious plants
6.	*Datura stramonium* L.	T	Plants of wide distribution
7.	*Erigeron annuus* Pers	T	Cultivated and adventitious plants
8.	*Galinsoga parviflora* Cav.	T	Cultivated and adventitious plants
9.	*Oxalis stricta* L.	H	Cultivated and adventitious plants
10.	*Phytolacca americana* L.	G	Cultivated and adventitious plants
11.	*Portulaca oleraceae* L.	T	Plants of wide distribution
12.	*Sorghum halapense* L.	H	Plants of wide distribution
13.	*Veronica persica* hort ex. Poir.	T	Plants of wide distribution
14.	*Xanthium strumarium* L. ssp. italicum	T	Cultivated and adventitious plants

The analysis of the life forms of the weed flora of the vineyards of Bosnia and Herzegovina shows the presence of four life forms. The dominance of therophytes in 9 invasive species is noticeable, i.e. the presence of 65%, while the others are present in significantly lower numbers (Figure 2).

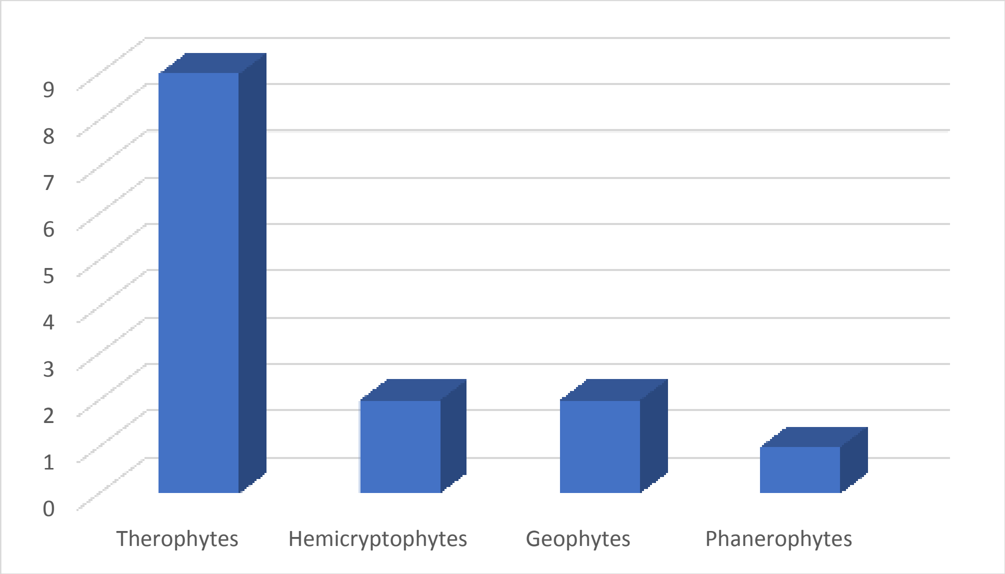

Figure 2. Life form of invasive species in the vineyards of Bosnia and Herzegovina

The analysis of flora elements, that is, the phytogeographical analysis of the weed flora, shows the presence of two flora groups, which was expected.

With 10 species, cultivated and adventive species dominate, which were mainly introduced by humans, intentionally or unintentionally, from other continents, as ornamental, cultivated, and have more or less adapted there. The four invasive species are widely distributed plants, characteristic of anthropogenic habitats and weed flora. (Figure 3).

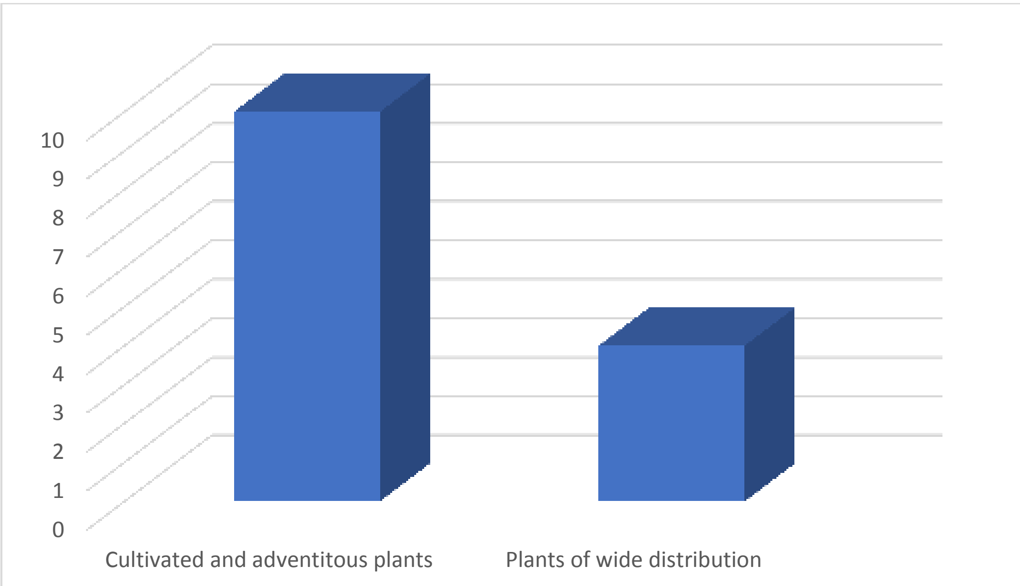

Figure 3. Analysis of flora elements of invasive species in the vineyards of Bosnia and Herzegovina

The control of invasive plant species, as well as the mitigation of their impact on native species and habitats, represents one of the most significant challenges of nature protection.

The list of invasive plants in the vineyards of Bosnia and Herzegovina was compared with the list of invasive plants of neighboring countries in the region. From the list of invasive species of vineyards in Bosnia and Herzegovina, the greatest coincidence is with the Republic of Serbia, where there is a match with 13 species, Croatia with 12 species, while 11 species of the same type are present in Montenegro. According to the status of invasiveness in the Republic of Slovenia, only 5 invasive species match the invasive species in the vineyards of Bosnia and Herzegovina (Table 2).

Table 2. Invasive species list in correlation with countries

	Invasive species in BiH	Invasive species in Slovenia	Invasive species in Croatia	Invasive species in Serbia	Invasive species in Montenegro
1.	*Ailanthus altissima* (Mill) Swingle	+	+	+	+
2.	*Amaranthus albus* L.	/	/	/	/
3.	*Amaranthus retroflexus* L.	/	+	+	+
4.	*Ambrosia artemisiifolia* L.	+	+	+	+
5.	*Asclepias syriaca* L.	+	+	+	+
6.	*Datura stramonium* L.	/	+	+	+
7.	*Erigeron annuus* Pers.	+	+	+	+
8.	*Galinsoga parviflora* Cav.	/	+	+	+
9.	*Oxalis stricata* L.	/	/	+	/
10.	*Phytolacca americana* L.	/	+	+	+
11.	*Portulaca oleraceae* L.	+	+	+	/
12.	*Sorghum halapense* L.	/	+	+	+
13.	*Veronica persica* hort ex. Poir.	/	+	+	+
14.	*Xanthium strumarium* L. ssp. italicum	/	+	+	+

Conclusion

Comparing the list of obtained weed species with the list of invasive plant species in FBiH from 2019, it was recorded that there are 14 identified invasive species in vineyards on the list. Among these 14 invasive species, the 9 species are therophytes. Then there are geophytes and hemicryptophytes with 2 species each, and phanerophytes with 1 species. Analysis of flora elements, i.e. phytogeographic analysis of invasive flora, shows the dominance of cultivated and adventitious species and a lower representation of widespread invasive species. When comparing the list of invasive plant species, the largest number of the same invasive plant species is 13 found in the Republic of Serbia, 12 in the Republic of Croatia, 11 in the Republic of Montenegro. The smallest similarity in terms of the presence of invasive plant species in vineyards was with the Republic of Slovenia, in whose vineyards only 5 invasive plant species out of 14 registered in Bosnia and Herzegovina were identified

Two species from the list are on the List of Invasive Alien Species of Union Concern (List of Invasive Alien Species of Union Concern): *Ailanthus altissima* and *Asclepias syriaca* are present in all the mentioned countries. The identification of invasive plant species in vineyards is useful for assessing their occurrence, planning, selecting and applying appropriate and timely control measures.

Encouraging and raising awareness against foreign and invasive alien species on an international level, in cooperation with neighboring countries, can significantly contribute to solving this problem in the field of environmental protection.

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STRUCTURE OF AGRICULTURAL LAND LOSSES IN FORMER YUGOSLAVIA COUNTRIES OVER THE PERIOD 1990-2018

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Abstract

The paper shows the losses of agricultural land in former Yugoslavia countries according to the Pan-European CORINE Land Cover (CLC) database in the period 1990-2018. It is one of the most well-known databases based on the analysis of Landsat and Sentinel satellite images covering 39 European Environment Agency (EEA) countries. Using CLC Changes (CLC CHA) database the conversion of agricultural land that occurred in the periods 1990-2000, 2000-2006, 2006-2012, and 2012-2018 were monitored.
According to CLC 2018 database Serbia has the most agricultural land with around 53% of the total territory, followed by Croatia with 39.7%, North Macedonia with 35.9%, Slovenia with 34.3% and Montenegro with only 16.1%. Analyzing the structure of agricultural land losses, it can be concluded that the main driver for changes are anthropogenic activities. The urban sprawl has taken place mainly at the expense of agricultural land, as well as the construction of transport communication infrastructure and mining. Natural processes are also an important factor in agricultural land losses. In Serbia anthropogenic activities causes 66% of agricultural land losses while natural processes caused by the abandonment of agricultural land amounted 34%. This ratio in Croatia amounts 56.6% to 43.4%, B&H 76.4% to 23.6%, while in Slovenia it is even 93.2% to 6.8%. The aim of this research is to find out the causes of agricultural land losses and areas with the most intense pressure. Results will be compared by countries in order to see what are the similarities or differences.

Keywords: *agricultural land losses, drivers, anthropogenic activities, natural processes.*

Introduction

After the dissolution of the former Yugoslavia, six new states were established: Serbia, Croatia, Slovenia, Bosnia and Herzegovina (B&H), Montenegro and North Macedonia. Each of them had a different history and its own development path, but common to almost all countries is that they face serious demographic issues. Experiencing both a falling birth rate and rampant emigration, all the countries are facing a major population decline. According to the census from 1991, the number of inhabitants of the former Yugoslavia was around 23.5 million (SORS, 1991), while censuses from the period 2011-2022 shows the total number of inhabitants in newly created states was about 20.8 million. Today it is even less, for example B&H which had around 4.5 million in 1991, has not held a census since 2013, when it had about 3.5 million inhabitants (Agency for Statistic of B&H, 2016). Furthermore, 11 years later it is estimated to have significantly less than 3 million. Only Slovenia (about 2 million) and Montenegro (about 620.000) have kept their population since independence.
Until recently, it was generally assumed that in the long-term human activity had little lasting effect on the land thanks to nature's ability to restore itself. This view remained prevalent for a long time even though farming practices have been causing irreversible damage in certain

areas for centuries (EEA, 1994). The conversion of agricultural land to forest may be the result of natural forest expansion or tree planting. Forest and semi-natural areas expansion may occur when agricultural land is abandoned, for example when a rural population declines, land becomes sufficiently degraded that it becomes unproductive as agricultural land, or more productive agricultural land becomes available elsewhere (FAO, 2016). Urbanization in developing countries in comparison with developed countries has been accelerating and this has been caused by unplanned physical expansion resulting in more harmful undesirable effects on natural environments and the agricultural lands around and the trouble which is happening a serious treatment of urban-rural perspective (Parsipour et al, 2019). Urban sprawl can affect landscapes through three main processes: transformation, degradation, and fragmentation. The transformation of formerly pristine or agricultural lands into built‑up areas is visually the most obvious effect. Valuable habitats and agricultural soils are lost (Scalenghe & Marsan, 2009). Soils need to be conserved. Soils are non‑renewable resources and the loss of agricultural land has major impacts on biodiversity. As an example, in the period 2012-2018, the most land in Europe was taken from agricultural areas (47% of all land taken), with a loss of 1.694 km², and from pastures (36% of all land taken), with 1.276 km² being converted to artificial areas (EEA, 2021).

The process of urbanization in former Yugoslavia countries has intensified because the population is concentrated in and around big cities, while rural areas remain almost uninhabited. This type of migrations causes two processes that endanger agricultural land, on the one hand, the expansion of urban areas and infrastructure takes place mainly over agricultural land, and on the other hand, previously cultivated agricultural land in rural areas remains uncultivated and over time grows into bushes and other semi-natural areas. Given the different characteristics of individual states, each of the states has gone through different stages of development since independence, so the question arises which of these two processes is dominant and where? This is perhaps the most important goal of this research, to answer the question of which driving forces affect the loss of agricultural land in former Yugoslavia states.

Materials and methods

The Copernicus Land Monitoring Service (CLMS) provides geographical information on land cover and its changes, land use, vegetation state, water cycle and Earth's surface energy variables to a broad range of users in Europe. Pan-European database provides land cover and land use (LC/LU), land cover and land use changes and land cover characteristics. The images acquired by Earth observation satellites are used as the main source of data to derive the land cover information. In 1985, the CLC databases and several of its programs were taken over by the EEA with the aim of collecting, coordinating, and ensuring the consistency of information on natural resources and the environment. During the time, the EEA members expanded from EU 28 (EU plus Great Britain) to EEA 39 (including the former Yugoslavia cooperating countries).

CLC nomenclature comprises three levels: the first level (5 items) indicates the major categories of land cover on the planet, the second level (15 items) is for uses on scales of 1:500.000 and 1:1.000.000, and the third level (44 items) is for uses on a scale of 1:100.000 (EEA, 1995). Main categories at the first level are: Artificial surfaces (code 1), Agricultural areas (code 2), Forest and seminatural areas (code 3), Wetlands (code 4) and Water bodies (code 5). Second level representing more detailed land cover types (e.g., 2.1 code is related to Arable land, 2.2 is Permanent crops etc.) while third level is most detailed (2.1.1 code is related to Non-irrigated arable land, 2.1.2 to Permanently irrigated arable land, etc). In 2019, the EEA published an updated illustrated guide to the nomenclature of land cover types with a structural classification at three hierarchical levels and a differentiated level of detail (details and codes at EEA, 2019a).

Mapping of CLC-Change is carried out by applying the “change mapping first” approach. A minimum cartographic unit for the CLC-Change map (CHA) is set to 5 ha which is more detailed comparing to CLC map with 25 ha. In the CHA database can the internal structure of changes and conversions from one type of land cover to another be monitored. For instance, if there was a conversion of Non-irrigated arable land (2.1.1) into Discontinuous urban fabric (1.1.2) there were urban expansion at areas previously covered by agricultural land, which is counted as a loss. The single-date Landsat TM used in CLC 2000 was replaced by two satellite images (usually taken by IRS and SPOT-4) acquired in two different seasons (Büttner & Cosztra, 2017). Multi-temporal images (each location was captured by at least two satellite images) were useful in separating some land cover classes (e.g., Arable land and Pastures). The interpretation was supported by ancillary data such as national orthophotomaps, BDOT10K (available in the WMS format), Google Earth, and city maps (Hościło & Tomaszewska, 2015).

Data for the former Yugoslavia countries were extracted using GIS software and changes in the form of polygons for all the periods were visualized and then exported to Microsoft Excel and classified by type of conversion. Using the Sort & Filter and SUBTOTAL SUM tools, individual sum of areas of all land cover types and changes that occurred by periods were calculated. With regard to the aim of the paper, the changes related to agricultural land have been singled out and particularly analyzed.

Results and Discussion

In the EEA 39 countries, agricultural land occupied 42.01% of the total territory in 2000 and 41.76% in 2018, which represents a loss of 0.25%. At the same time agricultural land in the EU 28 countries covered 48.6% in 2000 and 48.2% in 2018 with losses of 0.4%. The largest amount of conversion/loss of Arable land (2.1) in EEA 39 was related to Industrial, commercial and transport units (1.2) in the amount of 1402 km^2, while urban expansion accounted for 626 km^2 (EEA, 2019b). Trends are similar compared to former Yugoslavia; all the newborn countries are recording losses of agricultural land (Table 1).

Table 1. Agricultural land area in former Yugoslavia and EU28 countries in 2000-2018

Country	Serbia	Croatia	B&H	N. Macedonia	Slovenia	Montenegro	EU 28
CLC 2000 (%)	53.20	39.75	33.33	36.09	34.31	16.12	48.58
CLC 2018 (%)	53.03	39.69	33.12	35.89	34.25	16.07	48.17
Losses 2000-2018	-0.17	-0.06	-0.21	-0.20	-0.06	-0.05	-0.41

Source: EEA, 2019b

Table 1 shows that Serbia has the most agricultural land, about 53% of the territory, which is more than the EU 28 average. Croatia, North Macedonia, Slovenia and B&H have 33-39%, while Montenegro is the poorest with only 16.07%. Compared to the EU 28 average of 0.41%, the agricultural land losses were slightly lower in the countries of the former Yugoslavia. The biggest losses were in B&H (0.21%), followed by North Macedonia (0.20%) and Serbia (0.17%). When it comes to the structure of losses, by analyzing CHA data, it can be determined that the biggest losses are caused by the expansion of artificial surfaces over agricultural land (conversion of code 2 to code 1). The next significant factor for reduction of agricultural land is conversion to forests and semi-natural areas (code 2-3). The losses were proportional to the size of the countries and the area of agricultural land. Serbia had the biggest losses, followed by Croatia and B&H (Table 2).

Table 2. Main causes for agricultural land by countries and periods (km^2)

	1990-2000		2000-2006		2006-2012		2012-2018		Total	
Changes types*	(2-1)	(2-3)	(2-1)	(2-3)	(2-1)	(2-3)	(2-1)	(2-3)	(2-1)	(2-3)
Serbia	53.28	47.32	41.68	5.16	50.87	17.52	44.91	28.18	190.74	98.18
Croatia	34.21	47.17	33.12	3.36	16.54	15.08	19.75	13.75	103.62	79.36
B&H	no data	no data	72.78	16	12.1	7.3	19.84	9.05	104.72	32.35
Slovenia	1.04	0.38	4.2	0.06	1.61	0	4.6	0.39	11.45	0.83
N. Macedonia	no data	no data	22.17	9.23	8.27	2.55	16.41	5.07	46.85	16.85
Montenegro	1.54	1.79	1.91	0.07	2.75	0	2.71	1.16	8.91	3.02
EU28	no data	no data	817	236	4013	1806	42	34	4555	1988

* Code changes 2-1 represent expansion artificial surfaces over agricultural land. Code changes 2-3 means conversion of agricultural land to forest and semi-natural areas.

EEA has described sprawl as the physical pattern of low-density expansion of large urban areas, under market conditions, mainly into the surrounding agricultural areas (EEA, 2006). Agricultural land-rich areas are prone to construction and soil sealing if they are located close to existing urban areas because they are inexpensive and are under pressure to be transformed into residential or commercial areas (Mann, 2009; Grigorescu et al., 2012).
The biggest pressure on agricultural land was urbanization and migrations during the wars in the former Yugoslavia, when numerous refugees were forced to build their homes in the suburbs of large cities, mostly over former agricultural land. It can be noted by analyzing the CHA data, because the biggest losses were recorded in the period 1990-2000 in Serbia and Croatia. During that period, agricultural land losses were mainly related to urban expansion. According to EEA (2006), the price of agricultural land is universally much lower than the price of land zoned for housing or the development of services (EEA, 2006). In Serbia, urban sprawl accounted for 65.5% of all agricultural land losses, and in Croatia 66.3%. It was similar in B&H, however, data for the period 1990-2000 are not available, but in the period 2000-2006, urban expansion accounted for 78% of total agricultural land losses.
However, speaking about the most powerful drivers of agricultural land cover change, the situation differs from one country to another. In Serbia, the ratio between artificial surfaces expansion over agricultural land and natural processes caused by the abandonment of agricultural land amounted to 66% to 34%, in Croatia 56.6% to 43.4%, B&H 76.4% to 23.6%, while in Slovenia it is even 93.2% to 6.8% (in Slovenia it was because of industrial, commercial and transport units activities)(Table 3).

Table 3. Agricultural land (code 2) changes by Artificial surfaces (code 1) and Forest and seminatural areas (code 3)(in %)

Changes type	Serbia	Croatia	B&H	Slovenia	N. Macedonia	Montenegro	EU28
(2-1)	66.02	56.63	76.40	93.24	73.55	74.69	69.62
(3-2)	33.98	43.37	23.60	6.76	26.45	25.31	30.38

Natural processes are much slower and can be observed by visualization over long periods (Drašković et al, 2021). The concentration of population around large cities has left rural areas sparsely inhabited. The young population prefers to live in urban areas and agricultural land has remained uncultivated over time, gradually turning into seminatural areas and bushes. However, compared to the loss of agricultural land through the process of urbanization, these losses are reversible. By cultivating semi-natural areas, it can become suitable for agriculture again.

Conclusions

Trends in the loss of agricultural land were recorded in the countries of the former Yugoslavia as well as in other countries of the EEA 39 and EU 28. By analyzing the structure of changes according to the CHA database, the two main driving forces for conversion/loss of agricultural land are the processes of urbanization and deagrarization caused by reduction of the population in rural areas.

Due to the turbulent historical processes and wars in former Yugoslavia, the pressure on agricultural land was intense, especially in Serbia, Croatia and B&H. Accelerated urbanization and the large number of refugees who settled around the big cities caused significant agricultural areas to be lost. Sprawling cities also threaten to consume the best agricultural lands, displacing agricultural activity to both less productive areas. The biggest losses occurred during the period 1990-2000 in Serbia and Croatia and in period 2000-2006 in Bosnia and Herzegovina. On the other hand, rural areas are affected by labour shortages, which causes the conversion of agricultural land into semi-natural land.

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THE LIFE CYCLE OF THAUMETOPOEA PITYOCAMPA (DENIS & SCHIFFERMÜLLER, 1775) ON THE PINE TREES OF THE CITY OF MOSTAR AND THE HEALTH RISK

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Abstract

The pine beetle, *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) is mentioned in the scientific literature as a pest of forest ecosystems. The female of this butterfly lays a large number of eggs near the tops of the pines, needles of which are food for the pest in the next stages of development (five (5) stages of development). It is particularly harmful in the larval stage (caterpillar), which body is covered with poisonous hairs that cause severe itching with painful inflammation of the skin in humans and pronounced defoliation in pines. The harmfulness of this insect is emphasized by its geographical spread and good adaptability, under the influence of internal and external factors such as global warming and the increase in the area of coniferous forests. The Pine beetle was first identified in Bosnia and Herzegovina in the area of the Neretva River canyon (Konjic, Prozor) in the late 70s of the last century. The goals of the research were the identification and monitoring of communities of this insect, familiarization with the development cycle, ecology and ethology of the pest and pine defoliator, as well as the degree of infection and forest destruction. Field research was carried out in the city of Mostar at five selected locations during seven months in 2023. The results of the work confirmed the presence of the typical form of the pest and defoliator *Thaumetopoea haumetopoea* pityocampa in the selected localities. The greater extent of infection and defoliation of pines as well as harmfulness to humans and animals were also confirmed.

Keywords: *University campus, Thaumetopoea pityocampa, pine, defoliator pest, health risk.*

Introduction

The Mostar basin belongs to the climatogenic community of low forest and thickets of the Rusco-Carpinetum orientalis white hornbeam community. Those forests, in ancient times, covered large areas of today's karst. The barrenness of the karst and changing climatic and edaphic conditions caused a regression in the composition of the forest vegetation.
This part of Bosnia and Herzegovina has true forms of nature, both living and inert, whereas the abundance of steno-endemic and relict plant and animal species gives importance to this area in terms of global biodiversity. The Rakitnica Canyon is among the most beautiful canyons in this area, while the refuges of Prenj, Cvrsnica and Cabulja are heartlands of development and survival of endemic species. Endemic species of deciduous, light-conifer and hygrophilic forests and shrubs, including those of willows, aiders, poplars and planes, and especially unique plant species in the ruptures of carbonate rocks and rock creeps give special importance to these colorful refuge biotopes (Redžić, Barudanović, Radević, (eds.), (2008).

The pine moth (*Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) belongs to the order Lepidoptera (butterflies), a family of small, stocky (wingspan of 3-4 cm) and unsightly butterflies. The female butterfly lays a large number of eggs near the tops of pine trees, whose needles will be food during development. It swarms during the month of July, at lower altitudes in the first half of July, and at higher altitudes in the second half of July (Živojinović, 1968).
Its harmfulness is determined by its geographical spread and expansion in recent decades. Bearing in mind the harmfulness of the pine beetle to pine trees, we investigated its presence in the area of Mostar and the degree of infection of the already small area of pine trees in this biogeographical area.

Geographical location

According to the morphogenesis of its origin, the Mostar basin represents a tectonic tertiary basin located between the Prenja mountain in the north, Velež and the Podveležja plain in the east, and Raška gora, an outcrop of the Čabulje mountain and the low Brotnja plain on the west side.
The altitude of the southern part of the basin is 40-70 m (100 m), while the altitude in the northern part of the basin is 60-300 m (480 m), (Picture 1).

Picture 1. Location of the investigated localities

Material and methods

The research was carried out in two phases: field and laboratory. Field research was carried out in February, April, and August of 2023, in which the development stages of the pine beetle were confirmed in order to monitor all the ecological and phenological characteristics of the insect in the investigated localities.
We took photos of the situation on the ground, determined the altitude, the exposure of the tree trunks, the number of infected trees and the number of trunks per zone of the tree, the type of infected tree, weather conditions, temperature, and other observed phenomena important for the work.
In laboratory conditions, we monitored the egg clutches and after one month witnessed the eclosion of caterpillars of the 1st developmental stage of *Thaumetopoea pityocampa* Schiff.
The following literature was used during the preparation of this work: insect identification, data on previous research, biology, and ecology of the pest, as well as control measures taken

from Androić (1950 &1955 & 1957 & 1969); Dautbasic et al. (2017 & 2018); Zivojinović (1968). On adverse effects on human and animal health taken from Moneo et al. (2003). Data from the Federal Hydrometeorological Institute of Bosnia and Herzegovina for the fall of 2022 were used for the analysis of the influence of the temperature from the previous year on the greater infection.

Results and Discussion

Field research was carried out in the period from February to September 2023 at five locations in the City of Mostar: Location 1.: Campus of the University "Džemal Bijedić" in Mostar (north of the city center), Location 2.: Park-šuma Trimuša, (western part City of Mostar - (the site covers the area of the Partisan memorial and Park-forest Trimuš), Site 3.: Imperial vineyards (Carski Vinogradi) - Blagaj, (southeast of the City of Mostar, on the eastern edges of Bišća polje all the way to Blagaj), Site 4.: Road to Rujište (Put za Rujiste) -Humi, (north of the City of Mostar) and Locality 5.: Fortica, (east of the City of Mostar).

Initial research was carried out in February 2023, when the presence of the pine moth (*Thaumetopoea pityocampa* Schiff) was recorded in all localities, as well as the last larval stage of this butterfly - the 5th stage, which represents a health risk for humans and animals.

At the first location, the University campus in Mostar, 9 pine trees (black pine - *Pinus nigra*, red pine - *Pinus resinosa* and Aleppo pine - *Pinus halepensis*) were recorded with characteristic nests of the southern-facing pine warbler. 42 winter nests (cocoons) were observed on 9 trees. One infected tree is located in front of the Rectorate building of the University "Džemal Bijedić" in Mostar, then one in front of the Faculty of Economics and one in front of the Mostar Medical School. On the black pine tree - *Pinus nigra* (in front of the Rector's office) 9 cocoonc were observed (Chart 1.).

At locality 2, Park - Forest Trimuša, there are Aleppo pines (*Pinus halepensis*), which are weakly infected with pine beetle, and it is about the appearance of only one cocoon per tree, with a position at the top of the crown itself. By the way, when making silk cocoons (nests), pine beetle caterpillars look for a position in the canopy that is well-sunlit, and the fact is that this site is located on the western, less sunny side, which favors the cocoons in the top of the canopy. Through research, we have proven that Aleppo pine is more resistant and less exposed to pine beetle infestation. (Chart 1).

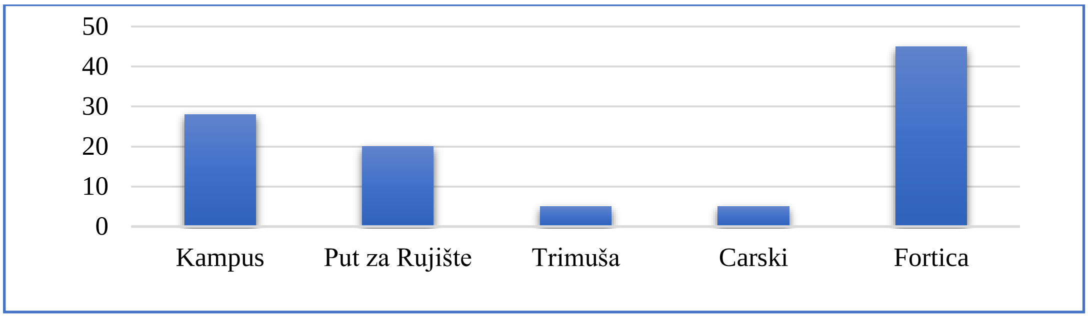
At Locality 3 (Carski vinogradi – Blagaj), larvae of pine beetle caterpillars were observed, and the intensity of the infection is similar to that at Locality 2. On one Aleppo pine tree, there are two larvae nest (cocoons) each, in the top and central part of the crown with southern exposure (Graph 1).

A higher degree of pine beetle infestation is found in the fourth location, Put za Rujište - Humi. It is a locality that is sheltered from the wind by the nearby hills and that is well sunlit, so that the pest has favorable ecological conditions for life. The dendroflora of this area consists of black pine and mountain pine (*Pinus nigra* and *Pinus mugo*), on which 8 cocoons were recorded on one tree. The cocoons were in all zones of the crown (lower, middle, and top) with southern exposure. Young trees are affected by the infection.

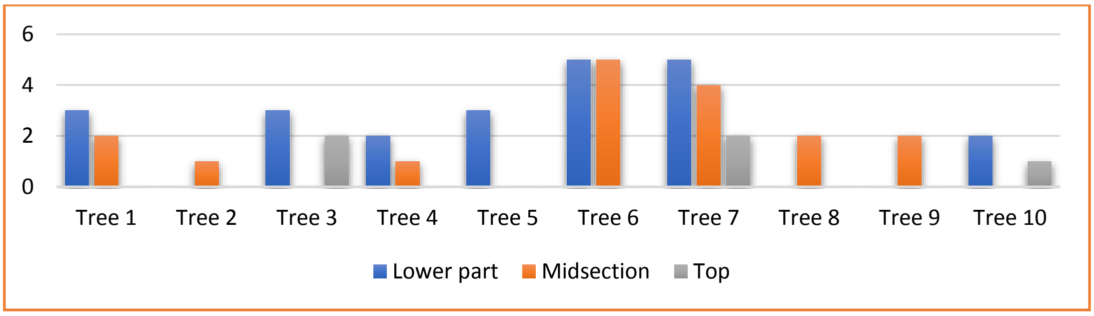
In the last marked location, Fortica, there are black and Aleppo pine trees (*Pinus nigra* and *Pinus halepensis*) where a high degree of infection with pine beetle with the highest number of larvae cocoons is noticeable (Graph 1).

During April 2023, repeated trips to the fields were made, where significant damage was observed to the observed trees, which, due to weakness as a result of pest attacks, were also susceptible to other diseases.

Field research at Site 5. Fortica, which constitutes the "viewing surface- sample area", will continue in August 2023. The largest number of cocoons, in a sample of 5 conifer trees, is found in site 5, with representation in all zones of the tree. A particularly frequent appearance of the cocoon is in the lower zone (Graph 2). Due to the appearance of the largest number of cocoons in that locality - the "sample area", we also followed the life cycle of the pine beetle.



Graph 1. The number of observed pine beetle eggs in cocoons in the researched area



Graph 2. Display of the number of cocoons by zones of the tree

The position of the cocoons on the "viewing surface- sample area" is a sign that after eclosion the caterpillars did not have to migrate to a more favorable place, towards the top of the tree, i.e. the lower zones of the tree also had a favorable temperature and insolation. Although these are organisms that feed at night, we found 4th and 5th stage caterpillars in the daytime nibble, with a favorable external temperature of 15 degree C. Considering the temperature averages in the fall of 2023, which were significantly higher than the reference series 1991-2020 (Data from the Federal Hydrometeorological Institute of Bosnia and Herzegovina), these caterpillars had at their disposal a whole year's nibble, without the resting phase that occurs in unfavorable environmental conditions, and concerning are low temperatures and abundant rainfall. The optimal temperature for the development of pine beetle is 24 degree C (Androić, 1957).

In that area, black pine and Aleppo pine trees were about 90% infected, with the black pine having a higher degree of infection with noticeable damage and other diseases caused by continuous multi-year attacks.

All developmental forms of the mentioned pest were confirmed: egg, larva-caterpillar, pupa-pupa and imago-adult butterfly), as well as five stages in the development of the caterpillar itself. Stages 3, 4, and 5 pose a health risk to humans and animals due to toxic hairs containing the allergen thaumetopoein (Moneo et al., 2003).

In April 2023, the cocoons were already in the stage of degradation, without caterpillars, which indicated the next stage in the development of the butterfly-pupa. The pupal stage typically takes place in the ground, near pine trees at a depth of 15-20 cm and lasts for about 3-4 months. Sometimes, the diapause in development can last for a couple of years, so that the butterfly emerges only the following year.

Research into the life cycle of the pine beetle continued in August 2023, with the intention of looking for butterfly egg clutches, which are hard to see and very similar to the central pine needles.
During that tour of the field, several egg clutches were found at a height of 1-1.5 m from the ground, in the central part of the conifer between the needles, wrapped around two needles and covered with gray-reddish scales, which the female carries on the abdominal plate.
The egg litters were collected and transferred to home terrarium areas, and events were intensively monitored for 24 days. In September 2023, we observed the beginning of the eclosion of the caterpillars of the 1st stage in the development of the pine beetle. The hatching of the larvae took 3 days, and they were very mobile. Knowing that this is a social development stage, immediately after eclosion they formed characteristic columns (processions, processions), "head-tail-head", following the pheromone trail and the silk trail. Gathering into groups was also observed. After that period of confinement, in the absence of food, for 24 hours, they all died (Picture 2).

Picture 2. Caterpillars of the 1st stage of pine beetle

In parallel with these events, in laboratory conditions, we performed microscopic examination of the scales from the egg beds and, based on the characteristic appearance of the scales, determined the "typical form" of the pine beetle, already described earlier according to Androić, 1957.
For coniferous forests, damage in the form of defoliation and reduction of the assimilation zone is caused by caterpillars that feed on pine needles.
Knowledge about the biology and ecology of this insect was confirmed much earlier with the analysis of environmental conditions that benefit the pest: the advantage of high temperatures and insolation, exposure of the cocoons, as well as genetic factors and susceptibility to diseases, i.e. the presence of species parasites. A pine beetle is a nocturnal butterfly that lives very briefly in the imago stage, so we were not able to find a sample of an adult butterfly as the last stage of the life cycle (Androić, 1957).

Harm to humans and animals.

Pine beetles are dangerous for humans and animals. Contact with caterpillar hairs causes a dermatological reaction in the form of redness and rashes. People may experience allergic reactions or respiratory problems, and in extreme cases, even blindness.

Bearing in mind the above, as well as the fact that pine beetle caterpillars are found in our immediate vicinity (University Campus, Fortica Nature Park), we can say that human and animal health is exposed to the danger of allergens, which are an integral part of the hairs on the caterpillar's body.

Conclusions

Based on the results of research in the area of the City of Mostar, which included the locations of the "Džemal Bijedić" University Campus in Mostar, Park - Trimuša Forest, Imperial Vineyards - Blagaj, Road to Rujište - Humi and Fortica, it can be concluded that the pine beetle - *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775), a pest and defoliator of pines present with greater or lesser intensity of infection.

Black and Aleppo pines, as well as the population and animals that inhabit these areas, are particularly exposed to the attack of the pine beetle.

For the City of Mostar area, the health and survival of every conifer tree is a priority due to the beneficial effect on the soil, temperature, amount of oxygen and other positive effects on the environment, and the spread of infection by this harmful insect must be taken seriously with the finding of adequate solutions for its suppression.

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ALLERGENIC WOODY PLANT SPECIES IN THE COURTYARDS OF SCHOOLS IN THE CITY OF MOSTAR IN BOSNIA AND HERZEGOVINA

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Abstract

As part of the green infrastructure of the city, schoolyards changed over the historical periods, they acquired new features and meanings. In the last 10 years, there has been an increased interest in horticultural decoration of schools. When arranging the school yard, besides taking care that it should not be overcrowded with plants, gardener should also take into account the allergenicity of certain plant species. The most significant environmental aeroallergen is pollen.
The analysis of the presence of allergenic woody plant species was carried out during two growing seasons (2022 and 2023) at the locations of educational institutions in the city of Mostar in Bosnia and Herzegovina. The goal of the research was to determine the current state of allergenic woody flora in yards. The research covered 22 locations, 13 primary school locations and nine secondary schools. Twenty species of allergenic woody flora from 12 families were recorded at the investigated locations, the courtyards of elementary schools. In the courtyards of secondary schools, 15 species of allergenic woody flora from nine families were recorded. Given that it is a question of woody allergenic species, the analysis of life forms shows the absolute dominance of phanerophytes (P = 100%). Cultivated and adventitious floral elements are most represented.

Keywords: *Schoolyards, Allergenic woody plant, City of Mostar.*

Introduction

Schoolyards have a significant ecological, educational, pedagogical and didactic-methodical function in the work practices of primary and secondary schools. It is a place for the development of work habits, social and motor skills that encourage the development of responsibility, nature conservation, strengthen self-confidence and concentration. It is quite difficult to accurately determine the beginning of the establishment of school gardens, because they are not uniformly understood (Munjiza, 2003).
In the beginning, the school gardens were a kind of botanical gardens, which represented a source of lightness in classes and a place for school walks and excursions. Later, a production-economic function was added to school gardens, after which they were assigned a pedagogical function (Munjiza, 2003).
The wild and horticultural flora of cities, including elementary school gardens in the city, has multiple useful roles: absorption of particles from the air (dust, heavy metals, smog), binding of carbon (IV) oxide and production of oxygen, reduction of heating and noise, preservation of biodiversity, adoption of aesthetic role and importance for rest and recreation (Jarić, 2019).
Despite differences in the frequency, intensity and structure of allergies in different countries, the incidence and prevalence of allergic diseases, including allergic asthma, rhinitis and

conjunctivitis, show an increasing trend in recent decades (Peat, 1996). The biggest burden of this increase refers to the occurrence of allergic diseases in children.
D'Amato (2007) states that allergological studies show that the pollen map of Europe is changing as a result of human factors, colonization and climate change, and that the length of the growing season in Europe has increased by an average of 10-11 days over the last 30 years.
In the last few decades, the number of people suffering from pollen allergy has increased. Allergies to pollen (pollinosis) are the most common types of allergies and occur during the flowering of allergenic plants. They usually start in early spring and end in autumn. It is certainly important to emphasize the meteorological conditions, because longer periods of rain reduce the spread and concentration of pollen in the air. Hay fever combines allergic rhinitis and allergic conjunctivitis. The symptoms are: itching, burning and watery eyes, sneezing, sniffling, a feeling of fullness and blockage of the nose, itching of the tip of the nose, more abundant watery discharge and reduction or lack of sense of smell.
The more pronounced the symptoms are, the greater the amount of pollen in the air, which means that they worsen towards the peak of the flowering period in nature. Pollen concentration is higher in the morning, on sunny and windy days. In these conditions, dry and light pollen can be blown by the wind to a great distance. Conversely, at the beginning and end of the flowering season and during wet and rainy weather, the concentration of pollen in the air is significantly lower.
People allergic to the pollen of one plant (monosensitization) very quickly become allergic to the pollen of other plants (polysensitization) due to decreased immunity and hypersensitivity.
The aim of this work was to visit courtyards of schools in the city of Mostar and list the plant species found in the schoolyards. Also, the aim was to separate allergenic herbs and determine their allergic potential.

Material and methods

The analysis of the presence of allergenic woody species was carried out during two growing seasons (2022 and 2023). The survey included 22 locations in the city of Mostar, 13 primary schools and 9 secondary schools (Table 1).

Table 1. List of analyzed locations

Location number	Location	Coordinates
1.	Primary school Antun Branko Šimić Mostar	43°33'79"N 17°80'20"E
2.	Elementary school Ilići	43°34'43"N 17°77'56"E
3.	Third primary school	43°34'24"N 17°80'19"E
4.	Elementary School of Ivan Gundulic	43°35'42"N 17°79'98"E
5.	Osnovna škola Petra Bakule	43°34'79"N 17°79'96"E
6.	Osnovna škola Silvija Strahimira Kranjčevića	43°34'99"N 17°79'16"E
7.	Osnovna škola Ilije Jakovljevića	43°35'23"N 17°80'33"E
8.	Osnovna škola Kruševo	43°28'57"N 17°75'35"E
9.	Osnovna škola Polog	43°35'10"N 17°69'03"E
10.	Osnovna škola Bartola Kašića Rodoč	43°31'62"N 17°81'69"E
11.	Osnovna škola Marina Držića Buna	43°24'36"N 17°84'22"E
12.	Osnovna škola Cim	43°35'08"N 17°78'24"E
13.	Elementary school for children with special needs	43°34'19"N 17°80'70"E

14.	Gymnasium Mostar	43°34'27"N 17°80'71"E
15.	Gymnasium fra Grga Martić	43°34'83"N 17°79'91"E
16.	Sisters of Mercy Secondary Medical School	43°33'77"N 17°80'71"E
17.	High School of Economics Joze Marinovića	43°34'71"N 17°80'01"E
18.	Ruđer Bošković High School of Electrical Engineering	43°34'71"N 17°80'01"E
19.	Secondary traffic school	43°34'71"N 17°80'01"E
20.	Juraj Dalmatinac Secondary School of Construction	43°34'56"N 17°79'97"E
21.	Faust Vrančić High School of Engineering	43°34'56"N 17°79'97"E
22.	Gabrijel Jukić High School of Fine Arts	43°34'99"N 17°79'16"E

The following literature was used to determine the plants, their taxonomic affiliation, life form, origin: Bird (2012), Borzan (2001), Brickell (2006), Domac (1994), Erhardt et al. (2014), Idžojtić (2009), Karavla (2007), Nikolić et al. (2014). Also, the following websites were used: Flora Croatica Database (Nikolić T. ed. (2021), Missouri Botanical Garden, Plants of the World online.
Allergenicity of plant species was determined according to D'Amat et al. (2007) who classify the level of pollen in the air as: low (1), moderate (2), high (3) and very high (4).

Results and discussion

During a two-year survey of allergenic flora in the courtyards of primary and secondary schools in the city of Mostar (Bosnia and Herzegovina), 20 species of allergenic flora of primary schools classified into 12 families and 15 species of allergenic flora of secondary schools classified into nine families were recorded. The *Pinaceae* family stands out with the largest number of species. Given that it is a question of woody allergenic species, the analysis of life forms shows the absolute dominance of phanerophytes (P = 100%).
The analysis of flora elements, that is, the phytogeographical analysis of the allergenic flora of elementary schools in the city of Mostar shows the presence of eight flora groups (Horvatić 1963 and Pignatti 1982) with the highest representation of cultivated and adventitious elements with 8 species. Then follows the Eurasian flora element with 4 species, species represented by three species. Other flora elements have one type of allergenic plant each (Table 2).
The analysis of flora elements, that is, the phytogeographical analysis of the allergenic flora of secondary schools in the city of Mostar shows the presence of six flora groups (Horvatić 1963 and Pignatti 1982) with the highest representation of cultivated and adventitious elements with 8 species. Other flora elements occur with one or two species (Table 3). Cultivated and adventive species were mainly introduced by humans, intentionally or unintentionally, from other continents as ornamentals, cultivated, and more or less adapted there.

Table 2. Representation of allergenic plants in the courtyards of primary schools in the city of Mostar

Family	**Species**	A life form	Floral elements	OŠ. A. B. Šimića	OŠ. Ilići	OŠ. Ivana Gundulića Mostar	Treća osnovna škola	OŠ. Ilije Jakovljevića	OŠ. Silvija Strahimira Kranjčevića	OŠ Petra Bakule	Osnovna škola Cim	OŠ. Bartola Kašića Rodoč	OŠ. Marina Držića Buna	OŠ. Polog	OŠ. Kruševo	OŠ. za djecu sa posebnim potrebama
Betulaceae	*Betula pendula* Roth	P	euro asiat	+	+						+	+				
	Carpinus betulus L.	p	euro asiat			+										
Cupressaceae	*Cupressus sempervirens L.*	P	adv	+	+	+			+	+	+	+	+	+	+	
	Thuja occidentalis L.	P	adv	+									+		+	
Fabaceae	*Robinia pseudoacacia* L.	P	adv	+		+			+			+	+		+	
Fagaceae	*Quercus pubesceus Willd.*	P	Europ-pont							+						
	Quercus petraea (Mattuschka) Liebl.	P	c-europ												+	
Hyppocastanaceae	*Aesculus hippocastanum* L.	P	se - europ	+												
Juglandaceae	*Juglans regia* L.	P	adv						+			+			+	
Malvaceae	*Tilia sp.*	P	c-europ	+		+		+			+	+	+			
	Tilia cordata Mill.	P	c-europ	+		+	+	+			+	+				
Oleaceae	*Olea europaea* L.	P	medit		+	+		+	+	+			+	+		+
Pinaceae	*Abies alba* Mill.		europ					+			+					
	Picea abies (L.) Karsten	P	adv	+	+		+	+	+	+			+		+	
	Pinus sylvestris L.	P	euro asiat			+		+	+	+	+	+			+	+
	Pinus halepense Miller	P	adv	+										+		
	Larix decidua Mill.	P	adv	+	+								+		+	
Platanaceae	*Platanus sp.*	P	adv	+	+					+	+					
Salicaceae	*Salix alba* L.	P	euro asiat					+								
Ulmaceae	*Ulmus sp.*	P	cosmop	+		+							+		+	

Table 3. Representation of allergenic plants in the courtyards of secondary schools in the city of Mostar

Family	**Species**	A life form	Floral elements	Sisters of Mercy Secondary Medical School	Jozo Marinović High School of Economics	Ruđer Bošković High School of Electrical Engineering	Gymnasium Mostar	Gymnasium fra. Grga Martić	Secondary traffic school	Gabrijel Jukić High School of Fine Arts	Jurije Dalmatinac Secondary School of Construction	Faust Vrančić High School of Engineering
Cupressaceae	*Cuppresus sempervirens* L.	P	adv	+	+	+	+	+	+	+		
	Thuja occidentalis L.	P	adv								+	+
Fabaceae	*Robinia pseudoacacia* L.	P	adv	+			+			+		
Juglandaceae	*Juglans regia* L.	P	adv	+					+	+		
Malvaceae	*Tilia sp.*	P	c-europ		+	+	+	+	+		+	+
	Tilia chordata Mill.	P	c-europ								+	+
Oleaceae	*Olea europaea* L.	P	medit	+	+	+			+	+	+	+
Pinaceae	*Abies alba* Mill.	P	europ						+			
	Picea abies (L.) Karsten	P	adv		+	+				+		
	Pinus sylvestris L.	P	euro asiat				+	+	+	+	+	+
	Pinus halapense Miller	P	adv				+					
	Larix decidua Mill.	P	adv		+							
Platanaceae	*Platanus sp.*	P	adv				+	+			+	
Salicaceae	*Salix alba* L.	P	euroasiat		+							
Ulmaceae	*Ulmus sp.*	P	cosmop				+				+	

As for allergenic species, out of a total of 22 analyzed locations, all have some allergenic woody species in their yard (Tables 2 and 3). The most common allergenic species is *Cuppresus sempervirens* in 17 locations, and pollen is most often present from January to the end of June. Cypresses produce pollen in large quantities, with measured allergenic potential.
Then the olive (*Olea europaea*) in a total of 15 locations, sometimes causes very strong allergic symptoms that are present in sensitive people throughout the year. *Tilia sp., Pinus sylvestris, Picea abies* are also significant for their presence in yards. If the representation of allergenic species is observed according to the type of educational institution, the most examples of allergenic woody plants were recorded on green areas within primary schools.

Conclusion

Plants have a different growing season when they produce pollen, so during pollination there are large concentrations of pollen from these plants in the air. Of the 22 analyzed locations in the city of Mostar, 11 of them recorded a high presence of woody allergenic plant species. The species *Cuppresus sempervirens* has the highest representation in 17 locations.
It is often grown as a horticultural product and planted as a decoration of city green areas. Cultivation of urban areas contributes to the spread of large amounts of allergenic pollen, so exposure to cypress pollen has been steadily increasing in recent decades. Then *Olea*

europaea in 15 locations, olive pollen has a strong allergic potential and is one of the most important causes of allergic diseases of the respiratory system in the Mediterranean area.
Tilia sp. and *Pinus sylvestris* were recorded in 13 locations, and Picea abies in 11 locations. Such a large number of allergenic species on the green areas of primary and secondary schools in the city of Mostar speaks of the need to pay more attention when choosing decorative plant species for the design of green areas whose users are children.

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EFFECT OF FERTILIZATION AND TYPE OF GRASS-LEGUMINOUS MIXTURE ON GRASSLAND COVER ON TECHNOGENIC SOIL

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Abstract

The grassland covering or crop density is an important feature in the cultivation of grass-leguminous mixtures. This parameter is particularly important on technogenic soil in order to reduce water erosion. The research of biological phases of soil reclamation by seeding the grassland has been conducted on the Deposol at internal disposal area for overburden from Raskovac open pit in Stanari coal mine (Republic of Srpska, Bosnia and Herzegovina). The survey task refers to measurement of impact of fertilization and type of the grass-leguminous mixture on technogenic soil cover. The survey was expended through three-year period (2011–2013). Three grass-leguminous mixtures and one grass mixture were studied; altogether four treatments of various doses of mineral fertilizer and lime. Statistical analysis of measuring quantitative properties of grassland cover was conducted by method of ANOVA, 3x4x4. At the beginning of research, the sandy-loam Deposol had unfavorable physical and chemical properties. The applied treatments and interactions had positive impact on measuring quantitative properties of researched mixtures and properties of Rekultisol. The highest average coverage after crop emergence (2011) was achieved with the treatment b_4 (TDS-4) mixture (73.1%), and the lowest with the treatment b_1 (TDS-1) mixture (68.6%). The treatment b_3 (TDS-3) mixture had the biggest covering at the beginning of vegetation period (82.01%) and at end of vegetation period (92.2%) in 2013. The biological reclamation in researched agro-ecological conditions was successfully conducted by seeding grasslands, raising selected species in grass-leguminous mixtures and by application of optimal agromeliorative measures.

Key words: *open pit, soil reclamation, grassland covering, Stanari.*

Introduction

Main objective of the reclamation of degraded areas, caused by open pit mine exploitation of the ore reserves, is to establish the management functions on these newly created technogenic soils (technosols). The main task of all these man-built terrestrial ecosystems is to stabilize and revive production and ecological functions of a technogenic soil, ie Deposol (the surface layer of the disposal area for overburden).

The fertility of the Deposol and most other types of technogenic soils (e.g. mine soils) is usually low. The concentration of the Deposol with the basic biogenic elements (N, P and K) is within or below the minimum concentrations (Dvurechensiy and Seredina, 2015; Pivić et al., 2011; Sheoran et al., 2010; Coppin and Bradshaw, 1982; Antonović et al., 1978). In addition to the deficit of nutrients, technogenic soils have low content of pedobi'os and organic matter, and poorly developed adsorptive complex (Rasulić et al., 2005). Shukla et al. (2004) states the following disorders in technogenic soil: loss of aggregate and soil structure, decrease in soil C concentration, increase in volume, and decrease in porosity.

The results of the past physical and chemical analyzes of Deposol at disposal area for overburden at the Stanari mine found that they have favorable physical - mechanical but unfavorable chemical properties (Malić et al., 2017; Malić, 2015; Malić et al., 2021; Malić and Marković, 2021). The same authors state that, based on the content of organic matter, the researched Deposol belongs to the class of low and medium content, while there is no pure humus and nitrogen. According to the content of P_2O_5 and K_2O in the Deposol, they are classified as very poorly secured by these elements. The Deposol is characterized by a non-carbonate substrate, a strong unsaturation with base cations, a medium and highly acidic chemical reaction. The fact that the content of organic matter is very low and acidic chemical reaction make are the biggest problems in Stanari Deposols.
A significant part of the agricultural reclamation refers to the establishment of artificial grassland. Studies on the methods of grassland establishment in the reclamation process and potential yields have started in 2008 at the Deposols of Raškovac open pit external disposal area in Stanari (Malić and Lakić, 2011). The grassland covering or crop density is an important feature in the cultivation of grass-leguminous mixtures (Čolić et al., 2013). This parameter is particularly important on technogenic soil in order to reduce water erosion. Crop density is also important for biomass production, which is the basis of the circulation cycle of organic matter in the soil (Malić, 2015).
Pioneer species in agricultural reclamation include species from the families of Poaceae and Fabaceae. Seeding grasslands establishment through seeding grass-leguminous mixtures and pure cultures of certain grass species is significantly present at mine in Bosnia and Herzegovina, Serbia and abroad. Since the earliest reclamation works in the USA, a vast expanse of recultivated areas has been under seeding meadows and pastures (Thorne, 2010, Skousen and Zipper, 2010, Lyle, 1987, Vogel and Berg, 1968). Normally, most commonly sown species during reclamation process are grasses (family Poaceae) because they are producing a large amount of biomass and quickly adapt to specific and harsh environmental conditions. For biological reclamation most commonly used grass species are from the following genus: *Poa*, *Festuca*, *Lolium*, *Panicum*, *Agrostis*, *Phleum*, *Dactylis* (Malić and Lakić, 2011, Smith et al., 2002). Participation of leguminous in mixtures depends on the type and characteristics of Deposols used for reclamation. In addition to the potential yields of forage and hay, multiple significance of all types of grasslands is reflected in the changes of basic physical, chemical and biological properties of Deposol by increasing its fertility. The aim of this survey refers to implementation of biological phase of reclamation and improvement of technogenic soil fertility.

Material and method

The coal basin Stanari is located between 44°40' and 44°50' N and 17°45' and 18°00' E, in the northern part of the Republic of Srpska / Bosnia and Herzegovina. Research on biological reclamation of a direct type was carried out at the experimental field (GPS Coordinates: y = 6.486.822.33; x = 4.957.645.63; altitude 220 m a.s.l) within internal disposal area for overburden of the excavation from the open pit Raškovac in the lignite coal basin Stanari: "Power Plant Stanari". The survey was conducted in a three-year period (2011–2013). Part of the disposal area for overburden where the experimental field is located was formed during 2010.
The three-factorial experiment was set by the random block method in four repetitions. The experiment plots area is 10 m^2 (5×2 m). The distance between the plots is 50 and 80 cm, and between the blocks is 1 m. Plots are without inclination.
The first factor of research refers to the year (factor A), with three treatments. The second factor refers to various grass and leguminous species in mixtures (factor B), with four

treatments. The third factor is agromeliorative measures (fertilization, liming and mulch, factor C), with four treatments (Table 1).
Statistical analysis of measuring quantitative properties of grassland cover was conducted by method of ANOVA, 3x4x4.
Establishment of the seeding grassland was carried out during the spring sowing period in 2011. Seeding rate amounted to 45 kg/ha. The measurements of grassland cover were carried at the beginning of vegetation period (May 2011, March 2012 and 2013) and at end of vegetation period (October 2011, November 2012 and October 2013). The coverage of the surface was measured according to the Rulebook on methods of testing grass varieties (Official Gazette of the Republic of Serbia, No. 92/12), and according to a scale from 1 to 9:

- grade 1 – no coverage,
- grade 3 – up to 25% surface coverage,
- grade 5 – 25% - 50% surface coverage,
- grade 7 – 50% - 75% surface coverage,
- grade 9 – 75% - 100% surface coverage.

Table 1. Treatments three-factorial experiment

Factors	Treatments			
Factor A (three years)	a_1 (2011)	a_2 (2012)	a_3 (2013)	
Factor B (four mixtures)	b_1 (TDS-1): *Festuca arundinacea* Schreb. 25%, *Festuca rubra* L. 20%, *Dactylis glomerata* L. 10%, *Phleum pratense* L. 10%, *Trifolium repens* L. 10%, *Trifolium pratense* L. 10%, *Medicago sativa* L. 10%, *Poa pratensis* L. 5% b_2 (TS-2): *Festuca arundinacea* Schreb. 40%, *Festuca rubra* L. 20%, *Poa pratensis* L. 20%, *Dactylis glomerata* L. 10%, *Phleum pratense* L. 10% b_3 (TDS-3): *Festuca rubra* L. 50%, *Poa pratensis* L. 30%, *Lotus corniculatus* L. 10%, *Trifolium repens* L. 10% b_4 (TDS-4): *Dactylis glomerata* L. 30%, *Phleum pratense* L. 30%, *Lotus corniculatus* L. 20%, *Arrhenatherum elatius* (L.) Mert. et Koch. 20%			
Factor C (four agromeliorative measures)	c_1: $N_{90+54+54}P_{90}K_{90}$	c_2: $N_{60+54+54}P_{90}K_{90}$+ 8 t ha^{-1} $CaCO_3$	c_3: $N_{60+40+40}P_{60}K_{60}$	c_4: $N_{90+40+40}P_{90}K_{90}$ + wheat straw mulch

The third factor (C) represents dosages of mineral fertilizer (pure nutrients) applied during sowing period (doses: $N_{90}P_{90}K_{90}$, $N_{60}P_{90}K_{90}$ and $N_{60}P_{60}K_{60}$) and spring supplementation with nitrogen fertilizer (doses: N_{54+54} and N_{40+40}). The mineral fertilizer NPK 15:15:15 was used before sowing, and KAN (27% N) during supplementation phase. Limestone was added during the primary tillage of the Deposol (treatment c_2). After the sowing, wheat straw in the form of mulch was used on treatment c_4.
The determination of the studied types of technogenic soil was carried out according to Resulović and Čustović (2007), and the WRB classification (2014). According to the soil classification in Bosnia and Herzegovina, the newly discovered soils mine belong to the class of technogenic soils (types Deposol and Rekultisol). Deposol represents the type of surface layer of disposal area for overburden prior to biological phase of the reclamation. Rekultisol is a layer of soil where reclamation measures have been carried out and the initial processes of humification and mineralization begin. The researched Deposol and formed geogenic Rekultisol are of a silicate subtype. According to the WRB classification (World Reference

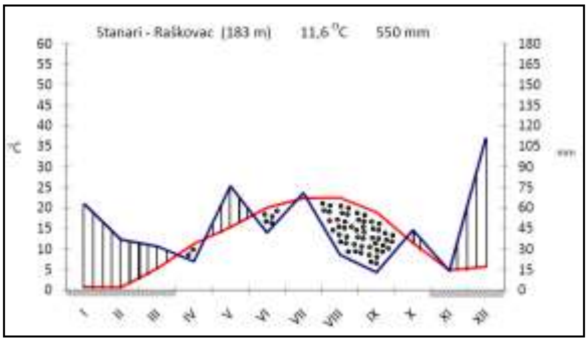
Base for Soil Resources, 2014), these soils are determined as technosols (Epiarenic and silicit material).
For the purposes of laboratory pedological research, the average samples of the Deposol were taken before the study at the beginning of 2011. The samples were taken from a depth of 0-20 cm. The analysis of the technogenic soil included the examination of the following parameters:

1) pH in H_2O and KCl;
2) content of organic matter in %, dry burning method at 550 ^oC;
3) humus content in %, colorimetric method, in a wet-burned sample with 1N $K_2Cr_2O_7$ and concentrated H_2SO_4;
4) total N in %, semimycro Kjeldahl method;
5) plant available P_2O_5 and K_2O, in mg per 100 g of soil, AL-method.

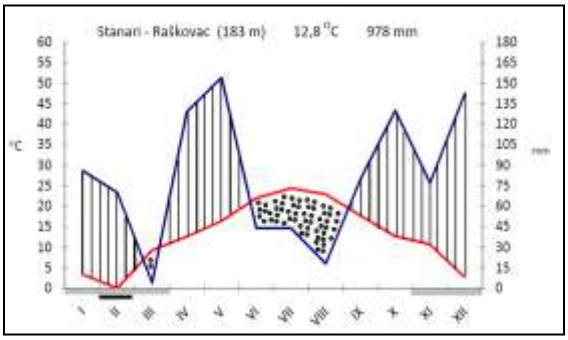
Table 2. Results of the analysis of chemical properties of Deposol

No. of sample	pH		Organic matter	Humus	Total N	AL - P_2O_5	AL - K_2O
	H_2O	KCl	%			mg/100g soil	
1	6.2	4.9	2.1	0.0	0.0	0.0	1.3
2	5.8	4.5	1.2	0.0	0.0	0.5	2.2
3	5.2	4.0	1.6	0.0	0.0	0.6	2.7

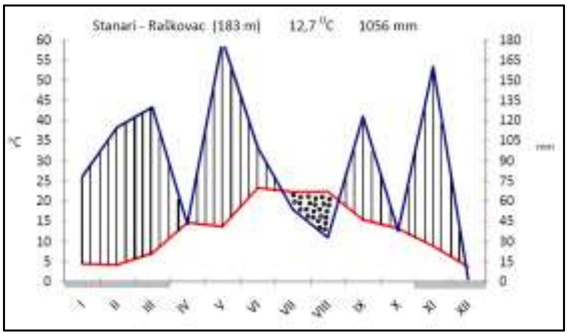
The basic climate indicators (precipitation and air temperature) in the studied three-year period are shown in the following graphs (climate diagram to Walter and Lieth).



Graph 1. Climate diagram in 2011



Graph 2. Climate diagram in 2011



Graph 3. Climate diagram in 2013

Results and discussion

Three-year research and application of the intensive agrotechnical measurements in the implementation of the biological phase of reclamation (by the dominance of the anthropogenic factor) resulted in formation of a stable grassland on the surface layer of the technogenic soil. The coverage of the surface at the beginning of the growing season in 2011 was carried out after the emergence of the plants, which also represents the assessment of the emergence, because it is the year of sowing.

The crops were mulched so the dry mass stayed on the Deposol surface in the process of reclamation. Table 4 shows the average values of the covering at the end of vegetation period. The identified interaction effects are given in Graph 4 and Graph 5.

Table 3. Average covering at the beginning of vegetation period (%)

		Treatments basic factors		Factor B (mixtures) b_1	b_2	b_3	b_4	$\bar{x}_C$
Factor A (years)	a_1 (2011)	Factor C (agromeliorative measures)	c_1	58.7	63.9	65.0	70.1	64.4
			c_2	71.8	69.7	72.7	73.6	71.9
			c_3	71.7	70.4	73.6	72.6	72.0
			c_4	72.3	74.6	72.4	73.1	73.8
			$\bar{x}_B$	68.6	69.6	70.9	73.1	70.5
	a_2 (2012)	Factor C (agromeliorative measures)	c_1	85.1	85.6	92.8	90.8	88.6
			c_2	96.0	90.6	92.8	90.7	92.5
			c_3	96.6	91.0	94.5	95.4	93.6
			c_4	92.6	95.6	97.6	97.5	95.8
			$\bar{x}_B$	91.8	90.7	94.4	93.6	92.6
	a_3 (2013)	Factor C (agromeliorative measures)	c_1	68.8	68.2	76.2	65.5	69.6
			c_2	75.3	61.9	82.9	74.4	73.6
			c_3	73.2	82.7	81.5	71.5	77.2
			c_4	74.7	77.4	82.1	73.1	76.8
			$\bar{x}_B$	73.0	72.5	80.6	71.1	74.3
			$\bar{x}_{BC}$	77.8	77.6	82.01	79.27	79.18

ANOVA		A	B	C	A×B	A×C	B×C	A×B×C
$F_{calc.}$		189.71**	5.20**	11.80**	1.95$^-$	0.34$^-$	1.19$^-$	0.91$^-$
Lsd	0.05	2.36		2.73		-	-	-
	0.01	3.11		3.59		-	-	-

With the researched mixtures, cover during the research were variable in some of the treatments. The results in Table 3 indicate the increase of the average covering at the beginning of vegetation. The average value of the cover in the first year was 70.5%, in the second 92.6% and in the third 74.3%. The analysis of the variance shows the statistically significant influence of the basic factors of the year (A), different mixtures (factor B) and agromeliorative measures (C).

The highest average coverage after crop emergence (2011) was achieved with the treatment b_4 (TDS-4) mixture (73.1%), and the lowest with the treatment b_1 (TDS-1) mixture (68.6%). The highest average coverage at the beginning of vegetation in 2011 and 2012 was achieved with the treatment b_3 (TDS-3) mixture (94.4% and 80.6%), and the lowest with the treatment b_2 (90.7%) in 2012 and treatment b_4 (71.1%) in 2013.

The highest average yield of green of green mass in the first swath (average value of factor C treatment) for the entire three-year period (4.8 t ha^{-1} , 16.7 t ha^{-1} and 25.8 t ha^{-1}) was measured with the TDS-3 mixture (Malić et al., 2017). The lowest average yield of green mass in 2011 was measured with the TDS-1 mixture (2.9 t ha^{-1}), in 2012 with the TDS-4 mixture (12.07 t ha^{-1}) and in 2013 with the TS-2 (18.6 t ha^{-1}).

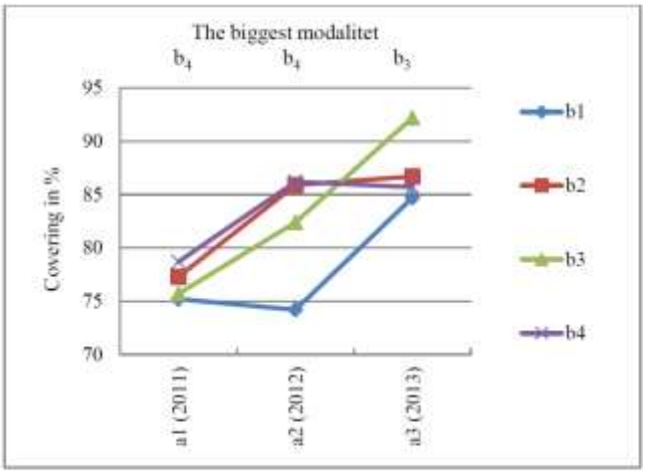
Table 4. Average covering at the end of vegetation period (%)

Treatments basic factors				Factor B (mixtures) b_1	b_2	b_3	b_4	$\bar{x}_C$
Factor A (years)	a_1 (2011)	Factor C (agromeliorative measures)	c_1	66.4	76.6	69.6	75.8	72.1
			c_2	77.7	76.6	75.8	78.7	77.2
			c_3	78.2	76.2	79.7	78.7	78.2
			c_4	78.8	79.9	77.9	81.6	79.5
			$\bar{x}_B$	75.2	77.3	75.7	78.7	76.7
	a_2 (2012)	Factor C (agromeliorative measures)	c_1	69.7	73.6	72.8	80.1	74.0
			c_2	76.4	90.8	83.8	85.2	84.0
			c_3	76.4	90.6	90.9	88.5	86.6
			c_4	74.3	88.7	82.1	91.1	84.0
			$\bar{x}_B$	74.2	85.9	82.4	86.2	82.2
	a_3 (2013)	Factor C (agromeliorative measures)	c_1	81.1	82.6	90.6	82.0	84.8
			c_2	86.3	84.2	93.1	86.9	87.6
			c_3	84.8	89.3	93.7	88.8	89.1
			c_4	86.7	91.0	91.3	82.1	87.7
			$\bar{x}_B$	84.7	86.7	92.2	85.7	87.3
			$\bar{x}_{BC}$	78.06	83.34	83.34	83.54	82.09

ANOVA		A	B	C	A×B	A×C	B×C	A×B×C
$F_{calc.}$		13.08**	8.87**	20.89**	5.4**	2.27*	0.87^-	0.78^-
Lsd	0.05	2.01	2.32		4.02		-	-
	0.01	2.64	3.05		5.28		-	-

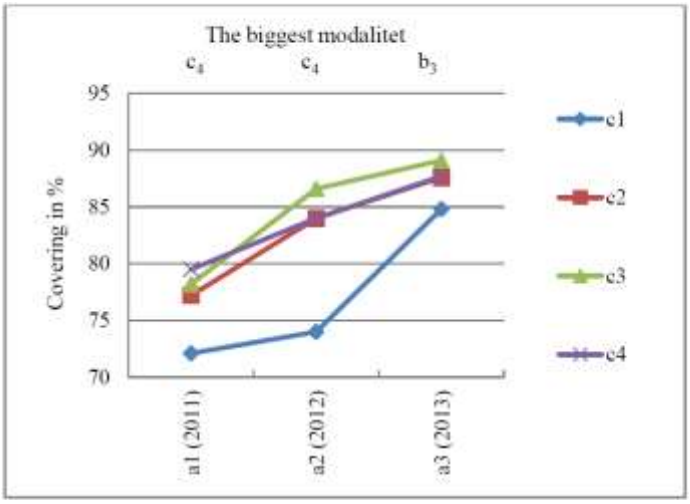
The results in Table 4 indicate the increase of the average covering at the end of vegetation. The average value of the cover in the first year was 76.7%, in the second 82.2% and in the third 87.3%. The analysis of the variance shows the statistically significant influence of the all basic factors, interaction of factor A and B (year × mixtures), while the interaction of factor A and C (year × agromeliorative measurements) is significant.

The highest average coverage at the end of vegetation period in 2011 and 2012 was achieved with the treatment b_4 (TDS-4) mixture (78.7% and 86.2%) and in 2013 with the treatment b_3 (TDS-3) mixture (92.2%). The lowest average coverage at the end of vegetation period was achieved with the treatment b_1 (75.2% in 2011, 74.2% in 2012 and 84,7% in 2013).



Graph 4. Interactive effects of research year (factor A) and mixture (factor B) on the cover at the end of vegetation in %

The analysis of the interaction effect of the year × mixture (A × B) on the average values of cover at the end of vegetation shows the variations between the individual mixtures in the third year compared to the first and second year of the study. The mean value of the surface coverage under the TDS-3 mixture in 2013 had a higher value compared to the other mixtures and these differences are statistically highly significant. The differences between the mean values average of the surface coverage at the end of the vegetation period in the first year are not significant, while in the second year of the research the mean average value of the surface coverage under the TDS-1 mixture is lower than the values of the coverage under the other mixtures and these differences are statistically highly significant. The mentioned differences are explained by the fact that the climatic coditions in 2013, as well as the biological growth of cultivated grass and leguminous species, had a positive effect on the increase in the surface coverage of treatments b_3 (TDS-3: *Festuca rubra* L., *Poa pratensis* L., *Lotus corniculatus* L., *Trifolium repens* L.). This modality had an approximately linear growth trend.



Graph 5. Interactive effects of research year (factor A) and agromeliorative measures (factor C) on the covering at end of vegetation (%)

The analysis of the interaction effect of the year × agromeliorative measures (A × C) on the average values of cover at the end of vegetation shows that the average value of the surface coverage under the mixtures in treatment c_1 is lower than the value of other treatments of factor C and these differences are highly significant in 2011 and 2012. At the end of the growing season of last year of the study (2013), the observed differences in surface coverage between mixtures from different treatments of agromeliorative measures were reduced, and statistically significant differences were present only between treatments c_1 and c_3.

Conclusion

The establishment of a seeded grassland with the sowing of grass-leguminous and grass mixtures, with the application of agromeliorative measures, concludes the following:

- The chemical properties of the researched Deposol are characterized by the non-carbonate and non-humus substrate, the strong unsaturation of the base cations, the medium and highly acidic chemical reaction, the low content of organic matter and basic biogenic elements.
- The ecological conditions during the research and applied agro-technical measures have had an impact on the growth and development of grass and legumes, as well as on the changes in the surface layer of the researched technogenic soil.

- The highest average coverage (mean value for factor C treatments) after emergence was achieved with the treatment b_4 (73.1%), and the lowest with the treatment b_1 (68.6%).
- The highest average coverage at the beginning of vegetation period was measured with the b_3 treatment in 2012 (94.4%) and 2013 (82.01%), and the lowest average values (90.7% and 77.6%) in the same same years with the b_2 treatment.
- The highest average values of surface coverage at the end of vegetation in 2011 (78.7%) and 2012 (86.2%) were measured with the b_4 treatment, and in 2013 (92.2%) with the b_3 treatment.
- The lowest average values in all three measurements (75.2%, 74.2% and 84.7%) were recorder with the treatment b_1.

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SANITARY WASTEWATER FROM CAMPS AT HIGHWAY CONSTRUCTION SITES

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Abstract

The goal of wastewater treatment is to obtain treated effluent of a quality that will not harm the flora and fauna of the recipient. The treatment criteria are determined for each specific situation according to the conditions of the plant and environmental requirements. The system at the construction site is used for treating sanitary wastewater. It is a closed system. The wastewater treatment is carried out by an aerobic biological treatment process with activated sludge. The entire process is automated. The plant's capacity is 200-300 inhabitants, and it is classified as a small plant. This paper aims to conduct a physico-chemical and microbiological analysis of the effect of treating sanitary wastewater, with the hypothesis that the composition of the treated water meets the defined ecological requirements. The results confirmed that the water at the outlet of the treatment plant meets all the requirements for discharge for redistribution to recipients as second-category water. The system represents a positive example of how contractors think about protecting natural watercourses and the environment. Since the ecological priority is to prevent water pollution, there is a great responsibility and duty to develop as efficient water treatment procedures as possible.

Key words: *wastewater, recipient, microbiological analysis, physico-chemical analysis.*

Introduction

Wastewater represents waste that, according to the classical approach, must be treated so that when it returns to natural receivers, it does not cause their degradation (Povrenović *et al.*, 2013). In accordance with environmental protection requirements, world standards and criteria have been set that wastewater must meet before being discharged into receivers (USEPA 2000). To achieve this, in addition to mechanical and physico-chemical processes, wastewater is also treated with biological processes. The choice of treatment method depends on the characteristics of the wastewater and the required level of treatment (Rajaković et al. 2003). The biological process is used to break down organic matter, which is the main component of sanitary wastewater. However, it is also important to clarify the wastewater as it participates in the water cycle in nature and the built urban water system (Đurin *et al.*, 2017). At construction sites, several types of wastewater occur, all of which end up in watercourses near the construction sites, so it is particularly important to manage them in the best possible way. In practice, for treating municipal and sanitary wastewater, the most widely used method is the activated sludge process. This process provides an active mass of microorganisms capable of aerobically breaking down organic pollutants present in wastewater. Their removal from the water is based on biooxidation in the cells of activated sludge microorganisms in the presence of sufficient dissolved oxygen (Mojović, 2004). However, the high cost of obtaining activated carbon and its loss during regeneration significantly limits the use of this method (Pap, 2017). After bioaeration, the water with suspended activated sludge is transported for sedimentation, where the sludge separates from the water. Due to its high adsorption capacity, some difficult-to-decompose substances can also be removed (Malešević *et al.,* 2021). At wastewater treatment plants, the input and output quantities and the physico-chemical composition of the wastewater are examined, and values for a range of indicators such as temperature, pH, biological oxygen demand (BPK_5), chemical oxygen demand (HPK), suspended solids, dry matter, oxygen, phosphorus, nitrogen, etc. are determined.

Description and Location of the Construction Site

Due to inaccessibility, facilitating work, and saving time, camps are built for highway construction, housing office space and living quarters for workers. Depending on capacity, these camps daily accommodate 200 to 300 employees, who require all the accompanying elements of a settlement. Within such camps, wastewater treatment systems are built. These systems handle sanitary water and water from kitchens (Figure 1). Sanitary water pollution can be of mineral, organic, inorganic, or combined origin. Organic pollution originates from animal, human, or plant sources and appears as solutions, colloids, and suspensions. The construction of this plant has prevented the degradation of the Morava River, which is the main recipient of wastewater from the construction site.

Figure 1: Construction camp for accommodating employees, offices, and workshops

The construction of the treatment system was based on literature data on sanitary wastewater (Massoud *et al*., 2009), with the concentration of these parameters being a function of the amount of water used in the camps. Also, for the construction of the plant within the EU regulations, the 1991, EEC Directive of the European Council on wastewater treatment is of particular importance (Council Directive 91/271/EEC 1991). Table 1 shows the input design parameters of the system.

Table 1. Input Design Parameters of the Biological Treatment Plant

Parameter	Value
Capacity	200-300
Total daily amount of wastewater	30- 45 m^3/day
Degree of treatment, %	95%
Working temperature of wastewater, ^0C	20
Organic load of wastewater, BPK_5 /day	300 ·0,06 kg BPK_5/day = 18 kg
Suspended solids load	2,1 kg suspended solids/day
Type of waste water	Sanitary - fecal
Treatment technology	Gravity and biological degradation
Installation method	Buried in the ground

The project task required a treatment efficiency of over 90%, with an effluent BPK_5 quality of 20 mg O_2/L and suspended solids of 30 mg/L, which is a slightly stricter criterion than provided by the EU Directive.

Technological Process of Wastewater Treatment from the Camp

Various systems are used at construction sites for wastewater treatment. One of the most efficient is the biologically aerated wastewater treatment plant. It is a flow-through device made of waterproof reinforced concrete and plastic with a very large volume for the designed amount of wastewater (30-45 m³/day), making it reliable in operation at maximum flows up to 45 m³/day (Figure 2.). It is 100% buried in the ground, allowing any type of traffic to pass over it. On the surface, several openings are visible: the inlet manhole, the outlet manhole with a sampling point and a chlorine tablet place, and a sludge pump-out manhole. The aesthetic covers are provided by the investor depending on the landscaping.

Figure 2: Wastewater treatment system on the Preljina-Požega highway

The treatment is carried out mechanically by gravitational sedimentation and biological degradation of organic matter through anaerobic and aerobic processes, where air is pumped into the device under pressure according to a specific regime. The device consists of several chambers with biofilm carriers and blowers for air injection. Surface atmospheric waters are prohibited from being introduced into the device. The advantage of this system is that the device is buried in the ground below the freezing point, making it usable in areas with very low temperatures. Wastewater is brought to the treatment plant in a receiving manhole with a coarse grid. The manhole height is 180 m. Before entering the bioreactor, the pH value of the untreated water is regulated within the necessary limits of 6.5-7.5. The bioaeration basin (bioreactor) is a closed type with three inspection openings to prevent the spread of unpleasant odors. The biological degradation process of organic matter operates on the principle of deep aeration. It is equipped with air diffusers that work together with an air compressor to evenly distribute dissolved oxygen throughout the bioreactor to support aerobic processes (Ilić-Stamenković *et al.,* 2018). Additionally, the bioreactor is fed with recycled activated sludge from the secondary clarifier and with wastewater from multi-media filters. Activated sludge recirculation is performed in series to maintain active biomass in the bioreactor (Markanović *et al*., 2014).
During the biological treatment process, after sludge sedimentation and recirculation in the bioaeration basin, excess activated sludge is produced. Therefore, a sludge manhole is adopted for the value of dry matter production of activated sludge of 0.80 kg of dry mass/kg of decomposed BPK_5. Treated water after clarification in the clarifier enters sand filters using crushed basalt and quartz sand as the filtration medium, designed to remove suspended

organic and inorganic particles from the wastewater (up to 20 μm in size). The plant includes units for neutralization, coagulation, and flocculation. They are used as needed to achieve the required quality of treated water. pH correction and sedimentation are performed with $Ca(OH)_2$ with the addition of a coagulant ($Al_2(SO_4)_3$) and a flocculant (PAA). The coagulant $Al_2(SO_4)_3$ is added at a concentration of 10-20% (Nakomčić-Smaragdakis *et al.*, 2012). Aluminum sulfate behaves differently in removing water turbidity at different temperatures (Kukobat *et al.*, 2014). The flocculant is a cationic polyelectrolyte in a solution concentration of 0.1 – 0.2%.

Experimental Part

The analysis of sanitary water is a more complex process than the analysis of surface and groundwater. Monitoring the operation and efficiency of the system is performed through specific parameters. Sampling was carried out at two locations: Sample I - water taken at the inlet to the purifier. The water sample is pale yellow in color, has an unpleasant odor, with barely noticeable floating matter, air temperature 27.2 ^{0}C, water temperature 21.5 ^{0}C; Sample II - the sample from the recipient is taken 50 m downstream from the place where the water from the purifier flows into the recipient. The water is clear, odorless, and free of floating matter, air temperature 26.6 ^{0}C, water temperature 19.8 ^{0}C.
Physical-chemical and bacteriological analyses of wastewater samples were performed at the Institute of Public Health, Center for Hygiene and Human Ecology. For water sampling, the SRPS ISO/IEC 17025 standard method was applied (Official Gazette of RS, 33/2016). The required tests were performed according to the Regulation on Limit Values of Polluting Substances in Surface and Groundwater (Off. Gaz. of the R of Serbia 67/11 and 48/12).

Results and Discussion

Tables 2. and 3. show the results of physical-chemical and microbiological tests of wastewater before and after treatment.

Table 2. Results of physical-chemical water tests

No.	Parameter	Units	Sample	
			I	II
1.	Color	/	Cloudy	Clear
2.	Odor	/	Slightly unpleasant	None
3.	Visible waste materials, NTU		None	None
4.	pH value	/	8.2	8.0
6.	Total dry residue mg/L	mg/L	984	324
7.	Ammonium ion, NH_4^+	mg/L	1,77	<0,04
8.	Nitrites, NO_2^-	mg/L	0,5	0,06
9.	Nitrates, NO_3^-	mg/L	2,53	1,91
10.	Chlorides , Cl^-	mg/L	34,9	15,5
11.	Sulfates, SO_4^{2-}	mg/L	29	23,4
12.	Sediment materials for 2 h	mg/L	4	0,2
13.	Detergents	mg/L	<0,1	<0,1
14.	Electrolytic conductivity at 20°C	μS/cm	710	430

*Source: Author's processing based on the results of the analysis

The biological device for the treatment of sanitary wastewater showed a high level of efficiency (Table 3). For all physical-chemical parameters, the efficiency rate was above 90%. The values of the parameters were interpreted in relation to the standard defined in category III of municipal wastewater.

Table 3. Wastewater treatment efficiency

Measured parameters	Before the device	After the device	Emission limit values	Efficiency percentage
Consumption КмnO$_4$ (mg/L)	1736	133,3	-	92,3
Suspended solids at 103-105 ^{0}C (mg/L)	4560	332	100 mg/L	92,7
Biochemical oxygen demand, BPK$_5$ (mg O$_2$/L)	1258	50	80mg/L или 75%	96
Chemical oxygen demand, HPK (mg O$_2$/L)	6420	207	7%	96,8
Total phosphorus, P (mg/L)	9,78	0,653	-	93,3
Total inorganic nitrogen (mg/L)	46,28	4,8	-	89,6
Total coliform bacteria in 100 mL	>1209800	706800	10000	41,6
Fecal coliform bacteria in 100 mL	>1209800	244200	2000	79,8
Fecal streptococci in 100 mL	>2419,6	>2419,6	400	

*Source: Author's processing based on the results of the analysis

Namely, for a biological device with a capacity of 200 PE, emission limit values are applied for plants with an equivalent population (PE) of less than 600. The values of the parameters biochemical oxygen demand (BPK$_5$) and chemical oxygen demand (HPK) were reduced with an efficiency greater than 90%. According to Josimov-Dunđerski *et al.*, (2006), the BPK$_5$ value for sanitary polluted water is up to 400 mg O$_2$/L. Therefore, the load of wastewater with organic compounds, according to the BOD5 indicator, is extremely low. Measured values at the system inlet are in 75% of cases below the average concentration in municipal wastewater from the population (Malešević *et al.*, 2022). The reduction of BPK$_5$ is over 95%, with a concentration in the effluent of up to 50 mg O$_2$/L. The abundance of microorganisms depends on the content of organic matter and ecological factors, primarily water temperature (Jarak and Čolo, 2007; Josimov; Dunđerski *et al.*, 2006). Based on the obtained results of physical-chemical and microbiological analyses of wastewater samples, and downstream from the point of wastewater discharge into the recipient, i.e., the Morava River, the surface-river water is classified as Class II water, with good ecological status (Official Gazette of RS, 74/11). The degree of wastewater treatment also affects the quality of the recipient, where depending on the amount and composition of detritus, there is often the possibility of self-purification of the water (Ilić-Stamenković *et al*., 2016). In the outlet channel of wastewater treated in the wastewater treatment system, cattail, a marsh plant that further effectively purifies all impurities remaining after exiting the system, is planted.

Conclusions

The systems used for wastewater treatment in highway construction can be very efficient if users adhere to the guidelines provided by the system manufacturers. All facilities and equipment are enclosed or minimally exposed to the atmosphere (grates and pumping station), which has numerous ecological advantages. After the disinfection process, the treated wastewater is discharged into the recipient, the Morava River, which belongs to the second category. The results indicate that such plants show a high degree of efficiency. The advantages of the system are reflected in the marsh plant (cattail) planted around the system, whose activity further improves the quality of water flowing into the West Morava River. It is recommended to introduce biodegradable cleaning agents for toilets and kitchens, so as not to disrupt the reproduction of microorganisms in the system.

This work is another reflection on the general problem of globalization and industrialization in the environment and an attempt to point out the "price" paid by all people if modern procedures, which are practiced throughout the developed world, are not applied here as well, to improve the environment.

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EVALUATION OF SERUM CYSTATIN C, SYMMETRIC DIMETHYLARGININE (SDMA), CREATININE AND UREA IN CLINICALLY HEALTHY GERIATRIC HORSES

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Abstract

In recent years, veterinary practitioners pay more attention to detect acute kidney injury at its earlier stages. The subclinical nature of the disease in horses makes it easy to be missed and failure to diagnose it early can be detrimental for the patients’ health. Nowadays, researchers investigate the use of more perspective renal biomarkers in order to optimize the diagnostics of equine kidney disease. One such biomarker is serum cystatin C. Its use as kidney biomarker is well documented in human medicine but its usefulness in equine practice is not well established. There is some research regarding serum cystatin C levels in horses but there is a lot more that needs to be done until it can be used in clinical practice. Authors believe that cystatin C holds potential as a proper renal biomarker that can be used in horses but up to date available literature is too scarce to support its use. In this article we evaluate serum cystatin C in clinically healthy geriatric horses along with serum SDMA, Creatinine and Urea which veterinarians already use for diagnosing kidney disease in horses. The aim of the study is to establish serum cystatin C levels in this age group of horses and compare it to parameters that are used widely in practice. There is no evidence in literature if cystatin C levels vary among horses of different age. There is also no data regarding serum cystatin C levels in geriatric horses.

Keywords: *Horses, Geriatric, Cystatin C, Creatinine, Urea.*

Introduction

Acute kidney injury (AKI) is a common disorder in companion animals such as dogs and cats and in humans. Moreover, it is associated with an increase in the cost of treatment as well as an increase in morbidity and mortality in patients (Chen et al., 2017). Over the past few decades, mortality rates of human patients with AKI in intensive care units are as high as 70% (Waikar et al., 2008; Chen et al., 2017). The use of renal replacement therapies in veterinary medicine is limited thus, the possibility for effective therapy is reduced and this suggests that there is a need for early recognition of this disease process.

In equine medicine, kidney injury used to be underrecognized in previous years. Much attention is now paid to this disease process and the ability to diagnose it early in its course. According to one study the prevalence of AKI in horses was 14,8% (Sagave et al., 2019). The study included horses that were presented to Rossdales Equine Hospital (August 2015 – January 2019) and were admitted mostly (92,3%) as an emergency cases. Even though, this percentage is much lower than that in human and small animal patients, it is important to note that the prevalence of AKI and its severity in hospitalized patients reduces their chance for survival. In one study it was established that hospitalized horses with intrinsic renal injury were 3 times more likely to die or to be euthanized than horses with pre-renal azotemia (Groover et al., 2006). Later, Savage et al. (2019) reported that the overall survival of a population of hospitalized horses was 87,7% and for those with azotemia survival was 71,4%.

Based on its etiology AKI is pre-renal, post-renal or there in an intrinsic renal injury. If persistent, both pre- and post-renal factors can lead to intrinsic renal failure. A study which was performed at UGA-VTH (2000-2003) by Groover et al. (2006), showed that persistent azotemia due to intrinsic renal failure led to poor outcome for the patients. This proves for the necessity to diagnose kidney injury in its early stages and treat it promptly.
The widely used biomarker for renal injury – serum Creatinine (sCr) proves to be insensitive for early detection of kidney injury. In human medicine it is known that small and even transient increase in sCr levels is detrimental (Chen et al., 2017). This led to the increased interest among nephrologists to find more specific and sensitive biomarkers of kidney injury. In this article we pay attention to the use of cystatin C, serum symmetric dimethylarginine (SDMA), sCr and blood urea nitrogen (BUN) in horses.
SDMA use is becoming popular among equine practitioners. Also, the scientific evidence on its use is increasing. It is shown that its levels strongly correlate with sCr (Siwinska et al., 2020) and moreover, its sensitivity is better than sCr because its levels increase when there is 25-40% decrease of renal functional mass (Hall et al., 2014). Another useful thing for practical reasons is that its levels have been evaluated in horses with kidney injury (Siwinska et al., 2020; Siwinska et al., 2021), dehydration (Lo et al., 2021), horses of different age, sex, breed, body weight, body condition score (Siwinska et al., 2020; Schott et al., 2021; Bozorgmanesh et al., 2021; Lo et al., 2021; Siwinska et al., 2021; Gough et McGovern, 2022), and horses undergoing extreme exertion (Ertelt et al., 2021). This is important because some parameters could be influenced by such factors. More or less, it is now known enough for the usefulness of SDMA as an equine kidney biomarker.
Another potential equine kidney biomarker might be serum Cystatin C (sCysC). It is a cysteine-proteinase inhibitor and is constantly produced by nucleated cells (Abrahamson et al., 1990; Tenstad et al., 1996; Iwasa et al., 2018). In human medicine, serum and plasma cystatin C is considered superior to sCr in detecting kidney injury (Dharnidharka et al., 2002; Chen et al., 2017). Cystatin C is considered a marker of the GFR (Almy et al., 2002; Ghyl et al., 2014) because it is filtered through the glomeruli, then it is reabsorbed and catabolised by tubular epithelial cells in a way that it does not return to the systemic circulation. Moreover, it is not secreted by the renal tubules (Tenstad et al., 1996; Filller et al., 2005; Kaseda et al., 2007; Iwasa et al., 2018).
The currently used blood parameters for the estimation of the GFR – creatinine and urea, are affected by extrarenal factors such as feeding, body weight, muscle mass, and breed (Prause and Grauer, 1998; Braun et al., 2003; Freeman et al., 2003; Medaille et al., 2004; Parker and Freeman, 2011; Iwasa et al., 2018). In this article we focus on evaluating the levels of sCysC in healthy geriatric horses and compare them to the levels of serum SDMA, Cr and Urea. The aim of the article is to establish if age in old horses will affect its concentration. Up to date, little is known about the use of sCysC in equine medicine.

Materials and Methods

We collected blood samples from 35 randomly chosen clinically healthy geriatric horses. Individuals included in this study were all from Central Southern Bulgaria. Horses were from cold- and warmblood breeds. Their age was between 17 and 36 years old. All of the horses were clinically examined before sample collection. We estimated the horses’ general health by measuring basic clinical parameters such as heart rate, respiratory rate, body temperature, mucous membrane color and moisture, capillary refill time, mandibular lymph node palpation and peristalsis auscultation.
For the sample collection we used closed technique. The site for blood collection was from the jugular vein. We used 20G $1^{1/2}$” needles (Vacutest® KIMA S.R.L., Italy) and 6 ml

vacuteiner tubes (Vacutest® KIMA S.R.L., Italy) with clot activator – red top tube. After blood collection we separated the serum (1 mL) in Eppendorf tubes and froze them at -20ºC for no more that 4 weeks from the day of obtaining the sample. The period for sample collection was from the 1st of March to the 28th of March 2024.
Samples were evaluated at an external laboratory – Laboklin GmbH & Co. KG, Germany. The assay of all 4 parameters (Cystatin C, SDMA, Cr, and Urea) was performed via photometric studies.
Data was statistically analyzed using commercial software system. We used one-way ANOVA, and the level of statistical significance was calculated according to the Tukey-Kramar test ($P<0.05$). The results are presented as mean ± standard error of the mean (SEM).

Results and Discussion

Results from the clinical examination are shown in table 1. All parameters were within the normal reference intervals and the horses were considered suitable for the purpose of this article.

Table 1. Clinical parameters

Median age (years old), (range), (n)	**Median heart rate (bpm), (range)**	**Median resp. rate (bpm), (range)**	**Median Body Temperature (ºC), (range)**	**Mucous membranes**	**CRT (s)**	**Mandibular lymph node**	**Peristalsis**
25,11±0,68 (17-36), n=35	32,23±0,45 (28-36)	9,77±0,28 (8-14)	37,33±0,04 (36,9-37,8)	Pale pink, moist	< 2 s	Not reactive	Present in all quadrants

*Authors’ elaboration based on the obtained results.

The results of the Cystatin C, SDMA, Cr and Urea in regards of the horses’ age are shown in table 2.

Table 2. Cystatin C, SDMA, Cr, and Urea values

Median age (years old), (range), (n)	**Median heart rate (bpm), (range)**	**Median resp. rate (bpm), (range)**	**Median Body Temperature (ºC), (range)**	**Mucous membranes**	**CRT (s)**	**Mandibular lymph node**	**Peristalsis**
25,11±0,68 (17-36), n=35	32,23±0,45 (28-36)	9,77±0,28 (8-14)	37,33±0,04 (36,9-37,8)	Pale pink, moist	< 2 s	Not reactive	Present in all quadrants

*Authors’ elaboration based on the obtained results.

The horses included in our study were 25,11±0,68 (range 17-36) years old and had mean serum cystatin C levels 0,42±0,02 mg/L (range 0,3-0,7 mg/L). Mean serum SDMA was 0,45±0,02 µmol/L (range 0,13-0,72 µmol/L), creatinine was 102,94±4,08 µmol/L (range 72-157 µmol/L), and urea 6,55±0,27 mmol/L (range 3,4-10,6 mmol/L). In all horses all of the evaluated blood parameters were within the normal reference range intervals, except for 16 horses that had slightly elevated urea levels. However, the mean value of the parameters selected were all within the reference range intervals.
SDMA is a novel renal biomarker that is already used in clinical practice. Studies support that SDMA is a more sensitive measure of the glomerular filtration rate (GFR) than sCr (Hokamp and Nabity, 2016; Relford et al., 2016; Schott et al., 2021). According to the literature, SDMA concentration is not affected by extrarenal factors (Galen et al. 2022).
Within the past two decades, cystatin C has been introduced as an early diagnostic biomarker for renal compromise (Karami et al., 2018; Ahmadpour et al., 2020). In horses there are not

many studies on cystatin C. The first study on cystatin C in horses used human cystatin C test but this test did not work in horses (Arosalo et al., 2007). In another study performed by Siwinska et al. (2021), it is reported that serum cystatin C levels are similar to those in dogs and cats (Braun et al, 2002; Poswiatowska-Kaszczyszyn, 2012; Pelander et al., 2019).

Up to date it is not known if cystatin C can be used as kidney biomarker in equine practice. Siwinska et al. (2021) report that horses with AKI had increased serum cystatin C. However, this concentration was lower than that of other species with renal disease, except for cats (Poswiatowska-Kaszczyszyn, 2012; Siwinska et al., 2021). Ahmadpour et al. (2020) report that horses with parasitemia due to Theileria equi infection had renal compromise. The interesting thing was that low rates of infection could not significantly influence urea and creatinine concentrations but led to major increase in cystatin C levels. Thus, sCysC levels might provide a more efficient means for early detection of renal malfunction in the early stages of T. equi infection.

According to some authors, in human patients cystatin C is influenced by factors other than the GFR such as inflammation, metabolic diseases, and the interval between feeding and serum collection (Cimerman et al., 2000; Braun et al., 2002; Stevens et al., 2009; Siwinska et al., 2021). Miyagawa et al. (2009) report that body weight in dogs can affect measured sCysC levels. In people, the overall sCysC levels seem not to be affected by factors such as gender, race, muscle, mass, and hydration status (Beker et al., 2018; Ahmadpour et al., 2020). In horses it is not known if extrarenal factors influence sCysC levels.

Reported values of sCysC in healthy horses are from 0,13 to 0,71 mg/L (Siwinska et al., 2021; Galen et al., 2022). In our study all of the horses had sCysC levels within this range. This suggests that increasing age in horses might have no influence on sCysC concentration in horses. None of the horses had azotemia or increased SDMA levels.

There is a lot to establish in horses before we begin using sCysC in clinical practice. There is no data on its concentrations in neonates and young foals, as well as in older and geriatric horses. To our knowledge this is the first study on sCysC levels in geriatric horses. It is not known whether age will affect cystatin C values in horses. In people, cystatin C values are higher in neonates (Bokenkamp et al., 1998; Cataldi et al., 1999; Renders et al., 1999; Seronie-Vivien et al., 2008). Also, cystatin C levels in serum of children is reported to be higher after birth (Bokenkamp et al., 1998; Randers, 1999). In humans it is shown that age has an impact on cystatin C values and there are studies that imply higher reference values for people over 50-60 years old (Norlund et al., 1997; Erlandsen et al., 1998; Finney et al. 2000; Galteau et al. 2001; Seronie-Vivien et al. 2008). Our study suggests that sCysC levels are not affected by age in horses >17 years old as they have the same values as in healthy horses that are previously reported (Siwinska et al., 2021).

In people, it is reported that cystatin C levels are not influenced by inflammation (Simonsen et al, 1985; Grubb et al., 1985; Randers, 1999). This has not been yet investigated in horses or other equids.

Serum Cr and urea continue to be used in clinical practice. However, their use is insensitive and this leads to the need for a better kidney biomarker in equine practice. In our study sCr levels were within normal reference limits for horses. Urea levels were variable but high levels of urea without concurrent increase in sCr levels is not suggestive of kidney disease. This is because urea concentrations are not specific for kidney damage but are dependent on factors such as age, diet, and the rate of its production (Koterba, 1990; Schott II, 2018).

Conclusion

So far it seems that cystatin C levels are not affected by extrarenal factors as much as Cr. However, more studies need to be performed in regards of age, sex, muscle mass, weight and

BCS, and various disease states in horses. Our study supports the statement that advanced age in horses does not influence serum cystatin C levels in this species. We still don't know if sCysC can be used in horses as early renal biomarker. It seems practical to evaluate a bigger group of horses in order to establish a reference range interval for horses. In this way we can better asses our findings and establish whether in geriatric horses sCysC levels are within the normal values.

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ULTRAFILTRATION SEPARATION AND APPLICATION OF WASTE POLYPHENOLS FROM OIL-BEARING ROSES

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Abstract

Crop production is a source of raw materials for various processing sectors. Technological processes are associated with the use of large amounts of water. As a result, significant amounts of wastewater are generated. In the composition of these waters, various organic compounds fall through the plant raw materials. A large group of them are classified as polyphenols. The interest in them is great due to the natural origin of their bioactive characteristics. Polyphenols are secondary plant metabolites. In addition to similar chemical nature, they have a common strong quality - a powerful antioxidant effect. There are also many other benefits, which opens up new possibilities for their use. The separation of waste polyphenols will help to purify water, but it is also an opportunity for their utilization in food, cosmetic, pharmaceutical, and other products. Their extraction and fractionation is possible by membrane methods. Membrane processes, in contrast to conventional ones, achieve increased yield and controlled selectivity, the possibility of continuous and intermittent operation, avoidance of filtration aids, easy cleaning and maintenance of equipment, microbiological purification, reduction of waste products and filtration time, etc. The aims of the study are the selective retention of polyphenols by ultrafiltration process of wastewater from the hydrodistillation treatments of flowers of an oil-bearing roses and the investigation of their potential biological and toxicological effects. It was found that 12.79 mgGAE/ml of 13.67 mgGAE/ml was retained from the total phenolic content of *Rosa alba L.* variety and respectively 10.82 mgGAE/ml of 11.30 mgGAE/ml from *Rosa damascena Mill. F. trigintipetala Dieck*. Computational methods for bioactivity and drug likeness has shown a high pharmacological potential the compounds gallic acid, quercetin, kaempferol, and ellagic acid, which is a prerequisite for their valorization. A current trend is to turn them into healthy products. The effective and purposeful separation of waste polyphenols from the processing of oil-bearing roses is an opportunity to increase their value.

Keywords: *Roses, Waste polyphenols, Membrane separation, Ultrafiltration, In silico methods.*

Introduction

The industrial cultivation of different varieties of rose crops is a consequence of the benefits and quality of the products from the processing of their flowers [Georgieva *et al*., 2021]. Due to the globally recognized high qualities of Bulgarian products, and especially Bulgarian rose oil, it is entered in the European Register of Protected Geographical Indications [COUNCIL REGULATION (EC) No 510/2006; Dobreva *et al*., 2013]. All this has turned the cultivation and processing of roses into a cultural tradition, and the rose - a national symbol. Like all plants, roses produce and accumulate as secondary metabolites polyphenolic compounds, which have a major physiological and morphological role in the entire life cycle. Plants require phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and other functions [Lattanzio *et al*., 2006; Šamec *et al*., 2021]. Together with other substances necessary for vital activity [Ercisli S., 2007], the quality and quantity of polyphenolic compounds depends on the variety, environmental conditions - climate, weather and soil conditions, the way of cultivation and harvesting [Boso *et al*., 2022]. Currently, the most used technology for processing fresh rose flowers is based on water steam distillation [Kovacheva *et al*., 2010]. The different products of rose processing have an irreplaceable and valuable bioactive composition, but a significant part of the total content remains in the wastewater [Gateva *et al*., 2022; Dobreva, 2011]. Environmental legislation mandates that waste bioactive components be isolated and water quality restored. If done selectively [Tapia-Quirós *et al*., 2022], substances will be able to find useful application and commercialization based [Grajeda-Iglesias *et al*., 2022; Bravo, 1998; Brezoiu *et al*., 2019] on the proven qualities of polyphenols in preventing good health and help restore it [Ramos *et al*., 2023; Hano *et al*., 2020; Hegde *et al*., 2022]. The aim of the study is the selective retention of polyphenols by an ultrafiltration process from wastewater from the hydrodistillation treatment of rose oil flower and to calculate the molecular properties and bioactivity scores of the compounds (gallic acid, quercetin, kaempferol, and ellagic acid) from oil-bearing roses by in silico method (Molinspiration Chemoinformatics software).

Materials and Methods

The researches were carried out on two types of wastewater from the hydrodistillation of a flower from an oil-bearing rose variety of the *Rosa damascena Mill. f. trigintipetala Dieck (R.D.)* and *Rosa alba L.(R.A.)*. The two types of water were subjected to a membrane ultrafiltration process carried out in laboratory conditions with a polyacrylonitrile (PAN) membrane (25 kDa) obtained by the phase inversion method. The membrane module (SM 165-26 “Sartorius”, England) was used with a perpendicular feed flow driven by a pressure controlled with a variable step of 0.1 MPa in the range of 0-0.5 MPa. The flow rate through 1 m^2 of the membrane is determined by the following equations:

$$J = \frac{V}{S \cdot \tau} \quad \textbf{(1)}$$

where: J –flux permeate through the membrane, l/m^2.h; V - volume of permeated flux, l; S - effective area of tested samples, m^2; τ – record time, h.

The total phenolic content (TPC) was determined by The Folin-Ciocalteu assay [Hellwig *et al*., 2020]. The results were evaluated as gallic acid equivalent (mg GAE/ml) by linear regression equation of the standard curve – y=2.4538x +0.1092 and R^2 = 0.9639 at 410 nm. Polyphenolic compounds were validated with UV-VIS spectrophotometer Evolution 300, Thermo Scientific (VISIONpro Software (ver. 4.5.0)).

Molinspiration Chemoinformatics software. Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. The products support also fragment-based virtual screening, bioactivity prediction and data visualization [ACD/Labs].

The Molinspiration software was used to estimate different physicochemical properties, namely LogP, Topological polar surface area (TPSA) and number of hydrogen bond donors

(HBD) and acceptors (HBA) for different compounds and prediction of bioactivity score for the most important drug targets (Gprotein coupled receptor (GPCR) ligands, kinase inhibitors (KI), ion channel modulators (ICM), nuclear receptors (NRL), protease inhibitors (PI) and enzyme inhibitors (EI)) [Molinspiration Chemoinformatic Software, 2020].
Drug-likeness evaluated by the Lipinski rule of five that deals four simple physicochemical parameter ranges (MWT $\leq$ 500, log P $\leq$ 5, H-bond donors $\leq$ 5, H-bond acceptors $\leq$ 10) associated with 90% of orally active drugs that have passed phase II clinical status. Molecules violating more than one of these rules may have problems with bioavailability. The rule is called "Rule of 5" because the border values are 5, 500, 2*5, and 5 [Lipinski *et al*., 1997; Lipinski, 2004].
Bioactivity of the drug can be checked by calculating the activity score as calculated drug likeness score of each compounds and compared with the specific activity of each compound, and the results were compared with standard drug. For organic molecules the probability is if the bioactivity scores (>0), then it is active, if (-5.0-0.0) then moderately active, if (<-5.0) then inactive [Molinspiration Chemoinformatic Software, 2020].

Results and discussion

The results of the laboratory experiment show close flux rates through the membrane of wastewater from the processing of the two varieties of rose flower. The results of the laboratory experiment show close flux rates through the membrane of wastewater from the processing of the two varieties of rose flower. The speed changes according to the pressure change (Figure 1), with the highest being recorded at 0.5 MPa. Higher permeability at pressures after 0.2 MPa is characteristic of the wastewater flow from the *R.A.* processing and reaches 23.43 l/m^2. h at the highest pressure. The reason is probably the difference in the component composition and their spatial volume geometry.

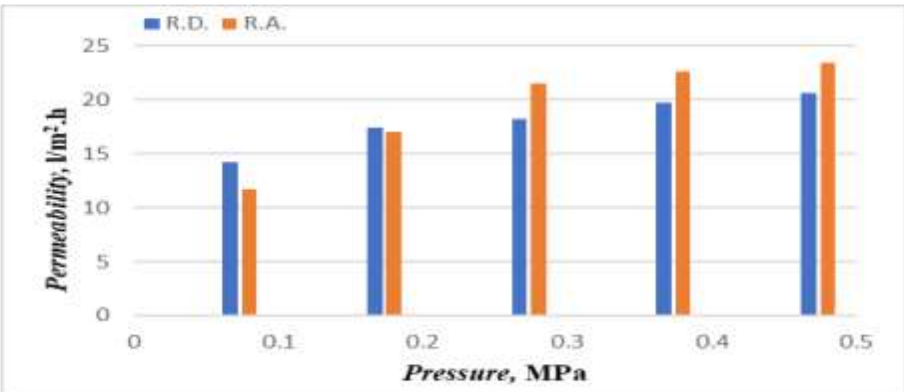

Fig. 1. Membrane permeability on the wastewater flows

The quantitative content of TPC in the wastewater from the processing of *R.D.* were found to be 11.30 mgGAE/ml, and for *R.A.* 13.67 mgGAE/ml, respectively. In membrane filtration, they are retained to varying degrees according to the applied pressure and depending on the type of treated wastewater (Figure 2). With increasing pressure, the amount of TPC from the wastewater that passed through the membrane into the filtrate reached a minimum value at a pressure of 0.3 MPa. These values are 0.48 mgGAE/ml in the membrane treated waters of R.D. and 0.88 mgGAE/ml in those of R.A. Respectively, at this pressure, a maximum TPC retention of 10.82 mgGAE/ml was recorded for the wastewater of *R.D.* and 12.79 mgGAE/ml for those of *R.A.* The studies of the polyphenol composition made with UV/Vis spectroscopy registered the characteristic peaks in the interval 200-400 nm, which could correspond to the compounds - gallic acid (1), protocatechin, quercetin (2), kaempferol (3), rutin, ellagic acid (4) [Lidia Solís-Oviedo *et al*., 2019].

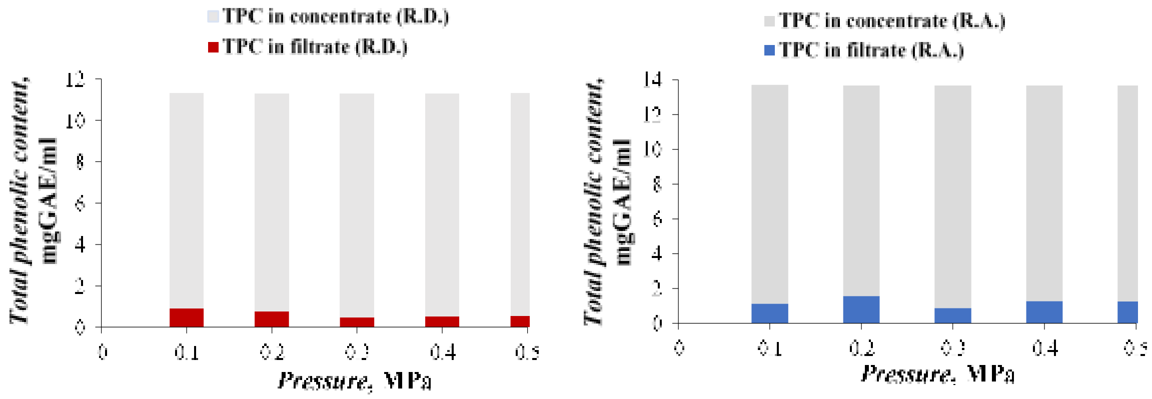

Fig. 2. The total phenolic content in membrane treated wastewater

The ultrafiltration membrane process guarantees the complete decontamination of the treated object, which in the case under consideration is a unique opportunity for direct investment in pharmaceutical, food, cosmetic products with a view to realizing the biopotential of the available compounds.
The Molinspiration software has been used for predicting parameters - molecular physicochemical properties (Table 1) and bioactivity score (Table 2) of the compounds (gallic acid, quercetin, kaempferol, and ellagic acid). Data of the calculation of molecular physicochemical properties of the compounds are presented in Table 1.

Table 1. Drug likeness score of the compounds

№	LogP[a]	TPSA[b]	n atoms[c]	MW[d]	nON[e]	nOHNH[f]	volume[g]	nrotb[h]	№ of violations[i]	*In silico* % absorption
1	0.59	97.98	12	170.12	5	4	135.10	1	0	75.20
2	1.68	131.35	22	302.24	7	5	240.08	1	0	63.68
3	2.17	111.12	21	286.24	6	4	232.07	1	0	70.66
4	0.94	141.33	22	302.19	8	4	221.78	0	0	60.24

a: LogP - Logarithm of partition coefficient between n-octanol and water (miLogP); b: Topological polar surface area (TPSA); c: Number of nonhydrogen atoms (natoms); d: Molecular weight (MW); e: Number of hydrogen-bond acceptors (O and N atoms) (nON); f: Number of hydrogen-bond donors (OH and NH groups) (nOHNH); g: Molecular volume (volume); h: Number of rotatable bonds (nrotb);. i: Number of Rule of 5 violations (nviolations).

All four molecules conform to Lipinski's rule, indicating that they may be orally active. The bioactivity scores of the compounds were calculated for different parameters such as binding to G protein-coupled receptor (GPCR) ligand and nuclear receptor ligand, ion channel modulation, kinase inhibition, protease inhibition, and enzyme activity inhibition [26]. All the parameters were calculated with the help of software Molinspiration. The bioactivity score is given in Table 2.

Table 2. Bioactivity score of the compounds

No	GPCR ligand	Ion channel modular	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	-0.77	-0.26	-0.88	-0.52	-0.94	0.17
2	-0.06	-0.19	0.28	0.36	-025	0.28
3	-0.10	-0.21	0.21	0.32	-0.27	0.26
4	-0.29	-0.27	-0.01	0.11	-0.18	0.17

The bioactivity of the compounds was evaluated against six different protein structures. The result of this study was found that the compounds is biologically active and has physiological effect. The bioactivity score of the four compounds against the six structures showed them to be active to moderately active.

Conclusion

During the ultrafiltration with a PAN membrane of wastewater from the hydrodistillation of flowers from rose crops, it was found that 12.79 mgGAE/ml of a total of 13.67 mgGAE/ml polyphenolic compounds were separated for *Rosa alba L.* variety and 10.82 mgGAE/ml from total 11.30 mgGAE/ml for *Rosa damascena Mill. f. trigintipetala Dieck*. The highest degree of selectivity above 90 % is achieved at a pressure of 0.3 MPa with a very good permeability of 23.43 l/m^2. h. The phenolic compounds that was studied for bioactivity and drug likeness by computational methods has shown a high pharmacological potential, which is a prerequisite for their valorization.

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CLIMATE NEUTRALITY AND THE ROLE OF EMISSIONS TRADING IN THE EUROPEAN UNION

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Abstract

As a response to the deepening climate changes, the EU has the ambition of achieving climate-neutrality and an economy with net-zero greenhouse gas (GHG) emissions by 2050. The Emissions Trading System (ETS) is a crucial tool in the EU's effort to combat climate change by reducing GHG emissions. Established in 2005, the ETS sets a cap on the amount of CO_2 emissions that can be released by participating industries, such as power generation, cement, and steel production, and allows them to trade their excess allowances or buy more from other companies. The EU has steadily decreased its GHG emissions since 1990, reaching 32.5% in 2022. In 2023, the EU adopted a set of Commission proposals to make its climate, energy, transport and taxation policies fit for reducing net GHG emissions by at least 55% by 2030. In February 2024, the European Commission, as a part of its assessment for a 2040 climate target for the EU, recommended reducing the EU net GHG emissions by 90% by 2040. It has many benefits, including increased levels of air quality and human health, conservation of biodiversity and energy security. EU ETS is the world’s first large-scale and still the largest GHG emissions trading scheme, allowing the reduction of GHG emissions cost-effectively. The main objective of the paper is to examine the result of the implementation of emissions trading in the ecological, economic and social aspect through a review of reports, analysis and official documents related to the emission trading system and its implementation.

Keywords: *emissions trading, greenhouse gas emissions, climate change, sustainable development, EU.*

Introduction

Rapid economic development, modernization of production activities and globalization of all spheres of public life have an increasingly tangible impact on the environment, leading to a continuous increase in pollution levels, depletion of natural resources, reduction of land productivity and overall deterioration of quality of life. Climate change can lead to unpredictable consequences such as reduction in the reserves of clean drinking water, droughts or floods, melting of glaciers, as well as changes in the climatic zones, including those for rural activity, and their displacement towards the poles (UN, 1992). Taking into account the constantly increasing population, it is imperative to take a number of measures and introduce a number of regulatory requirements that reflect the global commitment to achieve sustainable development and environmental protection. Regardless of these efforts, the world is witnessing a disturbing acceleration in the number, speed and scale of broken climate records, accompanied by devastating extreme events, which, according to the Intergovernmental Panel on Climate Change (IPCC), are just the beginning. Global GHG emissions and atmospheric concentrations of carbon dioxide (CO_2) also set new records in 2022 (UNEP, 2023). This necessitates the unification of global efforts to achieve the set goals.

Over the last decade, European surface temperatures were more than 2°C warmer than pre-industrial levels, while the summer of 2023 was globally the hottest summer on record and the fifth hottest for Europe. Every tonne of greenhouse gas saved contributes to mitigating the consequences of climate change, and the EU is committed to stepping up the efforts and taking decisive action on the path towards climate neutrality (EU, 2023). In this regard, the EU adopted a number of Commission proposals related to achieving GHG decrease by at least 55% by 2030, compared to 1990 levels with the ambition to reach a decrease of 90% by 2040 relative to 1990 and to become the first climate-neutral continent by 2050 (EU, 2019).
The EU's efforts to decarbonize are being led by the European Green Deal, which aims to reduce greenhouse gas emissions by at least 55% by 2030 and become climate-neutral by 2050. To achieve this, the EU has implemented a robust emissions trading system, also known as the Emissions Trading System (ETS). The ETS sets a cap on the total amount of greenhouse gases that can be emitted by participating countries, and companies are required to buy and sell emission allowances to comply with the cap. This creates a financial incentive for companies to reduce their emissions and invest in low-carbon technologies. Additionally, the EU has also introduced a Carbon Border Adjustment Mechanism (CBAM) to address carbon leakage, which is the practice of companies relocating production to countries with less stringent emissions regulations to avoid compliance costs. It would require importers to declare the carbon content of their goods and pay a carbon tax on imports from countries that do not have similar carbon pricing systems. This would encourage companies to invest in low-carbon production methods and reduce their carbon footprint, rather than relocating to countries with laxer environmental regulations. The CBAM would help to reduce emissions from international trade, promote sustainable development, and support the EU's climate neutrality goals. The United Nations Framework Convention on Climate Change (UNFCCC) (UN, 1992) is the first international document acknowledging the negative impact of greenhouse gas (GHG) emissions generated by anthropogenic activity. UNFCCC sets as its goal the stabilization of GHG concentrations in the atmosphere to a level that would not lead to deepening the levels of climate change and drastic adverse impacts on the environment.
The next step on the road to reducing GHG levels is the adoption of the Kyoto Protocol in 1997 and its entry into force in 2005 (UN, 1997). The Kyoto Protocol operationalizes the UNFCCC by setting binding emission reduction targets to an average 5 per cent emission reduction compared to 1990 levels over the first commitment period 2008–2012 (Article 3).
In 2012, with the adoption of the Doha Amendment of the Kyoto Protocol (UN, 2012), the second commitment period 2013 – 2020 starts, but the Amendment entered into force in December 2020 as of October 2020 when the needed number of instruments of acceptance was achieved. It includes additional commitments, a revised list of GHG to be reported on and updates to several articles related to the first commitment period.The Paris Agreement, adopted in 2015 (UN, 2015) and its entry into force in 2016 sets a goal of limiting the increase of global average temperature below 2°C above pre-industrial levels and pursuing efforts to limit its increase to 1.5°C above pre-industrial levels. This would lead to a significant reduction of climate change related risks and impacts and could be achieved by decreasing the GHG by at least 43 % by 2030. The main objective of the paper is to examine the result of the implementation of emissions trading in the European Union in ecological, economic and social aspects.

Materials and methods

This paper is based on a detailed review of reports, analysis and official documents as action plans, declarations, programs and data related to the emission trading system, its implementation and the policies aimed at supporting the transition towards achieving climate-neutrality and an economy with net-zero GHG emissions by 2050.

Emissions trading is a market-based instrument used to limit emissions. The total allowed emissions are expressed as an emissions cap divided in tradable emission permits that are allocated. Trading schemes occur at the intracompany, domestic and international levels, and may apply to CO_2, other GHGs or other substances. Emissions trading is also one of the mechanisms specified under the Kyoto Protocol (UNEP, 2023).
The levels of generated GHG are monitored by the Emissions Database for Global Atmospheric Research (EDGAR). EDGAR provides detailed country-level emissions statistics, including CO2, CH4, N2O, and other gases, allowing for accurate analysis of global GHG emissions trends and patterns. It is a multipurpose, independent, global database of anthropogenic emissions of GHG and air pollution on Earth operating since 2010 and providing independent emission estimates compared to what was reported by European Member States and by Parties under the UNFCC (EC, JRC, IEA, 2023).

Results and discussion

Since the beginning of the 21st century, global GHG emissions have grown steadily in comparison to the two previous decades. Based on the emission estimates for 2022 provided by EDGAR, global GHG emissions increased by 1.4% compared to 2021 and by 2.3% than in 2019. The global economy experienced a slowdown in 2020 due to the COVID-19 crisis followed by an increase in 2022 by 6.2% compared to the levels in 2020, and by 2.3% compared to the levels in 2019. In the longer-term, in the EU GHG emissions have decreased by 27.0% in 2022 than in 1990 and showing GHG emission decoupling from economic growth (Crippa et. al., JRC, EU, 2023).
The Kyoto Protocol (UN, 1997) only binds developed countries, and places a heavier burden on them under the principle of "common but differentiated responsibility and respective capabilities", because it recognizes that they are largely responsible for the current high levels of GHG emissions in the atmosphere. Its flexible market mechanisms for trading emissions permits were established with a focus on national efforts and with the option of additional support by three market-based mechanisms – International Emissions Trading; Clean Development Mechanism (CDM) and Joint implementation (JI).
As of 2005, after the adoption of Directive 2003/87/EC (L 275/32, 2003) the EU Emissions Trading System (ETS) was established. Through this mechanism, regulated entities could buy or receive emissions allowances, and at the end of each year, they must surrender enough allowances to cover all their emissions. If a regulated entity reduces its emissions, it can keep the "saved" allowances for future needs or sell them. A Market Stability Reserve, in place since 2019, stabilises the market by removing surplus allowances from it. Revenues from auctions from the existing ETS go mostly to member states' budgets and are mainly used to tackle climate change as member states are required to spend at least half of their auction revenues to help reduce GHG emissions, accelerate the transition towards renewable energy sources and improving energy efficiency.
Despite the positive results of emissions trading, with the entry into its fourth phase in 2021, a number of shortcomings have also been identified that must be taken into account (EU, 2020). The approach to allocate free allowances based on benchmarks provided significant incentives for improvement of energy efficiency, but there is scope to improve the application of these benchmarks as the quantity of free allowances continue to represent more than 40% of the total number of available allowances. As the specific rules for free allocation to modernize the electricity generation sector applying to the EU ETS phase 4 have been improved, free allocation to the power sector still needs improvement.
According to the European Commission (COM (2018) 773 final) the EU stands better in terms of decarbonisation than most other actors and has decoupled economic growth from

GHG emissions. A study by the International Renewable Energy Agency (IRENA) found that a transition to 100% renewable energy could add 24 million jobs globally by 2050, while reducing greenhouse gas emissions and improving air quality. Many factors have contributed to this, including energy efficiency, fuel switch policies, increased use of renewables and technological changes.

The expected increase in available job positions would have both economic and social impact as it would contribute to both individual wellbeing and economic development. On the other hand, improved energy efficiency and the transition towards more sustainable production and consumption models would have positive impact on ecological and economic spheres.

The total number of allowances issued in the EU each year is gradually being reduced, respectively by 1.74% between 2013 and 2020, and by 2.2% between 2021 and 2023. Between 2024 and 2027 it will be reduced by 4.3% per year, and from 2028 by 4.4% per year. The system of free allowances has been revised to tackle the issue of carbon leakage by focusing on sectors at risk of relocating their production outside of the EU.

The implementation gap, defined as the difference between projected emissions under current policies and full nationally determined contributions (NDC) implementation, has been reduced since the Paris Agreement. GHG emissions in 2030 with no policy updates for the last decade, would lead to an increase by 16%. Today, the projected increase is 3%. But unless emission levels in 2030 are brought down further, it will become impossible to establish low-cost ways to limit global warming to 1.5°C. High-income and high-emitting countries with greater capacity and responsibility for emissions will need to take more ambitious and rapid action and provide financial and technical support to developing nations by providing additional international financial assistance (UN, 2023).

Fig.1: Actors engaged in climate action: **A)** Total number of actors in all 194 countries engaged; **B)** Number of actors in Europe; **C)** Number of actors in EU
Source: The Global Climate Action portal; https://climateaction.unfccc.int/

At the same time, numerous initiatives are undertaken to support the transition to a low-carbon and sustainable economy worldwide. Progress is monitored by The Global Climate Action portal, launched in 2014 and companies, investors, organizations, regions, cities and countries can register their commitments to act on climate change. The portal represents the voluntary climate action undertaken by the Parties of the UNCCC – a total of 194 countries, 27 of which are the EU member states (about 15 % of all engaged countries).

The represented data support the EU leadership in climate action as 14 991 out of 39 404 of all registered actors (about 38 %) are based in the EU member states and 23 404 out of 39 404 actors are based in Europe (fig. 1). The share of companies, investors, organizations, regions and cities included, compared to the number of countries, also displays the EU at a leading position as more than 20 % of the companies, organizations and regions, more than 25 % of the investors, and more than 70 % of the cities engaged are in the EU.

As of 2022, the top 10 emitting countries of GHG emissions account for over 70% of total global emissions, with the United States, China, and India being the largest contributors. According to the International Energy Agency (IEA), the global energy-related CO_2 emissions in 2020 were 33.3 billion metric tons, with the power sector being the largest contributor. The CBAM is expected to increase the competitiveness of EU industries by reducing carbon leakage and incentivizing companies to reduce their emissions.

The consequences of climate change are far-reaching and devastating, with rising global temperatures causing more frequent and severe weather events such as heatwaves, droughts, and floods. This is leading to catastrophic damage to ecosystems, infrastructure, and human settlements, as well as increased risk of water scarcity, food insecurity, and mass migration. Additionally, climate change is also having a profound impact on human health, with increased risk of heat-related illnesses, respiratory problems, and the spread of disease-carrying insects.

The economic aspects of Environmental, Social and Governance (ESG) are related to the corporate sustainability of each business unit/enterprise, to the interaction and impact of the business unit's activity on its surrounding environment. Historically in the last 20 years, there has been an expansion in the scope of business units. In the beginning, this was a priority for large companies, while now small and medium-sized enterprises are actively involved. Through ESG, investors and other participants in the financial market can evaluate the activity of a given company not only on the basis of financial indicators, but also on the basis of non-financial data. ESG indicators aim to monitor the non-financial risks and opportunities associated with the day-to-day operations of companies. ESG is an integral part of the company's overall mission and strategy and runs through all business departments. In Bulgaria, business units, regardless of size and industry, include and apply environmental and social practices in their business models, thereby striving to strengthen their competitiveness and achieve long-term sustainability. The application of social and environmental practices is not limited only to the immediate production process, but throughout the supply chain of raw materials and realization of the finished product.

Reducing the levels of GHG emissions would contribute to achieving the EU goal for climate-neutrality and an economy with net-zero GHG emissions by 2050. It has undeniable benefits for both ecological, economic and social aspects of life as it contributes to limiting the pressure on environment, as well as for achieving sustainability. This, in turn, would lead to a decrease in the number and magnitude of extreme climate related events, destruction of ecosystems and deterioration of health and life of the population.

Conclusion

The unprecedented consequences of climate change call for joint efforts at the global level to mitigate them. One of the EU's most important instruments in this regard is the EU ETS. Regardless of its benefits it still should be improved and modernized to contribute to achieving the EU 2050 goal of net-zero emissions.

The EU has decoupled economic growth from GHG emissions and leads the way to decarbonization. Many factors like improving energy efficiency, fuel switching policies, increasing the use of renewable resources and technological changes have contributed to this, along with the EU ETS.

The EU member states are engaged in numerous initiatives related to the transition to a low-carbon and sustainable economy. Of all declared engagements of the 194 UNFCCC countries,

the share of EU companies, investors, organizations, regions and cities is more than 20 % as the EU member states represent about 15 % of all countries included.
Despite the efforts made so far, further reforms are needed to achieve the climate targets. It is necessary to implement mechanisms to further reduce free emission allowances, their better regulation, as well as to stimulate the transition to renewable energy sources.

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EVALUATION OF PHYTOREMEDIATION POTENTIAL OF *SIDA HERMAPHRODITA* GROWN IN CONTAMINATED SOILS WITH HEAVY METALS

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Abstract

Comparative studies have been carried out to evaluate the efficacy of *Sida hermaphrodita* for phytoremediation of contaminated soils with heavy metals. The field experiment was conducted on an agricultural field contaminated by the Non-Ferrous Metals Plant near Plovdiv, Bulgaria. The experimental plots were situated at 0.5 km distance from the source of pollution. Macronutrients (N, P, K, Mg, Ca), microelements (Fe, Mn, Cu), and heavy metal (Cd, Pb, Zn, Hg) concentrations in plant materials (roots, stems, leaves, and flowers) were evaluated at the end of the first growing season. *Sida hermaphrodita* is tolerant to heavy metals and can be grown on highly contaminated soils (1671.6 mg/kg Zn, 1694.8 mg/kg Pb, and 54.8 mg/kg Cd). The depot for accumulation follows the order: Pb, Zn, Hg, Cu, Mg – leaves > flowers > roots > stems, Cd – leaves > stems > roots > flowers, Fe, Mn, Ca – leaves > roots > flowers> stems, P – flowers > roots > leaves > stems, and K – leaves > flowers > stems > roots. Sida hermaphrodita does not accumulate Zn, accumulates Pb poorly, accumulates Hg and Cd at intermediate levels, and has no potential for phytoextraction. *Sida hermaphrodita* harvested from heavy metals contaminated soil may be used for energy production.

Keywords: *Sida hermaphrodita, polluted soils, toxic elements, bioaccumulation factor, translocation factor.*

Introduction

Soil pollution is one of the significant environmental problems worldwide, posing a severe risk to the environment and human health (Jiang et al., 2015). In the European Union, there are approximately 3 million sites with soils suspected to be contaminated and 250,000 sites known to need remediation (Europe Environmental Assessment Agency, 2007). Among these, soils contaminated with heavy metals such as Cd, Cr, Pb, Cu, Zn, Co, Ni, Se, and As account for more than 37% of cases (Europe Environmental Assessment Agency, 2007). Phytoremediation is a process by which plants effectively remove heavy metals by absorbing them from the contaminated soil (Chaney et al., 1997). Plants should have a rapid growth rate with a high potential for biomass production, a deep and branching root system, and be tolerant of biotic and abiotic stress.

A key factor hindering the widespread adoption of the technology is the disposal of large amounts of plant biomass contaminated with heavy metals accumulated during the remediation process (Sas-Nowosielska et al., 2004). When the contaminant content of plant biomass exceeds a certain level, the biomass is considered a potentially hazardous material and, therefore, should be stored as hazardous waste (Ghosh and Singh, 2005). An integrated approach involving soil remediation with subsequent conversion of biomass to energy and recovery of high-value elements has been proposed to improve the financial viability of phytoremediation and reduce the environmental impact of disposal of contaminated biomass (Jiang et al., 2015).

Sida hermaphrodita L. Rusby, also known as Virginia fanpetals and Virginia mallow, is a herbaceous perennial plant of the Malvaceae family (Cumplido-Marin et al., 2020). The plant was introduced to Europe from North America in the 1930s. Sida is cultivated in Central Europe, mainly for research centers and university experiments. Small areas for production are found in Austria, Romania, Lithuania, and Hungary, as well as in Poland (approximately 300 ha) and Germany - approximately 100-150 ha (Iglinski et al., 2015; Nahm and Morhart, 2018).
Sida hermaphrodita is a cold-tolerant (winter hardiness to minus 35°C) plant, tolerant of various soil conditions, can withstand droughts, and can be grown in sun and partial shade (Jablonowski et al., 2017). In the first year, it reaches 60-90 cm in height, very slowly; in the second year, the stems reach 2.5-4 m in height and up to 3 cm in diameter; and in the third year of planting, 20-40 stems grow from 1 plant. The leaves are simple 10 to 20 cm long. *Sida hermaphrodita* blooms with white flowers from July to September. The plant is characterized by a high regrowth ability maintained for up to 15-20 years.
Due to its phenotypic plasticity can be cultivated on degraded and marginal soils (Nabel et al., 2017). *Sida hermaphrodita* is one of the proposed species for soil stabilization, and remediation of contaminated and degraded soils (Kocon and Matyka, 2012; Krzywy-Gawronska, 2012a; Kocon and Jurga, 2017). According to Antonkiewicz et al. (2017) the plant has a high potential for phytoextraction of heavy metals (Ni, Cu, Zn, and Cd) compared to other species used as energy crops. Cultivation of *Sida hermaphrodita* on contaminated soils allows the use of this crop for biomass production for energy applications on the one hand and stabilization of such soils and affected areas on the other (Antonkiewicz et al., 2017; Suric et al., 2022).
The objective of this study is to conduct systematic investigations that will allow us to determine the content of heavy metals, micro and macroelements in vegetative and reproductive organs of *Sida hermaphrodita*, and the potential for using the plant for phytoremediation of heavy metal contaminated soils.

Material and Methods

The experiment was carried out on an agricultural field contaminated with Zn, Pb, and Cd, located 0.5 km from the source of contamination - a Non-Ferrous Metals plant (NFMW) near Plovdiv, Bulgaria. The soil used in this experiment was slightly alkaline (pH 7.6) with moderate organic matter (2.5%). The total Zn, Pb, and Cd contents were high (1671.6 mg/kg Zn, 1694.8 mg/kg Pb, and 54.8 mg/kg Cd, respectively) and exceeded the maximum allowable concentrations (MAC) (400 mg/kg Zn, 100 mg/kg Pb, 3.0 mg/kg Cd) (Table 1). The Hg content in soils is lower than the MAC.

Table 1. pH, organic matter (%) and total content of Pb, Zn, Cd (mg/kg), and Hg (µg/kg) in soil sampled from NFMW-Plovdiv

Parameter	pH	Org.matter, %	Pb, mg/kg x±sd	Zn, mg/kg x±sd	Cd, mg/kg x±sd	Hg, µg/kg x±sd
0.5 km	7.6	2.5	1671.6±3.1	1694.8±3.5	54.8±0.9	574.8±10

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation
MAC (pH >7.4) – Pb -100 mg/kg, Cd - 3.0 mg/kg, Zn -400 mg/kg, Hg -1.5 mg/kg

The field tests with the *Sida hermaphrodita* were set after the block method in four replications. The size of the test plot was 100 m^2. Plants were planted in May by planting the seedlings at 0.75 m intra-row and inter-row spacing. Whole plants (3 plants from each replicate) were analyzed in mid-November before frost fall. The plants were collected, and the

content of heavy metals and macro and trace elements in their different parts - roots, stems, leaves, and flowers were analyzed separately. The samples were dried at room temperature to obtain an air-dry mass and then dried at 105°C.
The total metal composition of the soils was determined according to ISO 11466.
Plant samples were processed using the microwave mineralization method. An inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used to determine the content of heavy metals and micro- and macroelements in plant and soil samples.

Results and Discussion

Accumulation of heavy metals in vegetative and reproductive organs of the plant
Table 2 presents the results obtained for the content of heavy metals in the vegetative and reproductive organs of the study energy crop. Most heavy metals and micro and macro elements accumulate in the leaves (except P).
The contents of heavy metals in roots were lower compared to the leaves. The Pb content of the roots reached 60.5 mg/kg, Zn - 97.1 mg/kg, Cd - 10.7 mg/kg, and Hg- 63.0 µg/kg. The content of micro and macro elements was also lower in the root system compared to the leaves. The plant's root system is an extensive, deep-reaching allowing access to water and nutrients.
The content of heavy metals and micro and macro elements in the stems was lower than in the root system (except for Cd, Ca, and K), showing that their movement through the conductive system was strongly restricted. The Pb content in the Sida hermaphrodita stems grown at 0.5 km from NFMW reached 19.97 mg/kg, Zn - 35.5 mg/kg, Cd - 11.7 mg/kg, and Hg - 30.07 µg/kg.
The movement of Pb from the roots to the above-ground parts of plants is typically limited. Once Pb enters the plant's roots, it promptly interacts with phosphates, carbonates, and bicarbonates in high concentrations within the intercellular spaces. This interaction causes Pb to precipitate as phosphates or carbonates, preventing its transport through the xylem (Kabata Pendias, 2001). Cd is an element that is very mobile and moves from the roots to the above-ground mass (Kabata Pendias, 2001), which is confirmed in our studies.

Table 2. Content of the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (N, P, K, Ca, Mg) element in vegetative and reproductive organs of Sida hermaphrodita

	Roots x±sd	Stems x±sd	Leaves x±sd	Flowers x±sd
Pb, mg/kg	60.5±0.8	20.0 ±0.5	321.4±1,5	72.4±0.8
Cd, mg/kg	10.7±0.5	11.7±0.5	24.4±1.0	6.1±0.3
Zn, mg/kg	97.1±1.0	35.5±0.5	381.2±2.5	114.6±1,1
Cu, mg/kg	13.7±0.2	4.2±0.1	69.7±0.5	23.5±0.2
Fe, mg/kg	566.4±1.4	78.2±0.3	641.7±1.4	189.3±0.6
Mn, mg/kg	35.2±0.9	12.3±0.5	120.9±1.8	32.6±0.8
P, mg/kg	1195.7±6.8	479.9±1.8	1413.3±7.4	4111.9±8.9
Ca, mg/kg	7396. 3±10.5	8275.3±10.8	15903.1±12.3	4211.7±10.0
Mg, mg/kg	2104.1±5.8	892.0±1.0	2576.5±6.1	1531.1±1.9
K, mg/kg	7109.5±9.9	8307.2±10.3	20759.8±20.4	15807.2±15.6
Hg, mg/kg	41.0±1	23.0±1	215.9±2	47.4±1

x- average value(mg/kg) from 5 repetitions; sd - mean standard deviation

The content of Pb in the leaves of plants grown at 0.5 km from NFMW reached 321.4 mg/kg, Zn - 381.4 mg/kg, Cd - 24.4 mg/kg, and Hg - 404.4 µg/kg. A probable reason is that the

leaves of the plant on the upper side of the leaf are fuzzy or hairy, which is a prerequisite for their aerosol contamination.
Significantly lower results were obtained by Krzywy-Gawronska(2012a) (3.78 mg/kg Cu, 0.33 mg/kg Cd, 50.5 mg/kg Zn, 2.50 mg/kg Pb). However, Antonkiewicz and Jasiewicz (2000) found that *Sida hermaphrodita* biomass accumulates significant amounts of metals, and it is possible to use this plant for soil remediation.
The order of accumulation of heavy metals and trace elements in *Sida hermaphrodita* is as follows: Fe>Zn>Pb>Mn>Cu>Cd>Hg.
K content in leaves reaches up to 20760 mg/kg, Ca - up to 15903 mg/kg, Mg - up to 2576.5 mg/kg, and P - up to 1413.4 mg/kg. Significantly lower results for K, Ca, Mg, and P in biomass were found by Krzywy-Gawronska (2012b), with the order of accumulation being Ca > K > N > S > Mg > P.
The accumulation of macronutrients in the stems of the plant, the content of which influences the quality of the biomass for direct harvesting, is essential. The obtained results show that the content of K in the stems reaches up to 8307 mg/kg, Ca - up to 8275 mg/kg, Mg - up to 892.0 mg/kg, and P - up to 479.8 mg/kg. It was found that the macroelements content in the stems varied in a very narrow range (P - from 400 to 2800 mg/kg, Ca - from 3400 to 22600 mg/kg, Mg - from 400 to 1900 mg/kg and K- from 2500 to 24700 mg/kg (Antonkiewicz et al., 2018; Sienkiewicz et al., 2018; Bilandzija et al., 2018).

Translocation (TF) and bioconcentration (BCF) factors
Translocation and bioconcentration factors were calculated to determine the phytoremediation potential of the plant. The translocation factor (TF) provides information on the ability of plants to uptake heavy metals through the roots and transport them to the above-ground mass (stems and leaves). TF values are more significant than 1 (5.31 Pb and 2.27 Cd, 3.93 Zn, 6.41 Hg) (Table 2).
BCF shoot is defined as the ratio of the metal concentration in the shoots of the plant and in the soil (BCF shoots=[Metal]shoots/[Metal]soils) and is a measure of the plant's ability to absorb and move the metals to the above-ground mass, which can be easily harvested. The results show that, concerning Pb, the BCFshoots reach up to 0.19, for Cd 0.44, Zn 0.02 and for Hg - 0.70 (Table 2).
The four-level scale was adopted as the basis for estimating the bioaccumulation of metals (Pachura et al., 2016). The scale ranges are BCF<0.01 with no accumulation, BCF 0.01-0.1 with low bioaccumulation, BCF 0.1-1.0 with medium bioaccumulation, and BCF >1.0 with high bioaccumulation.

Table 2. Translocation (TF) and Bioconcentration factors (BCF roots, BCF shoots) of *Sida hermaphrodita*

Coefficient	Pb	Cd	Zn	Hg
TF	5.31	2.27	3.93	6.41
BCF roots	0.036	0.20	0,009	0.11
BCF shoots	0.19	0.44	0,02	0.70

BCF_{roots}=$[Metal]_{roots}$/$[Metal]_{soil}$, TF=$[Metal]_{shoots}$/$[Metal]_{roots}$),
BCF $_{shoots}$=$[Metal]_{shoots}$/$[Metal]_{soils}$

Krzywy-Gawronska (2012a) found an intense accumulation in the plant biomass concerning Ni, Pb, and Zn, and medium for Cd, Cu, and Mn. Pachura et al. (2006) found that S. hermaphrodita does not accumulate Pb (<0.01), accumulates Zn at a medium level (0.1-1.0), and accumulates Cd at a high level (1.0), with accumulation for all metals studied being significantly higher for roots than for aerial parts, which is not confirmed by our results.

Obtained results show that *Sida hermaphrodita* does not accumulate Zn, accumulates Pb poorly, and accumulates Hg and Cd at intermediate levels. The plant accumulates small amounts of heavy metals in the leaves and has no potential for phytoextraction. This is contrary to the results of Kocon and Jurga (2017), who reported that *Sida hermaphrodita* is a species suitable for phytoextraction of Pb, Cd, Zn, which is also confirmed by the results of Pogrzeba et al. (2018).

Conclusions

Based on the obtained results, the following conclusions can be made:
1. The *Sida hermaphrodita* is tolerant to heavy metals. It can be grown in heavy metal-polluted soils (1671.6 mg/kg Zn, 1694.8 mg/kg Pb, and 54.8 mg/kg Cd) and successfully used in the phytoremediation of heavy metal-polluted soils.
2. There is a clear distinction in the accumulation of heavy metals, micro, and macroelements in the vegetative and reproductive organs of *Sida hermaphrodita*. The depot for accumulation follows the order: Pb, Zn, Hg, Cu, Mg – leaves > flowers > roots > stems, Cd – leaves > stems > roots > flowers, Fe, Mn, Ca – leaves > roots > flowers> stems, P – flowers > roots > leaves > stems, and K – leaves > flowers > stems > roots.
3. *Sida hermaphrodita* does not accumulate Zn, accumulates Pb poorly, accumulates Hg and Cd at intermediate levels, and has no potential for phytoextraction. *Sida hermaphrodita* can be classified as an excluder plant with BCF < 1.

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CHEMICAL PROPERTIES AND POTENTIALLY TOXIC ELEMENTS IN ANTHROPOGENIC SOILS ON FLYSCH DEPOSITS IN OLIVE GROVES OF KAŠTELA BAY, CROATIA

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Abstract

The study aimed i) to determine selected chemical properties of anthropogenic soils on flysch deposits; and ii) to assess soil pollution by potentially toxic elements (PTEs). A total of 30 samples were collected in the top-soil horizon (0-20 cm) of olive groves in Kaštela Bay in Middle Dalmatia, Croatia. The soil samples were analysed for particle size distribution and basic chemical properties: pH, total carbonate, P_2O5, K_2O, and soil organic carbon (SOC) content by standard analytical methods. Furthermore, PTEs (As, Cu, Pb, and Zn) were determined by the portable X-ray fluorescence method. The analysed soils were alkaline (pH_{KCl} 7.23-7.72) and highly calcareous (31.6-65.9% $CaCO_3$). Soil supply by physiologically active nutrients was very poor to poor for phosphorus (0.3-13.4 mg P_2O_5/100 g of soil) and poor to good for potassium (8.4-66.0 mg K_2O/100 g of soil). The SOC content was low (0.90-2.12%). Concentrations of As (4.5-8.8 mg/kg), Cu (19.8-107.1 mg/kg), Pb (7.3-27.0 mg/kg), and Zn (32.3-95.7 mg/kg) were below the maximum permissible concentrations of PTEs for agricultural soils, according to Croatian legislation. However, As and Cu concentrations exceeded threshold values indicating thus the need for further assessment of the area, as prescribed by some European countries. The results of the study revealed variability in soil chemical properties that could be attributed to anthropogenic impact (fertilization and use of plant protective agents). Furthermore, the results pointed to the need for improvement in soil fertility, while taking into account protection against soil pollution.

Key words: *carbonates, nutrients, olive groves, pH, SOC.*

Introduction

Flysch is a complex of lithologically heterogeneous clastic sedimentary rocks formed by turbidite flows or submarine sliding of sediments. It is characterized by successive alternations of fine-grained sediments such as marl, and sandstone, but breccias, conglomerates, and calcirudites also occur (Marinčić et al., 1971). Differences in mineralogical composition and weathering processes of flysch deposits resulted in variable properties of soils developed on this parent material. It is well known that agricultural production implies soil tillage, fertilization, and the application of pesticides that can significantly alter soil properties. It affects soil organic carbon (SOC) content (Aranda et al., 2011, Bensa and Jurković Balog, 2023), pH (Bogunović et al., 2009), and physiologically available nutrients (Parras Alcantara et al., 2013, Miloš and Bensa, 2019). Besides changes in basic chemical properties, an additional problem in agricultural soils is contamination by potentially toxic elements (PTE). Fertilizers and pesticides applied to agricultural soils are significant sources of PTEs (Kabata Pendias and Mukherjee, 2007). Due to the long-term application of Cu–based fungicides, the elevated level of Cu concentrations in soils under permanent crops is the most commonly observed problem in the literature (Ballabio et al., 2018). Furthermore, the enrichment of agricultural soils by As and Zn were also documented (Miloš and Bensa, 2019), as well as Pb accumulation in soils in urban areas (Buljac, 2012). The aim of the paper was: i) determination of the basic chemical properties of anthropogenic soils in olive groves developed on flysch deposits; and ii) assessment of their pollution by PTEs.

Material and methods

The study was conducted in the coastal area of Kaštela Bay in Middle Dalmatia, Croatia. According to the Köppen climate classification, the study area belongs to the Mediterranean climate (Csa), also known as the "olive climate." It is characterized by hot and dry summers and mild, rainy winters (Filipčić, 1998). Kaštela Bay coastal area is built of Eocene Flysch marls, sandstones, and siltstones with lenses of calcirudites and calcarenites (Marinčić et al., 1971). According to the Basic Soil Map of Croatia at a scale of 1: 50 000, section Split 3 (Čolak and Martinović, 1974), anthropogenic soils of orchards and vineyards on flysch deposits, terraced, are the dominant soil types. Mentioned soils can be classified sensu World Reference Base for Soil Resources (IUSS Working Group WRB, 2015) as Regosols (Calcaric, Siltic/Loamic, Escalic). In the past, this area has been an important agricultural resource associated with cultivating traditional Mediterranean plants, mainly vineyards and olive groves. Today, the olive growing in the study area characterizes dry farming, including traditional low-input and intensified olive growing systems.

A total of 30 top-soil samples (0-20) cm were collected as composite samples in olive groves with different intensities of anthropogenic activity (tillage, fertilization, application of plant protective agents). The disturbed soil samples were prepared for laboratory analysis according to ISO 11464:2006. The soil particle size distribution was analysed by the pipette-method (ISO 11277:2020). The soil pH was measured according to ISO 10390:2021. Total carbonate content was determined by a modified volumetric method (ISO 10693:1995) Physiologically available phosphorus and potassium were determined according to Egner et al. (1960). The humus content was analysed following the method of Tjurin (JDPZ, 1966). The humus content was then divided by the Van Bemmelen factor (1.724) to calculate soil organic carbon (SOC) content. Total concentrations of PTEs (As, Cu, Pb, and Zn) were determined by the portable X-ray fluorescence method according to the loose powder method (Takahashi, 2015). Statistical processing of analytical data included descriptive statistics parameters.

Results and discussion

Particle size analysis revealed the dominance of silt particles in the studied soils. These soils have mainly silty loamy (73% of samples), then silty clayey loamy (13%), clayey loamy (7%), and loamy texture (7%), Figure 1.

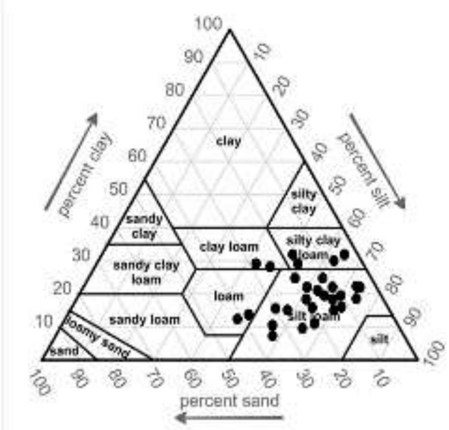

Figure 1. Distribution of soil samples on texture triangle (FAO/UNESCO, 1990)

The established soil texture of the studied soils corresponds to literature findings for soils on flysch deposits in Middle Dalmatia (Bogunović et al., 2009; Miloš and Bensa, 2019; Bensa and Jurkovć Balog, 2023). The dominance of silt particles points to the susceptibility of studied soils to erosion, which is related to their shallow depth (Miloš and Bensa, 2019). Furthermore, the diversity of soil texture (Figure 1) is the result of the great diversity and the lithological complexity of the Kaštela flysch deposits (Miščević and Roje-Bonacci, 2001, Polak et al., 2010). The studied soils had an alkaline reaction with $pH_{(KCl)}$ that varied from 7.21 to 7.72, with a mean value of 7.39, Table 1. The total carbonate content ranged from 31.6 to 65.9% indicating highly calcareous soils. The soil organic carbon (SOC) content in the studied soils was low, with an average of 1.53%. On average, the studied soils were poorly supplied with physiologically available phosphorus (4.72 mg P_2O_5/100 g of soil). However, P_2O_5 concentrations varied from 0.29 to 13.4 mg P_2O_5/100 g of soil, indicating a very poor to good supply of P_2O_5. The physiologically available potassium varied over a wide range (8.4 – 66.0 mg K_2O/100 g of soil), indicating a poor to rich supply of K_2O. The data distributions for pH, $CaCO_3$, and SOC content were approximately symmetric (skew < 0.5), Table 1. However, skewness for P_2O_5 and K_2O (0.86 and 1.04. respectively) pointed to a positively skewed data distribution.

Table 1. Descriptive statistic for basic chemical properties of studied soils

Statistical parameter	pH (H_2O)	pH (KCl)	$CaCO_3$ (%)	SOC (%)	P_2O_5 mg/100 g of soil	K_2O mg/100 g of soil
Min	7.63	7.21	31.6	0.90	0.29	8.4
Max	8.54	7.72	65.9	2.12	13.4	66.0
Mean	8.13	7.39	50.6	1.53	4.72	26.4
Med	8.13	7.40	51.1	1.58	4.55	22.0
SD	0.28	0.15	8.10	0.33	3.65	13.1
CV	3.4	2.0	16.0	21.3	77.4	49.7
Skew	-0.11	0.32	-0.40	0.01	0.86	1.04

Min- minimum; Max – maximum; Med – median; SD – standard deviation; CV – coefficient of variation; Skew - skewness

The established chemical properties of the studied soils agree with previous findings for anthropogenic soils derived on flysch deposits. Miloš and Maleš (1998) reported a mean $pH_{(KCl)}$ value of 6.99 and $CaCO_3$ content of 45.8% for the soils of the wider Kaštela Bay area. These values are slightly lower compared to mean values $pH_{(KCl}$ and $CaCO_3$ content in the current study (7.39 and 50.6 %, respectively) (Table 1). These differences can be explained by the higher proportion of soils developed on flysch deposits dominated by marl in the current study. Polak et al. (2010) highlighted the diverse lithological composition of flysch deposits in the region of Kaštela, the successive alteration of calcareous breccias, conglomerates, calcarenites, siltite, and marl. Also, the authors emphasized the different degrees of weathering of individual components of flysch. Low SOC content is a well-known problem in soils in the Mediterranean environment recognized in many studies (Aranda et al., 2011; Parras Alcantara et al., 2013; Parras Alcantara and Lozano Garcia, 2014). It can be attributed to high temperatures and low rainfall, exposure to erosion, torrential storms, and wildfires that frequently occur in this region (Certini et al., 2011). Furthermore, agricultural practices, such as soil tillage, enhance the mineralization of organic matter. The greatest variation among studied soils was established in physiologically available phosphorus and potassium (CV 77 and 50, respectively) (Table 1). It is related to different types of olive growing. In a low-input system, fertilization is rare and carried out with low doses of mineral fertilizers, and sometimes it is omitted. In a more intensive olive growing system, fertilization is most often carried out with much higher doses of fertilizers. Wide ranges of P_2O_5 and K_2O concentrations (1.4-31.2 and 9.5-160.0 mg/100 g of soil, respectively) were stated by Miloš and Maleš (1998) for anthropogenic soils on flysch under vineyards and olive groves in the wider Kaštela Bay area. The descriptive statistics for PTEs is shown in Table 2. The median values of As, Pb, and Zn (6.38, 11.7, and 63.3 mg/kg, respectively) were lower than the median values for Croatia (12, 33, and 88 mg/kg, respectively) according to the Geochemical Atlas of Croatia (Halamić and Miko, 2009). However, the median value of Cu (50.4 mg/kg) (Table 2), was higher than the median values for Croatia and the coastal area of Croatia (25.4 and 35.5 mg/kg, respectively), (Halamić and Miko, 2009). Furthermore, it was higher than the median values of Cu in flysch-derived soils in Istria (30.5 mg/kg) (Peh et al., 2009) and Kaštela Bay (40.7 mg/kg) (Buljac, 2012). The median Cu concentration in the current study was also higher than the median values for EU soils under olive groves reported in the LUCAS project (24.7 mg/kg), Ballabio et al. (2018). The wide range of Cu concentrations (19.8-107.1 mg/kg) (Table 2) and elevated concentrations can be attributed to different longstanding histories of anthropogenic influence. Some olives were grown all the time in monoculture orchards, some in mixed or consociate planting with grapes and other traditional crops such as figs, almonds, and vegetables, and some were planted on the terrain of former vineyards that no longer exist. The frequency of the Cu fungicide treatments and their duration influenced its concentration in soil due to the strong tendency of the Cu to accumulate and retain in soil. The data distribution of As, Cu, and Zn concentrations were approximately symmetric (skew<0.5), as shown in Table 2, while Pb concentrations had a highly positively skewed distribution (skew 1.75). Differences among Pb concentrations in studied soils (7.33-27.0 mg/kg) can be attributed to different anthropogenic sources, such as the combustion of fuels along roads. The Zn and As concentrations exhibit variation attributable to different applications of pesticides and mineral fertilizers containing the mentioned elements.

Table 2. Descriptive statistic for PTEs in studied soils

Statistical parameter	As	Cu	Pb	Zn
	mg/kg			
Minimum	4.53	19.8	7.33	32.3
Maximum	8.78	107.1	27.0	95.7
Mean	6.44	55.0	12.8	62.4
Median	6.38	50.4	11.7	63.3
Standard deviation	1.13	24.5	4.59	14.5
Coefficient of varation	17.5	44.6	35.8	23.3
Skewness	0.42	0.43	1.75	0.33

The concentrations of As, Cu, Pb, and Zn were below the maximum permissible concentrations of PTEs for agricultural soils (30, 120, 150, and 200 mg/kg), according to Croatian legislation (Official Gazette 71/19). However, different national systems had different approaches to defining risk levels associated with different concentrations of heavy metals in soils. The widely applied Finish standard values (MEF, 2007) represent a good approximation of mean values from EU countries (Carlon et al., 2007). This legislation sets different values, indicating the need for different actions if exceeded. The threshold value indicates the need for further assessment of the area, and the guideline value indicates contamination level that represents ecological or health risk (Table 3).

Table 3. Threshold and guideline values (mg/kg) for element in soils (MEF, 2007)

Element	Threshold value	Guideline value
As	5	50
Cu	100	150
Pb	60	200
Zn	200	250

According to these criterion As and Cu values (4.53-8.78 and 19.8–107.1 mg/kg, respectively), Table 2, were above threshold values. Therefore, some of the studied soils require further assessment regarding these elements.

Conclusions

The studied soils had a uniform alkaline reaction, low SOC, and high carbonate content. The supply of physiologically available phosphorus and potassium varied from poor to rich due to different anthropogenic impacts via fertilization. Concentrations of As, Cu, Pb, and Zn were below the maximum permissible concentrations of PTEs for agricultural soils, according to Croatian legislation. However, As and Cu concentrations exceeded threshold values prescribed by some legislation for European countries. The results of the study indicated the need for further, more detailed studies to improve the sustainability of this olive growing system in a fragile Mediterranean environment.

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SOIL PROPERTIES AND SOIL FERTILITY EVALUATION OF BUKOVICA REGION, CROATIA

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Abstract

The study aimed to: i) determine selected physical and chemical soil properties, ii) assess soil fertility and iii) provide recommendations for amelioration measures. Study area occupied 1833 ha in Bukovica region located in north-eastern Croatia. A total of 23 soil profiles were analysed for particle size distribution, soil density, porosity, water holding capacity, air capacity, pH, humus, P_2O5 and K_2O content by standard analytical methods. These analyzes were conducted in the following soil types: Fluvisol, Pseudogley, Pseudogley-gley, Hypogley and Amphigley. The texture of the analyzed soils was silty loam to silty clayey loam. Bulk and particle density varied in ranges 1.13-1.55 g/cm^3 and 2.51-2.71 g/cm^3, respectively. Water holding capacity was mostly moderate, while air capacity was low to moderate. The analyzed soils were acidic (pH KCl 3.9-6.7) with low to moderate humus content (0.3 – 3.8%). Soil supply by physiologically active phosphorus and potassium was very poor to rich (1.5-14.7 mg P_2O_5/100 g of soil and 6.6-16.3 mg K_2O/100 g of soil, respectively). The results of the study revealed variability in soil properties and fertility that could be attributed to natural causes (different parent material and geomorphological positions) and anthropogenic impact (tillage, fertilization). Recommendations for amelioration measures to improve soil fertility included: deep tillage, liming, mineral and organic fertilization and maintenance of hydro-amelioration systems.

Key words: *Hydromorphic soils, Soil properties and fertility, Amelioration measures.*

Introduction

Soil is a conditionally renewable natural resource representing a very complex, polyphase system. Soil fertility is affected by numerous agoecological factors, and the soil, with its physical, chemical, and biological properties, affects numerous soil processes that make it variably suitable for agricultural production (Rakesh et al., 2012). The most important soil properties are particle size distribution, water and air capacity, porosity density, pH, humus content, and the supply of plant-available nutrients. The mentioned properties are spatially variable and depend to a significant extent on pedogenetic factors and processes. The Panonnian agricultural region of Croatia, which includes the Bukovica area in the central part of Virovitica-Podravina County, is of special agricultural importance for the whole of Croatia. This area is dominated by hydromorphic and semi-terrestral soils formed on Pleistocene terraces and built of loess, leached loess, or marbled reprecipitated loam. These soils have numerous limitations for use in agricultural production due to their poor physical and/or chemical properties (Bogunović et al., 1991). The most common problems are unfavorable texture, compaction, poor sol water-air relations, as well as low humus and nutrient content, which represent a challenge for successful agricultural production (Akinbola et al., 2012). The application of agro- and/or hydroamelioration measures (deep tillage, undermining, fertilization, calcification, and drainage) affects the change of particular soil properties and consequently overall soil fertility. Therefore, the aim of this study was to: i) assess the fertility of the most common soil types in the Bukovca area based on the selected physical and chemical properties; and ii) to recommend agro and/or hydroamelioration measures necessary for the improvement of these soils.

Materials and methods

The study area (1833 ha) covers the area of the municipality of Nova Bukovica, located in the central part of Viirovitica-Podravina County, in northeastern Croatia. The studied agricultural soils were on the terrace and valley part of Podravina, dominated by the accumulation-tectonic type of relief with Quaternary loess and loess-like sediments (Bogunović et al., 1991). Pseudogley and pseudogley-gley soils have developed on the loess plains with slopes < 5%. In the former flood zones within Holocene accumulation retention, fluvial, hypogley, and amphigley soils developed. Detailed drainage of excess flood, runoff, stagnant precipitation water and groundwater was carried out using a combination of a system of open channels and pipe drainage, on most of the soils. The climate of the study area was moderately continental. In the period 1981-2020, the average air temperature was 11.4°C, and the average amount of precipitation was 835 mm. Systematic soil units, according to Husnjak (2014), and their distribution iin the Bukovica region are shown in Table 1.

Table 1. Systematic soil units and their distibution in the Bukovica region

Order	Class	Type	Subtype	Variety	Area(ha)
Hydromorphic soils	Initial	Fluvial	Non-calcareous, deep	Very deep	28
	Hypogleyic	Hypogley	Non-calcareous	Mineral	189
	Amphigleyic	Amphigley	Non-calcareous	Mineral	131
		Pseudogley-gley	Medium deep	Medium deep gleyic	308
Semiterrestric soils	Pseudo and stagnic soils	Pseudogley	On the plain; On the hillslope	Deep; Medium deep	1177

Disturbed and undisturbed soil samples (in 100 cm^3 cores) from topsoil and subsoil were taken from each pedosystematic unit from a total of 23 soil profiles. The disturbed soil samples were prepared for laboratory analysis according to ISO 11464:2009. The soil particle size distribution was analysed by the pipette method with wet sieving and sedimentation after dispersion with Na pyrophosphate (HRN ISO 11277:2009). Particle density and total porosity were determined according to HRN ISO 11508:1998, and bulk density according to HRN ISO 11272:2017. Soil water holding capacity was determined according to HRN ISO 11461: 2014, and air capacity was calculated as the difference between total porosity and soil water holding capacity. Soil pH was measured potentiometrically in a u 1:5 (v/v) suspension of soil and water and soil and KCl solution (c = 1M) (HRN ISO 10390:2005). The humus content was analysed following the method of Tjurin (JDPZ, 1966), and plant available phosphorus and potassium according to Egner et al. (1960).

Results and discussion

Average values for the basic physical and chemical properties of the studied soils are shown in Tables 2-4. Pseudogley soils have a silty loam texture with a predominance of silt fraction (57.9-75.4%) in topsoil, which represents the risk of erosion and crust formation, Table 2. The clay content in the topsoil of pseudogley soils varied from 18.9 to 19.6% and increased with depth, ranging from 23.1-26.2% in the subsoil. The vertical distribution and eluviation of soil particles are the result of combined actions of several factors: parent material (loess), which is subject to eluviation, leveled relief position; and the duration of the wet phase (stagnation of precipitation water) in the soil (Rubinić et al., 2015). Pseudogley-gley and amphigley soils have a silty clay loamy texture with > 30% clay in topsoil, Table 2. Pseudogley-gley soils occupy leveled valley positions between loess terraces and Holocene plains, where the drainage is weak and the influence of groundwater and stagnant precipitation water is great. Therefore, an illuvial horizon with a high proportion of clay particles was formed. Similar average clay content (37.4%) in drained pseudogley-gley soil of Middle Posavina reported Husnjak (2000). Fluvisols have the lowest clay content in topsoil and subsoil (14.6 and 17.0 %, respectively), as shown in Table 2 due to the flooding of river Voćinka.

Table 2. Average values of particle size distribution for systematic soil units in Bukovica region and soil texture

Systematic soil unit	Depth, cm	Particle size (%), with diameter (mm)					Soil texture*
		2.0-0.2	0.2-0.063	0.063-0.02	0.02-0.002	<0.002	
Fluvisol, noncalcareous, deep	0-34	5.8	12.0	37.6	30.0	14.6	SL
	34-88	2.3	6.7	44.2	29.8	17.0	SL
Pseudogley on the flat terrain	0-38	3.4	3.9	34.4	38.7	19.6	SL
	38-71	2.3	3.8	35.2	32.5	26.2	SL
Pseudogley on the hillslopes	0-34	2.2	3.5	43.1	32.3	18.9	SL
	34-82	1.4	2.6	42.3	30.6	23.1	SL
Pseudogley-gley	0-39	2.2	4.2	18.6	44.6	30.4	SCL
	39-79	3.8	4.7	24.8	33.1	33.6	SCL
Hipogley, noncalcareous, mineral	0-45	1.5	4.8	52.3	20.6	20.8	SL
	45-81	0.9	4.7	45.6	28.4	20.4	SL
Amphigley, noncalcareous, mineral	0-38	1.0	3.8	35.7	29.4	30.1	SCL
	38-69	0.9	2.4	35.5	31.9	29.3	SL

*Legend: SL- silty loam; SCL silty-clayey loam

Total porosity (P) in studied soils decreased with depth from porous topsoil (46.2-55.5%) to slightly porous subsoil (41.1-44.8%), Table 3. The highest porosity in pseudogley-gley and amphigley is the result of increased clay content and micropores in the soil (Husnjak, 2014). The majority of studied soils have medium soil water holding capacity (SWHC) in topsoil and subsoil (36.9 – 40.9% and 36.7 – 40.9%, respectively). The only exception is Pseudogley-gley which has high SWHC (47.3 % vol.) in the topsoil. Soil air capacity (SAC) desreased with depth and was generally moderate to low. The lowest SAC in topsoil was recorded in amphigley and the highest in fluvisol (7.7 and 10.7%, respectively), see Table 3. In subsoil, SAC ranged from 4.4 (fluvisol) to 8.0% (hipogley). Bulk density (ρb) increased with depth, ranging from 1.13 to 1.43 g/cm^3 in topsoil to 1.47 – 1.55 g/cm^3 in subsoil, Table 3. In addition to unfavorable relationship between micro- and macropores and high clay content, especially in pseudogley-gley and amfigleja, these factors affect soil compaction (Vopravil et al., 2017). These results indicate serious limitations of physical soil properties, especially in subsoil. Longer stagnation of precipitation water intensifies anaerobic processes, which negatively affect plant production. Although fluvisol has more favorable texture and water-air relationships in topsoil, due to the increased sand fraction, in the subsoil it also has limited percolation of water. Furthermore, the frequency and duration of flooding of the Voćinka river, as well as the dynamic of the groundwater level, affect its fertility, as highlighted by Husnjak (2014). Considering the physical properties of the studied soils, they can be ordered according to their production potential as follows: fluvisol > hypogley> pseudogley on hillslope > pseudogley on plain > pseudogly-gley > amphigley. In most of the studied soils, the duration of the wet phase should be reduced by deep loosening, which will help to establish more favorable water-air relationships. In micro-depressions terrain leveling should also be carried out with mandatory undermining. Fluvisols should be protected from floods, and in pseudogley-gley and amphigley care should be taken to maintain existing hydroamelioration systems.

Table 3. Average values of physical properties for systematic soil units in Bukovica region

Systematic soil unit	Depth, cm	ρb g/cm^3	ρp g/cm^3	P % vol.	Evaluation of P	SWHC % vol.	Evaluation of SWHC	SAC % vol.	Evaluation of SAC
Fluvisol, noncalcareous, deep	0-34	1.42	2.71	47.6	Porous	36.9	Medium	10.7	Medium
	34-88	1.55	2.63	41.1	Low porosity	36.7	Medium	4.4	Low
Pseudogley on the flat terrain	0-38	1.40	2.63	46.7	Porous	37.8	Medium	8.9	Medium
	38-71	1.47	2.63	44.1	Low porosity	36.8	Medium	7.3	Low
Pseudogley on the hillslopes	0-34	1.43	2.66	46.3	Porous	38.3	Medium	8.2	Medium
	34-82	1.51	2.71	44.3	Low porosity	38.0	Medium	6.3	Low
Pseudogley-gley	0-39	1.13	2.53	55.5	Porous	47.3	High	8.2	Medium
	39-79	1.47	2.60	43.7	Low Porosity	37.5	Medium	6.2	Low
Hipogley. non calcareous. mineral	0-45	1.41	2.61	46.2	Porous	37.3	Medium	8.9	Medium
	45-81	1.52	2.70	43.8	Low porosity	35.8	Medium	8.0	Medium
Amphigley. non calcareous. mineral	0-38	1.39	2.51	48.6	Porous	40.9	Medium	7.7	Low
	38-69	1.47	2.66	44.8	Low porosity	40.0	Medium	4.8	Low

*Legend: ρb – bulk soil density; ρp- soil particle density; P- soil porosity; SWHC- soil water holding capacity; SAC- soil air capacity

Studied soils are slightly to strongly acidic. Table 4. The lowest $pH_{(KCl)}$ value was measured in pseudogley on hillslope in topsoil and subsoil (4.0 and 3.9. respectively). The noticeable increase in pH value with depth is mostly the result of clay eluviation. as well as reduced humus content (Husnjak. 2014). Similar pH values for hydromorphic soil were reported by Bašić et al. (2000) in Middle Croatia and Lončarić et al. (2006) for soils in Eastern Croatia. Higher pH values were obtained in hipogley and amphigley due to a higher proportion of clay particles and the adsorption of base cations. Pseudogleys on hillslope have the lowest humus content in topsoil and subsoil (1.6 and 0.3 %. respectively). Table 4. whch is in line with data for pseudogleys of Eastern Croatia (Lončarić et al., 2006). The low level of humus in the mentioned agricultural soils can be attributed to soil tillage which enhances mineralization and absence of organic fertilization. However. higher humus content was determined in topsoil of hypogley. pseudogley-gley and amphigley (3.8. 4.0 and 4.1 %. respectively). The supply of studied soils with P_2O_5 was generally very low to low in topsoil and subsoil (7.5-14.7 and 1.0-9.2 mg P_2O_5 / 100 g of soil). Table 4. It can be attributed to insufficient rates of mineral fertilization. as well as phosphorous inactivation in soils with acid reactions (Lončarić et al., 2006). The supply of studied soils with K_2O was good in topsoil of fluvisol and pseudogleys (14.5-16.3 mg K_2O / 100 g of soil) and low in hydromorphic soils (11.6-12.1 mg

K_2O/ 100 g of soil). and decreased with depth. Table 4. The variability of K_2O content in the studied soils was caused by differences in fertilization and parent material. Soils formed on loess. rich in ilite. have a higher possibility for the adsorption of potassium (Rubinić et al., 2015). The obtained results of the chemical properties of the studied soils pointed to limited fertility. The improvement of the mentioned properties can be achieved by calcification and adequate fertilization with organic and mineral fertilizers. as highlighted by Rastija et al. (2009). The order of studied soils according to suitability of chemical properties for plant production is as follows: fluvisol > hypogley > amphigley > pseudogley-gley > pseudogley on plain > pseudogley on hillslope.

Table 4. Average values of chemical properties for systematic soil units in Bukovica region

Systematic soil unit	Depth cm	Soil pH		Evaluation of pH (KCl)	Humus %	Evaluation of humus	Content of available nutrients mg/100g of soil			
		H_2O	KCl				P_2O_5	Evaluation	K_2O	Evaluation
Fluvisol. noncalcareous. deep	0-34	5.4	4.2	Strong acid	1.8	Low	7.5	Low	14.5	Good
	34-88	6.1	4.9	Acid	1.3	Low	1.5	Very low	8.4	Low
Pseudogley on the flat terrain	0-38	5.5	4.3	Strong acid	2.5	Low	8.7	Low	16.3	Good
	38-71	5.7	4.5	Acid	1.0	Low	2.9	Very low	8.1	Low
Pseudogley on the hillslopes	0-34	4.9	4.0	Strong acid	1.6	Low	14.7	Good	15.3	Good
	34-82	5.2	3.9	Strong acid	0.3	Very low	9.2	Low	8.5	Low
Pseudogley-gley	0-39	6.1	4.8	Acid	4.0	High	10.3	Low	12.1	Low
	39-79	6.1	4.7	Acid	1.7	Low	1.0	Very low	9.0	Low
Hipogley. non calcareous. mineral	0-45	6.6	5.6	Slight acid	3.8	High	7.7	Low	11.6	Low
	45-81	6.9	6.1	Slight acid	1.6	Low	4.9	Very low	6.6	Very low
Amphigley. non calcareous. mineral	0-38	6.8	5.8	Slight acid	4.1	High	7.6	Low	11.7	Low
	38-69	7.6	6.7	Neutral	1.3	Low	4.5	Very low	8.1	Low

Conclusion

Most of the studied soils have a silty loam texture with a predominance of silt fraction. susceptible to erosion. Medium SWHC. low SAC. and increased bulk density (especially in subsoil) condition unfavorable soil water-air conditions. Studied soils are slightly (hypogley and amphigley) to strongly acidic (pseudogleys). They have low (pseudogleys) to high humus content (hydromorphic soils). Soils are generally poorly supplied with P_2O_5 and poorly to good with K_2O. Established properties are the result of different parent materal and anthropogenic impacts. Soils with the most favorable properties are fluvisols. while amphigleys have the worst physical. and pseudogleys the worst chemical properties. More favourable water-air conditions should be established in all studied soil by deep loosening. Furthermore calcification and adequate mineral and organic fertilization should be carried out.

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STUDY OF MICROPLASTICS IN SOIL AMENDEMENTS

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Abstract

Microplastics are a global environmental pollutant that have increasingly gained the attention of researchers. Although plastics in seas and oceans are in the main focus, the presence of microplastics in soil poses significant alterations in the soil microbiota and hence affects severely the terrestrial ecosystems. Soil amendments, play a crucial role in enhancing soil quality and productivity, however, their potential association with microplastics remains understudied. The aim of this research paper is the detection and quantitative determination of microplastics in commercially available agricultural products used as soil amendments. The samples tested were peat, organic compost, two types of chicken manure and three different types of vegetative soil, commercially available in retail and wholesale markets. Microplastics were separated and classified by size, shape, colour and abundance by stereoscopic and microscopic observations, in triplicates for each sample. Results showed that a significant number of microplastics were observed in chicken manure presented in greater abundance than in the rest of the samples. However, no plastics were identified in peat samples. Amendment of the agricultural soils with microplastics bearing products may initiate adverse effects to soil microfauna, alter the biochemical cycles of nutrients and potentially enhance the transfer of microplastics to agricultural food production.

Keywords*: Agriculture, Amendments, Manure, Microplastics, Soil.*

Introduction

In recent years, agriculture has been confronted with the risk of microplastics, due to anthropogenic debris. Microplastics are commonly defined as small plastic particles less than 5 micrometres in diameter. These particles predominantly originate from the degradation of larger plastic debris, known as macroplastics (Rillig & Lehmann, 2020). The rising concern over their presence in the environment has become a serious threat for scientists to manage at a global scale. Despite the increased efforts to reduce plastic production and promote new recycling methods, in 2022, the production of plastic reached 400.03 million tonnes (Mt), with 90.6% of it being fossil-based (PlasticsEurope, 2024). In general, plastics have a stable structure with high resistance against degradation, because they originate from petroleum, with their persistence and slow decomposition being particularly damaging, as it allows them to persist in the environment, causing devastating effects on the ecosystems and the organisms depending on them. Degradation of conservative plastics is achieved by some chemical processes such as photo-oxidative decomposition by UV rays of the sun, thermolysis, thermal oxidation, photolysis, radiolysis, etc. (Yousif & Haddad, 2013), or can be achieved through natural processes such as biodegradation using bacterial enzymes (Cai, et al., 2023). The most common form of plastic decomposition is photo-oxidative degradation (Wagner & Lambert, 2018) since exposure to UV radiation causes significant degradation in polymers and more specifically destroys polymer chains and molecular weight reduction, resulting in mechanical degradation of plastics in a short time. The mechanism of photo-oxidative degradation works, more specifically, by absorbing light and the molecules of plastics absorb the energy of photons. Polystyrene, one of the commonest polymers for plastics, when exposed to UV radiation conditions, undergoes yellowing, brittleness and the formation of hydrogen-free radicals, which oxidize and lead to fragmentation of the plastic (Yousif & Haddad, 2013). Therefore plastics, and by extension microplastics, are continuously released into the aquatic and terrestrial environment from a variety of sources, primarily from land-based sources, while water pollution comes from runoff plastics found in terrestrial areas or from paint residues from shipping and fishing (NOAA, 2024). Agricultural soils are polluted both directly from anthropogenic sources of pollution such as the application of excessive amounts of agricultural fertilisers, and indirectly as a result of flooding and atmospheric deposition. More specifically, the main sources of pollution in agricultural areas are pesticides, inorganic and organic fertilizers, agricultural waste and plastic materials used for land cover and in greenhouses (FAO, 2021). During decomposition, plastics do not allow crops to absorb the necessary nutrients and trace elements, therefore having big imprints in them. Bioaccumulation of toxic substances ingested by plants are highly harmful and cause serious damage to the crops, such as chlorophyll content, reduced leaf size and photosynthetic efficiency (Colzi, et al., 2022). Their presence may also block water circulation, causing water stagnation, waterlogging and total destruction of the crop. This is a result of soil losing its ability to drain the excess water properly and the oxygen supply to the plant roots is gradually reduced. As for the soil fauna, microplastics seem to cause weight loss of earthworms, reduce the survival rate and body length of nematodes, and lastly inhibit the growth of collembolan (Tian, Jinjin, Ji, Ma, & Yu, 2022). As a consequence, understanding the origin and function of plastics is important to achieve the introduction of polymers in agriculture and farming methods to avoid further pollution of grasslands and pastures.

Materials and methods

Peat, organic compost, factory-produced chicken manure, chicken manure obtained by a local producer and three different types of soil amendments were used as samples in the current study. The seven samples were obtained from various suppliers. The experimental procedure for the extraction of microplastics from the samples was conducted according to the DeFishGear protocol (Palatinus, Viršek, & Kaberi, 2015). Samples were air dried and treated with a saturated solution of distilled water and NaCl. 50g of each sample was treated in 400 ml of the sodium chloride solution for 24h, in order to separate the microplastics into the aqueous supernatant and the process was repeated twice. Further, samples were vacuum filtered and the filtrates were collected on 47mm glass Fiber filters. For the deterioration of organic residues in optical observations H_2O_2 was added in each sample. For the enumeration of microplastics, the samples were observed with a stereoscope, using different magnifications.

Results and Discussion

Microplastics were identified in almost all of the samples, with the exception of peat. The number of microplastics ranged from 0 to 38 per 50gr of sample (Table 1). Their size and length were also measured during the research process, with it being ranged from 5μm to 5000μm. The colours of the particles were distinct, most of them were blue or black fibres (Fig. 1). Only a small proportion of fibres and fragments were yellow, white, red or green. The presence of blue and black fibres can be attributed to the packaging material used for the storage and selling of soil amendments. Blue and black strings, wraps or cups are commonly used in the packaging of soil amendments found in the wholesale market. A common practice that could lead to the contamination of soil amendments with microplastics is covering with plastic materials such as bags and mulch films, while trying to protect it from the weather or animals (reNature, 2023).

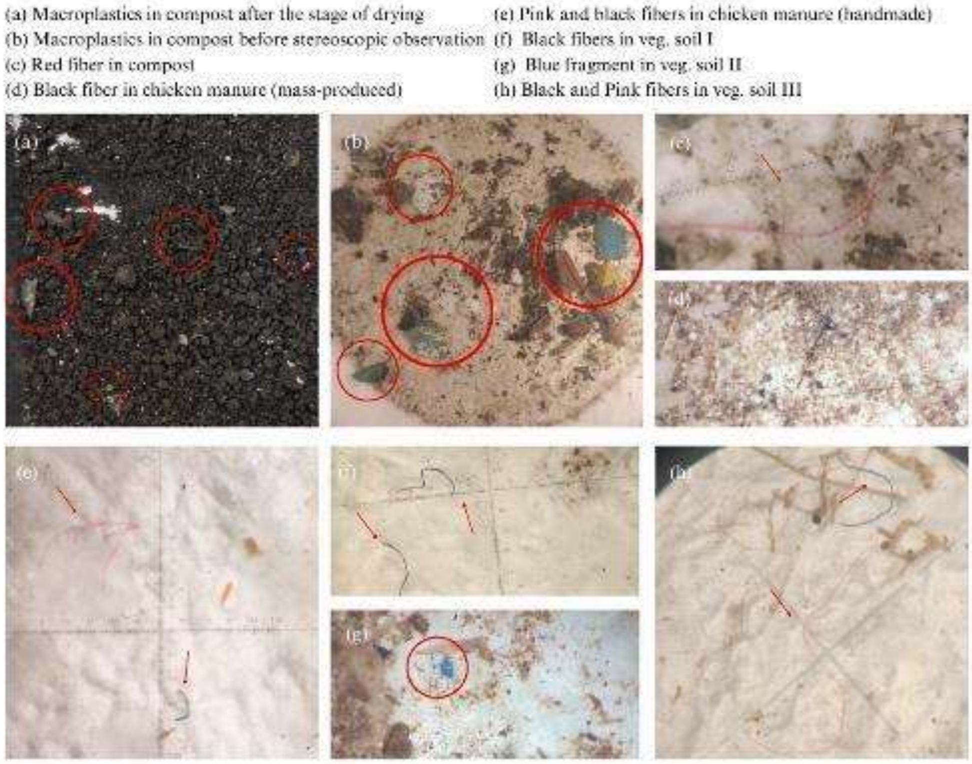

Figure 1. Examples of microplastics fragments and fibers in all samples.

Table 2. Average number of microplastics in 50gr of sample.

TYPE OF SAMPLE							
	Peat	**Compost**	**C. Manure M**	**C. Manure H**	**Veget. Soil I**	**Veget. Soil II**	**Veget. Soil III**
RESULTS:	0	93	38	25	15	31	17

In peat samples no microplastics were observed (Tables 1), since peat originates from botanical and geological processes (Stracher, Prakash, & Rein, 2015). It is also composed by organic matter and mostly plant material that had undergone specific procedures, such as waterlogging, oxygen deficiency and high acidity (IPS , 2024).

The largest quantity of fibres or fragments of microplastics was found in the compost samples. These, at some instances, were visible to the naked eye. It could be important to note that these fragments seemed to mostly come plastic wraps. In 50gr of compost sample, there was an average of 93 microplastics particles present (Table 1), while in 1kg of sample, it can be assumed that in 1kg of compost there would be 1866 particles (Table 2). These varied in size and colour, but they did not exceed the 5000μm length limit. Colour wise, the microplastic fragments varied and did not appear to follow a consistent pattern, suggesting the variability in their source and in the materials of their composition. While in the compost samples, contained most microplastic fragments and fibres, further research is necessary to make useful conclusions and suggestions regarding the avoidance of its use or the ways to make improvements in the product.

Researchers have suggested that the concentration of microplastics in compost can be attributed in the food waste products that are used (Porterfield, Hobson, Neher, Niles, & Roy, 2023). When these come from landfills, there is a high chance that they have come in contact with plastic packaging and other products. Although there are some ways to separate plastics from food waste, this is not always accomplished correctly. Furthermore, the lack of standardized methods of handling the waste materials makes studying it a difficult task (Porterfield, Hobson, Neher, Niles, & Roy, 2023).

Table 3. Average number of microplastics in 1000gr of sample.

	TYPE OF SAMPLE						
	Peat	**Compost**	**C. Manure M**	**C. Manure H**	**Veget. Soil I**	**Veget. Soil II**	**Veget. Soil III**
RESULTS:	0	1866	760	500	293	613	347

Regarding the production process of chicken manure, it is evident that that mass-production holds increased numbers of microplastics compared to individual production. Using manure as a way of fertilization is a common practice, considering the fact that between the years 2016-2019, the farming industry in the European Union (EU-27) and the UK produced approximately 1.4 billion tonnes/year of manure, while 90% of the production was directly re-applied to the crops as organic fertiliser (EuropeanCommision, 2021). The research on the presence of microplastics in manure production is still unexploited. A recent study, showed concentrations of $14{,}720 \pm 2{,}468$ items kg^{-1} (Zhang, et al., 2022). According to researchers, the possible reasons for microplastic contamination of the manure are varied but are mostly based on the feed of the animals that produced it. This can be attributed in the first place to the contamination of soil, plants and farmlands and the overall habitat that the husbandry activities take place. Animal feed may contain microplastics, as a result of feed composition and packaging, as they was found in 19 different farms in China (Wu, et al., 2021). Plastic nets, bottles, food wrappers, pipes for transporting feed etc. dominate in farming, resulting in them being mixed with animal food (Wu, et al., 2021). microplastics' presence in soil affects microorganisms like earthworms, a common prey for poultry.

All three vegetative soils exhibited low quantities of microplastics and mainly vegetative soil I and III. These two soils are being produced by the same company and they have similar characteristics. Vegetative soil II according to the manufacturer, contains more than 65% organic matter and it contains an undisclosed percentage of peat, organic compost, pelf, vegetables and animal manure.

Conclusions

The problem of microplastic presence in the agriculture and husbandry is still understudied. In the present study a preliminary and small-scale monitoring was conducted. However, the initial findings exhibited the presence of microplastics in compost, chicken manure, and vegetative soil. The diverse origin of each soil amendment used in the present study pose a significant finding for an emerging pollutant to agricultural soil. Microplastics may persist within soil, possibly impacting the crops and eventually entering the food chain. The continued accumulation of microplastics in the environment calls for further studies and the search for new techniques to minimise their presence in soils.

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PRODUCTION PRACTICES OF ECONOMICALLY IMPORTANT CROPS OF GREECE AS A POTENTIAL SOURCE OF MICROPLASTICS IN THE SOIL ECOSYSTEM

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Abstract

Over the last 70 years, the use of plastics in agrifood systems and food value chains has become pervasive. Low-cost and versatile plastic products have crept into every part of our food systems. Soils are one of the main receptors of agricultural plastics and are known to contain larger quantities of microplastics than oceans. Around one million metric tons of plastics is produced worldwide daily. Plastic contamination is aggravated when the particles reach sizes between 5 mm and 1 μm, giving rise to microplastics, which are omnipresent in the environment, especially in agroecosystems. Agricultural practices of economically important crops of Greece employ a wide range of plastic products to help improve productivity, such as: mulch films; tunnel and greenhouse films and nets; irrigation tubes and driplines; bags and sacks; soil-solarization plastic films; anti-hail protection nets; harvesting nets; permanent bedding nets. As the demand for agricultural plastics continues to grow, there is an urgent need to better monitor the quantities of plastic products used and that are released into the environment from agriculture. We have focused in the study of agricultural soils in Greece, comparing microplastic load between sites where plastics are used or not used as the result of specific farming practices for the production of economically important crops of Greece (olives, grapes, strawberries, kiwis, and vegetable). The scope of the study is: a) the identification of specific farming practices applied to important crops in Greece as potential or non-potential sources of microplastics in the agricultural environment. b) the modeling of microplastic pollution in major agricultural regions of Greece. c) the identification of more sustainable and microplastic – free cultivation techniques and materials. d) the public awareness of the farmers regarding the cultivation techniques as source of microplastic pollution.

Keywords: *soil pollution; microplastics; farming practices.*

Introduction

Plastics have become widely used in agrifood systems and food value chains over the past 70 years. Affordable and versatile plastic products of various types, shapes, and sizes are utilized in modern agriculture to safeguard crops and enhance yields. Low-density polyethylene (LDPE) stands as the most commonly used plastic in agriculture, primarily employed in diverse films for greenhouses, tunnels, and mulching. Following LDPE, polypropylene (PP) is the second most prevalent plastic, utilized for pipes, sheets, nets, and ropes. Finally, polyvinylchloride (PVC), the third most common plastic, is often present in pipes or tubes for irrigation systems and in semi-rigid sheets for greenhouse coverings (Kononov *et al*., 2022).
The production of plastic products has been steadily increasing, with global plastic production rising from around 1.5 million tons in 1950 to about 348 million tons in 2017 (Plastics Europe, 2020). Out of this total, 79% is released into the land and sea, 9% is recycled, and 12% is incinerated (Geyer *et al*., 2017).

Plastic has become pervasive in the environment, and although previous studies have mainly focused on the ocean and water systems, there is now a growing emphasis on soil, particularly in agricultural ecosystems where our food is produced. The majority of plastic products used in agriculture are disposable and can remain in the environment long after their use. The breakdown of large plastic waste has resulted in a significant rise in the prevalence of small plastic particles. These particles are capable of persisting in the environment for long periods, resisting natural decomposition and building up in large amounts becoming a major environmental concern in recent times.

Plastic debris is frequently categorised into subgroups based on particle size (Galgani *et al.*, 2013; Alimi *et al.*, 2018), distinguishing between macroplastics (> 25 mm), mesoplastics (5–25 mm), microplastics (1 μm–5 mm) and nanoplastics (< 1 μm).

Understanding the prevalence and distribution of MPs in soils is crucial nowadays. The majority of international studies regarding the presence of microplastics in soil focus on mulching, the addition of sewage sludge, compost and slow controlled release fertilizers.

The present work aims to the identification of specific farming practices applied on economically important crops in Greece (olives, grapes, strawberries, kiwis, and vegetable) as potential sources of microplastics in the agricultural environment. In addition, we aim to the development of a methodology for the creation of a forecast model of microplastic pollution in rural areas of the country, based on the total amount of plastics used and the systematic sampling, analysis and assessment of soil fields for microplastics. Specific farming practices that can act as a possible source of microplastics in Greek soil are presented in figures 1 to 3.

Figure 1. Farming practices as possible source of microplastics used in olive oil production: A) Harvesting nets made of HDPE, B) Harvesting plastic-fabric made of polypropylene, C) Plastic bags made of PP or HDPE and crates made of PP.

Figure 2. Farming practices as source of microplastic used in kiwi production: Anti-hail protection net made of HDPE.

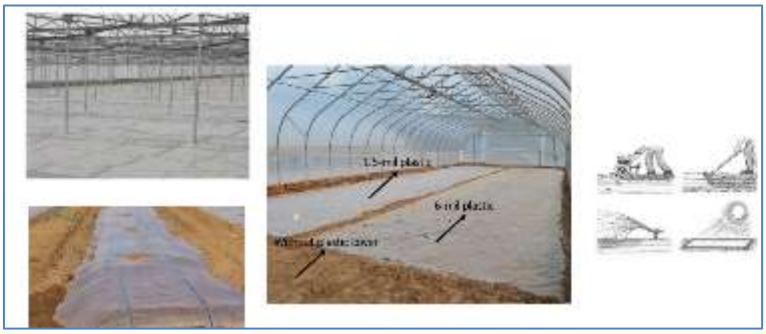

Figure 3. Farming practices as source of microplastics used in greenhouse vegetables production: Soil solarization – plastic film made of polypropylene or LDPE.

Materials and Methods

Thirty-six (36) soil samples were collected from olive and kiwi orchards, strawberry fields and vegetable grown fields, located in various Regional Units of Greece as can be seen in figure 4. Surface soils were collected from the top layer (0–20 cm depth) using a stainless-steel shovel, preventing sample contamination by plastics. A soil sample weighing approximately 2.0 kg was collected from three different sampling locations in each cultivated orchard/field. The soil samples were placed in covered aluminum containers and were transferred to the laboratory. After the samples were air-dried, they were sieved through a 5 mm stainless steel mesh. Furthermore, two (02) soil samples were collected from non-cultivated lands as blanks, using the same soil sampling procedure, to compare the results of microplastic items with those of the cultivated sites.

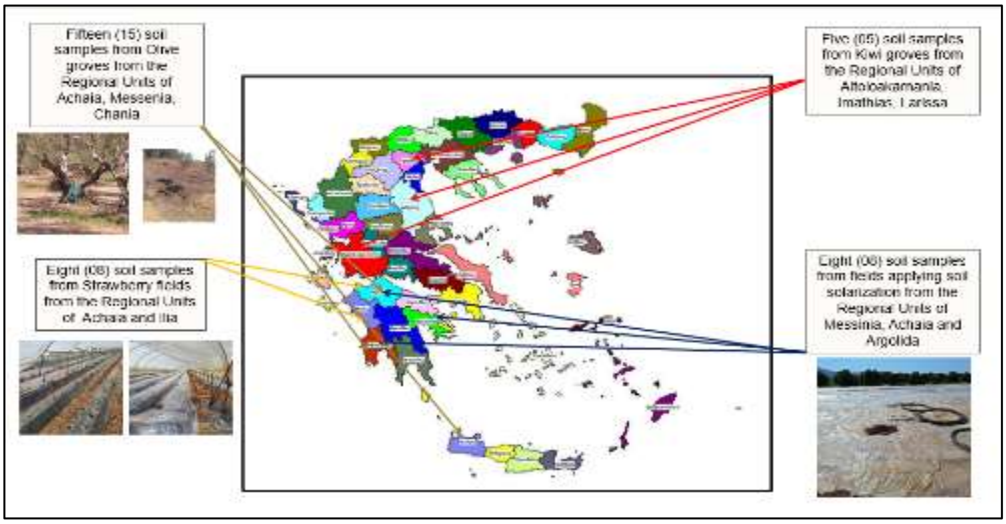

Figure 4. Map of Greece showing the various sampling sites.

For the separation of microplastics from the soil samples the density separation protocol, using NaCl, developed and described by Isari *et al, 2021* was used. A schematic representation of the MPs extraction procedure can be seen in figure 5.

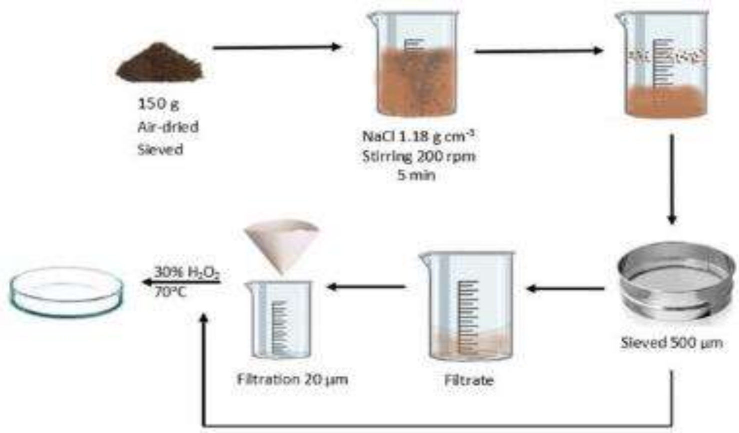

Figure 5. Schematic representation of the MPs extraction procedure by NaCl floatation (Isari *et al, 2021).*

The isolated floated items of the density extraction procedure are identified by Stereo microscope-MSL4000-Kruss, at 20x for the pre-screening of any observed particles that visually resemble microplastics. Identification of the origin and the type of the plastic material that was broken to microplastics was performed by attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) (Spectrum 100, PerkinElmer Inc., Shelton, CT, USA).

Results and Discussion

Despite the ubiquity of microplastics in agricultural soils used for food production, very few large-scale monitoring data from European soils are available. Most of the results describing microplastic abundance in soils refer to China and Eastern Asia. Preliminary results from our investigation showed that microplastic is a ubiquitous contaminant of farmland soils in the various regional units of Greece, posing a possible threat to human health and crop production. The concentrations of microplastics varied between the various land uses (Table 1).

Table 1. Microplastics (MPs) abundance in soil samples

Sampling site type	***MPs (items / kg)***
Vegetable growing fields (lettuce, broccoli, cabbage, cucumber, watermelons)	302 ± 145
Strawberries	353 ± 36

The accumulation of microplastics was higher in soils cultivated with strawberries and vegetables. Further assessment is needed for the quantification of MPs in olive and kiwi orchards.

Conclusion

To the best of our knowledge, research on the MPs contribution of farming practices of olive and kiwi orchards is very limited. Our study is currently in progress and more data are expected to enrich the scientific knowledge on microplastic pollution of agricultural soils in Greece in the next months.

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ENHANCING SOIL BULK DENSITY PREDICTION: A COMPARATIVE PRELIMINARY STUDY OF ML METHODS AND PEDOTRANSFER FUNCTIONS

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Abstract

Soil bulk density (BD) is a key indicator of soil health, influencing air and water availability through pore space. However, it is well known that direct field measurement can be labor-intensive and impractical. Pedotransfer functions (PTFs) that utilize readily measured soil properties offer an alternative approach. However, the accuracy of PTF-derived BD estimates can be hampered by uncertainty. This necessitates the development of more efficient and reliable BD measurement methods. The current study focuses on predicting BD in the areas of Kozani and Veroia, Greece, using Machine Learning (ML). Multiple soil samples, 85 for Kozani and 70 for Veroia, were collected in a single year (2023) and analyzed at the Soil & Water Resources Institute of ELGO-DIMITRA for bulk density, organic matter, silt, clay and sand. Two different ML models, Random Forests (RF) and Cubist (CB), were developed and assessed for their accuracy in predicting BD. Furthermore, a performance evaluation of well-known PTFs and ML methods was conducted. The preliminary results of this study suggest that ML methods may provide better accuracy than the PTFs. In Kozani, ML achieved improvements of 3.5% and 8.0% for RMSE and MAE compared to the average PTFs, while the overall fit (R^2) remained similar. Similar improvements were seen in Veroia, where ML surpassed PTFs by 10.2% and 7.1% for RMSE and MAE, respectively, with R^2 staying about the same. The study will be continued for a second year (2004) with new data, along with the exploration of more ML methods and pedotransfer functions.

Keywords: *soil data, bulk density, machine learning, pedotransfer functions.*

Introduction

Soil bulk density (BD) is a key indicator of soil health, significantly impacting air and water availability through its influence on pore space. Accurate BD measurement is essential for assessing soil quality, managing agricultural practices, and understanding ecological processes. However, direct field measurement of BD is often labor-intensive, time-consuming, and impractical, particularly for large-scale studies or challenging field conditions (Benites et al., 2007; Holmes et al., 2012; Quraishi & Mouazen, 2013). Pedotransfer functions (PTFs) provide an alternative by predicting BD from readily measurable soil properties such as organic matter content, soil texture (sand, silt, and clay), and other relevant factors. Although PTFs are widely used, their accuracy can be limited by site-specific conditions and the inherent uncertainty of the derived equations (Abdelbaki, 2018; De Vos et al., 2005; Kaur et al., 2002; Xu et al., 2015). This uncertainty highlights the need for more efficient and reliable methods for estimating BD. Recently, Machine Learning (ML) techniques have emerged as powerful tools for predicting complex soil properties, including BD. ML models can improve prediction accuracy by capturing non-linear relationships and interactions among soil properties that traditional PTFs may not adequately address (Alaboz et al., 2021; de Castro Moreira da Silva et al., 2023; Ramcharan et al., 2017; Xiao et al., 2022).

Material and methods

For the current study, soil samples were collected from two areas in Northern Greece: Kozani and Veroia. The specific Kozani area (Velvedos) is nestled near the shores of artificial Lake Polyfytos, boasts coordinates in the World Geodetic System of 1984 (WGS84) spanning approximately between latitudes 40°47′24.00″ N and 40°51′09.00″ N, and longitudes 21°41′24.00″ E and 21°45′39.00″ E. It has a flat terrain with an average elevation of 428 meters. The climate is temperate with cold winters and hot, dry summers. Average annual temperature is 10.8°C and precipitation is 700 mm. In contrast, Veroia, positioned in Central Macedonia, Greece, holds coordinates in the World Geodetic System of 1984 (WGS84) spanning approximately between latitudes 40°31′48.00″ N and 40°33′36.00″ N, and longitudes 22°11′24.00″ E and 22°15′36.00″ E. It has a more varied topography with hills and valleys. The climate is also temperate with milder winters compared to Kozani, but still experiences occasional freezing temperatures. Summers are warm with average temperatures around 14°C and 500 mm of precipitation.

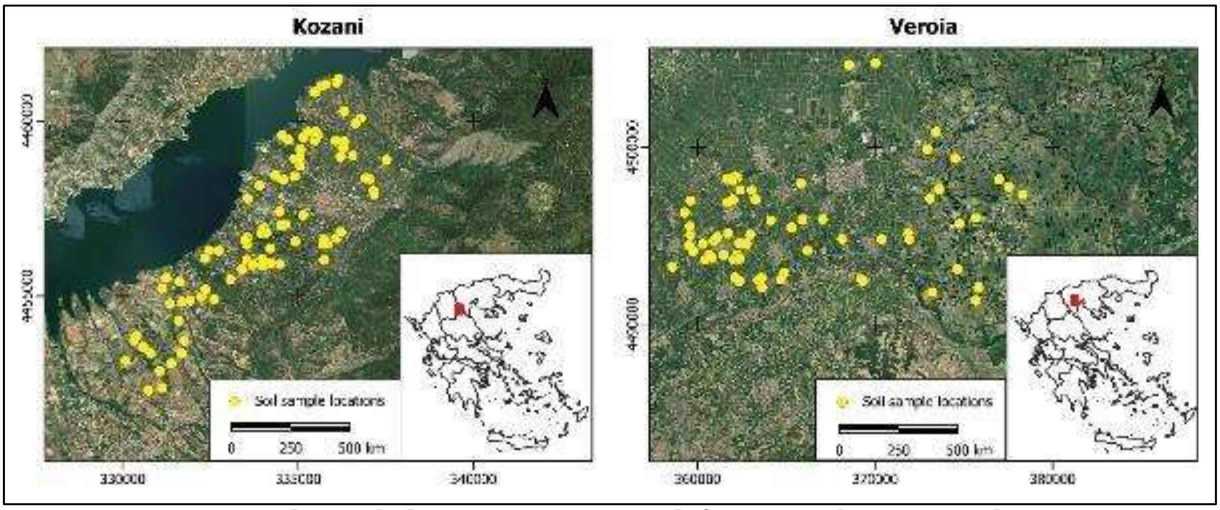

Figure 1. Soil sample locations in Kozani (left map) and Veroia (right map)

During the fall of 2023, a total of 155 soil samples were collected from randomly selected locations within the study areas. In Veroia, 70 samples were obtained, while Kozani contributed 85 samples and analyzed at the Soil & Water Resources Institute of ELGO-DIMITRA for bulk density, organic matter, silt, clay, and sand content. To ensure a representative sample of the top 30 cm of soil, composite samples from each field parcel were collected. These composites consisted of several subsamples taken throughout the 30 cm depth. Following collection, the samples were dried and analyzed at the Soil and Water Resources Institute laboratory in Thessaloniki, Greece. For spatial reference, the precise geographic coordinates of each sampling location were recorded using GPS devices.

Soil physical and organic properties were characterized for each sample. Particle size analysis using the hydrometer method determined the proportions of clay, silt, and sand, collectively defining soil texture. Organic Carbon (OC) content was quantified through Walkley-Black method. Undisturbed soil cores were collected from a depth of 10 to 20 centimeters. These cores were carefully extracted to maintain soil structure and minimize disturbance. Bulk density was subsequently determined on these undisturbed samples in the laboratory.

Table 4. Soil parameters measured in the study.

	Parameters	Category	Unit	Method analyses
1	Clay (C)	Soil	%	Particle size analysis with hydrometer
2	Silt (Si)	Soil	%	Particle size analysis with hydrometer
3	Sand (S)	Soil	%	Particle size analysis with hydrometer
4	Organic Carbon (OC)	Soil	%	Walkley-Black method
5	Bulk Density (BD)	Soil	g/cm^3	Intact soil samples using a core cylinder

RF and CB were selected as the ML algorithms for this study due to their proven performance in soil-related prediction tasks (Gunarathna et al., 2019; Nikou & Tziachris, 2022; Ramcharan et al., 2017; Xiao et al., 2022). In more detail, **RF** is an ensemble learning method that operates by constructing multiple decision trees during training. Each tree in the forest casts a vote for the most popular class (classification) or mean prediction (regression). The advantage of RF lies in its ability to handle high-dimensional data, reduce overfitting, and provide feature importance estimates. **The** CB is a rule-based model that combines decision trees with linear regression models. It creates a set of rules that partition the data into subsets, and then fits a linear regression model to each subset. CB offers a good balance between predictive accuracy and interpretability, as the generated rules can be examined to understand the underlying relationships between soil properties and BD. In both ML algorithms, the default hyperparameters were selected. PTFs establish empirical or statistical relationships between BD and other soil attributes such as particle size distribution, organic carbon content, and soil moisture. These functions are essential for bridging the gap between limited field data and the demands of large-scale soil modeling and environmental assessments. In this initial stage of the study, four PDFs were chosen from the literature since they are widely used and produce improved results (Abdelbaki, 2018; Post & Kwon, 2000; Rawls et al., 2004; Saxton & Rawls, 2006; Sevastas et al., 2018).

Table 5. Pedotransfer functions used in the study.

Authors	Abbrev.	Function
Abdelbaki (2018)	AB	$BD=1.449e^{-0.03OC}$
Post and Kwon (2000)	PK	$BD=100/[(OM/0.244)+((100-OM)/MBD)]$
Rawls et al. (2004)	R	$BD=1.36411+0.185628*(0.0845397+0.701658w-0.614038w^2-1.18871w^3+0.0991862y-0.301816wy-0.153337w^2y-0.0722421y^2+0.392736wy^2+0.0886315y^3-0.601301z+0.651673wz-1.37484w^2z+0.298823yz-0.192686wyz+0.0815752y2z-0.0450214z^2-0.179529wz^2-0.0797412yz^2+0.00942183z^3)$ $x=-1.2141+4.23123*(Sa/100)$ $y=-1.70126+7.55319*(Cl/100)$ $z=-1.55601+0.507094*OM$ $w=-0.0771892+0.256629x+0.256704x^2-0.140911x^3-0.0237361y-0.098737x^2y-0.140381y^2+0.0140902xy^2+0.0287001y^3$
Saxton and Rawls (2006)	SR	SWC-HPC Model

MBD = Mineral Bulk Density is set in 1.64 g/cm^3

These functions are using mainly Organic Carbon or Organic Matter, and soil texture as parameters to estimate BD.

Results and Discussion

To train and evaluate the models, each dataset was divided into two parts. The larger portion (80%) served as the training data, used to build the models. The remaining 20% became the testing data, used to assess the models' performance. We evaluated the accuracy of the different models by comparing the observed soil BD in the testing set with the models' predictions for the same data. This overall procedure was repeated 10 times with different training and testing dataset each time that were selected randomly.

Table 3. Comparison of Predictive Accuracy for BD using PTFs and ML in the study areas

Kozani

PTFs				**ML**			
Method	MAE	RMSE	Rsquared	Method	MAE	RMSE	Rsquared
AB	0.131	0.157	0.23	CB	0.099	0.129	0.24
PK	0.100	0.130	0.23	RF	0.096	0.129	0.30
R	0.099	0.128	0.27				
SR	0.092	0.121	0.34				
Average	**0.106**	**0.134**	**0.27**	**Average**	**0.097**	**0.129**	**0.27**

Veroia

PTFs				**ML**			
Method	MAE	RMSE	Rsquared	Method	MAE	RMSE	Rsquared
AB	0.087	0.108	0.52	CB	0.073	0.088	0.50
PK	0.078	0.099	0.52	RF	0.080	0.098	0.39
R	0.078	0.096	0.37				
SR	0.087	0.112	0.36				
Average	**0.082**	**0.104**	**0.44**	**Average**	**0.077**	**0.093**	**0.45**

As it is presented in the results based on the average results, ML appears to outperform PTFs for predicting soil BD both in the Kozani area and in Veroia area. In Kozani area while both methods have a similar explanatory power (R^2 of 0.27), ML achieves slightly lower Root Mean Squared Error (0.129 vs 0.134) and Mean Absolute Error (0.097 vs 0.106), indicating more accurate predictions. Similarly, in the Veroia area, ML superior performance in predicting soil BD compared to PTFs. While their explanatory power is similar, ML yields more accurate results with a lower RMSE (0.093 vs 0.104) and MAE (0.077 vs 0.082).

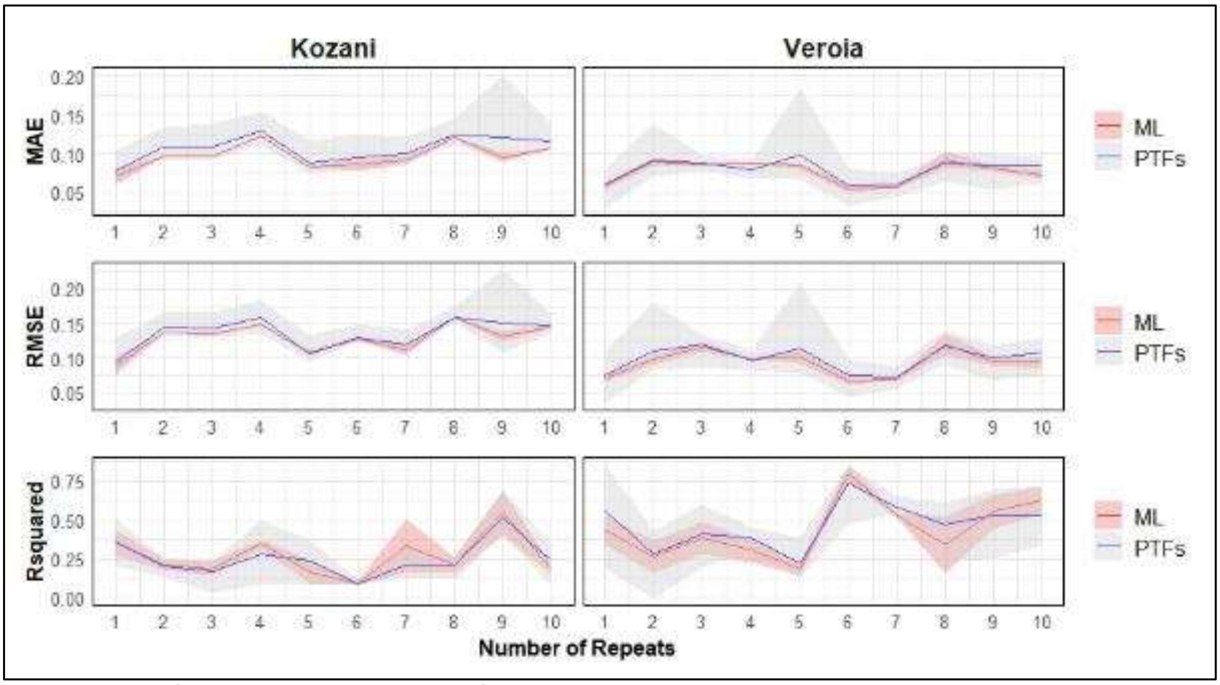

Figure 2. Performance comparison of ML and PTFs methods in predicting Soil Bulk Density (BD) for Kozani and Veroia areas

Finally, across all metrics, the Veroia area consistently achieved higher overall accuracy compared to the Kozani area. This difference will be further investigated when new data from 2004 is collected and incorporated into the dataset, potentially providing more insights into these spatial variations

In conclusion, the results indicate that machine learning (ML) methods are more accurate in predicting soil bulk density (BD) than traditional pedotransfer functions (PTFs) in both the Kozani and Veroia areas. In Kozani, ML models showed improvements of 3.5% in root mean squared error (RMSE) and 8.0% in mean absolute error (MAE) compared to the average PTFs, while maintaining a similar overall fit (R^2). Similar improvements were observed in Veroia, where ML models outperformed PTFs by 10.2% in RMSE and 7.1% in MAE, with R^2 remaining consistent.

Conclusions

This study demonstrates the potential of ML models in accurately predicting soil bulk density, a critical parameter for assessing soil health. Traditional methods, such as PTFs, while offering a convenient alternative to direct measurements, often exhibit limitations in precision. Our findings indicate that ML models, specifically RF and CB, outperform PTFs in estimating BD for the study areas of Kozani and Veroia, Greece.

The observed improvements in RMSE and MAE metrics highlight the superior predictive capabilities of ML in capturing the complex relationships between BD and soil properties. While the overall fit (R^2) remained comparable between ML and PTFs, the enhanced accuracy in error terms suggests that ML models offer a more reliable approach for BD estimation.

This study represents a preliminary investigation based on data collected in a single year (2023). To enhance the model's robustness and generalizability, we are currently expanding the dataset by collecting samples for 2024. Additionally, exploring a wider range of ML algorithms and PTFs, as well as incorporating spatial information and other relevant soil properties, will be essential for developing more accurate and precise BD prediction models.

The successful application of ML in predicting BD underscores the potential of data-driven approaches in soil science. These findings contribute to the development of efficient and accurate methods for assessing soil health and supporting sustainable land management practices.

Acknowledgement

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PRODUCTION TECHNOLOGY OF AN INNOVATIVE SOIL FERTILITY IMPROVEMENT MEANS

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Abstract

In addition to numerous factors, organic waste has a significant impact on increasing environmental pollution, which is an imperative for its solution. The goal of the work was to use the by-products of biogas production plants and cogeneration plants for the purpose of soil fertilization and fertilization, it allows to create a new innovative product from their mixtures. The preparation of the best mixtures applyed and evaluated in greenhouses under production conditions was carried out at the companies JSC "Ziedi JP" and "Pampali". The production scheme of soil liming and fertilizer was developed and approved at both companies. The effectiveness of the composition of the mixtures was first tested on soils of different acidity in a greenhouse. The set of machines and aggregates required for the preparation and spreading of the new type of fertilizer on the field was made. Digestate, after complete development in bioreactors, is fed to the mechanical screw press separator, where it is divided into solid (dry matter 25 %<) and liquid (dry matter 3%>) fraction. The digestate of solid fractions is mixed with wood ash in portions in a screw-type mixer equipped with electronic scales. The ingredients are poured in parts so that the mixer mixes a uniform mass. After mixing, the new fertilizer is discharged from the mixer onto a conveyor belt, and then into a pile, which is covered with a cover to reduce ammonium emissions. The use of the innovative soil fertility enhancer can be an effective way of recycling both products, and can also be an environmentally friendly alternative to mineral fertilizers.

Key words: *digestate, wood ash, mixtures.*

Introduction

Anaerobic digestion and biogas production plants are considered the center of the circular economy, where anthropogenic organic residues previously considered as waste can be converted into energy, organic fertilizers and other value-added components and materials. (Adekunle, et al., 2019)
The post-fermentation residue is called digestate and spreading the digestate on fields is a common practice in agricultural enterprises. Digestate spreading norms in nitrate-sensitive areas are limited to 170 kg N ha^{-1} per year. (Commission of the European Communities, 1991) Phosphorus spreading norms are not directly included in the Nitrates Directive, but many European countries have different phosphate spreading limits. Depending on the species of cultivated plants, the amount of phosphorus in the soil, and other variable factors, its spread is in the range of 0 - 250 kg P_2O_5 ha^{-1} per year. (Amery & Schoumans, 2014) If there are already enough nutrients in the soil, but the amount of digestate is more than necessary, it may be necessary to transport the digestate to further fields. Long transport distances justify economic investments in mechanical separation of the digestate. During separation, the digestate is divided into solid and liquid fractions. Sometimes the liquid fraction still contains high solids (up to 10% dry matter) and the solid fraction still has high moisture (15-45% dry matter). (Guilayn, 2018) Dividing the digestate into fractions allows to reduce the moisture content in the solid fraction, thereby reducing the costs of transporting and storing the solid

fraction. The liquid fraction is easy to pump and can be easily incorporated directly into the soil in the fields, thus significantly reducing nitrogen losses. (Fuchs & Drosg, 2013)
In the mechanical separation of digestate, nitrogen usually remains more in the liquid fraction, while phosphorus and potassium remain in the solid fraction. This leads to better nutrient management. (Möller & Müller, 2012)
Burning woody biomass for energy is of interest to many countries as they want to reduce their consumption and dependence on fossil fuels. (Perkiomaki & Fritze, 2005) Wood-fired cogeneration plants and other biomass-fired boiler houses generate more and more ash. Ash in wood cogeneration plants is a by-product. (Silva, 2018) Wood ash consists of inorganic compounds from burned biomass, sand and very small, incompletely burned organic parts. (Ingerslev, 2011) As a by-product of burning wood, phosphorus (P), potassium (K) is retained in the ash, but most of the nitrogen (N) is lost during combustion in the form of NO_x compounds, while the remaining N is strongly associated with organic, unburned residues and is in a non-absorbable form for microorganisms. During the combustion of biomass, various oxides are formed and the subsequent aeration leads to the formation of carbonates in the wood ash, making the ash very alkaline with a pH of 8 to 13. (Augusto, 2008) If the ash is not disposed of in landfills, but processed into plant fertilizers, all the ash is returned to the soil existing nutrients, and the pH of the soil is increased. (Pittman, 2006)
The aim of this research was (i) to create a technology to prepare an innovative fertilizer using two production by-products, biogas post-fermentation digestate and biomass cogeneration ash, (ii) efficiency of novel mixture fertilizer creation by available agricultural tools on the experimental farm

Material and Methods

The initial stage of the research is to check the chemical composition of the digestate and wood ash of different origins. Wood ash was mixed with digestate according to certain proportions, which are based on laboratory studies guided by the chemical composition of the raw materials.
The preparation of the best mixtures applyed and evaluated in greenhouses under production conditions was carried out at the companies JSC "Ziedi JP" and "Pampali" in Latvia. The production scheme of soil liming and fertilizer was developed and approved at both companies. The effectiveness of the composition of the mixtures was first tested on soils of different acidity in a greenhouse, using fast-growing plant species lettuce and cucumber. The set of machines and aggregates required for the preparation and spreading of the new type of fertilizer on the field was made.
Biogas post-fermentation digestate from cattle dung obtained by JSC "Ziedi JP" and "Pampali" was used for the experiments. Digestates were separated into solid and liquid fractions before preparing the new fertilizer mixtures. Wood ash from the cogeneration stations of LLC "Gren Jelgava" and LLC "Dobeles Eko" was used for the experiments analyses. The characteristics of solid fractions of separated digestates and wood ash are presented in Table 1.

Table 1. The research used the composition of digestate and wood ash

Indicators	Cattle manure digestate		Wood ash	
	From JCS “Ziedi JP”	From JCS “Pampali’	From JCS “Ziedi JP”	From JCS “Pampali’
Moisture, %	74.3	76.7	0.16	0.14
Total nitrogen, %	0.57	0.48	0.00	0.00
Total phosphor, %	0.46	0.53	3.5	3.46
Total potassium, %	1.24	1.47	11.6	8.52
pH	9.05	9.03	13.9	13.5

The content of macronutrients and heavy metals was also tested using standard methods and analyzed. The macronutrient content of the soil was also tested The tests were carried out before and after the use of the digestate. The analyzes were carried out by the Latvia University of Life Sciences and Technologies Biotechnology Scientific Laboratory (LBTU BZL).

Results and Discussion

So far, only a few studies have been conducted to mix biogas digestate with wood ash in a specific ratio. To prepare the new fertilizer from biogas digestate and wood ash, the equipment available on farms was used. After the correct technology, the number of days the digestate is kept in fermenters is long enough so that the digestate is fully developed and, leaving the post-fermentation, biogas is no longer released or comes close to it. An innovative technological scheme for the preparation of digestate and wood ash mixtures is shown in Figure 1.

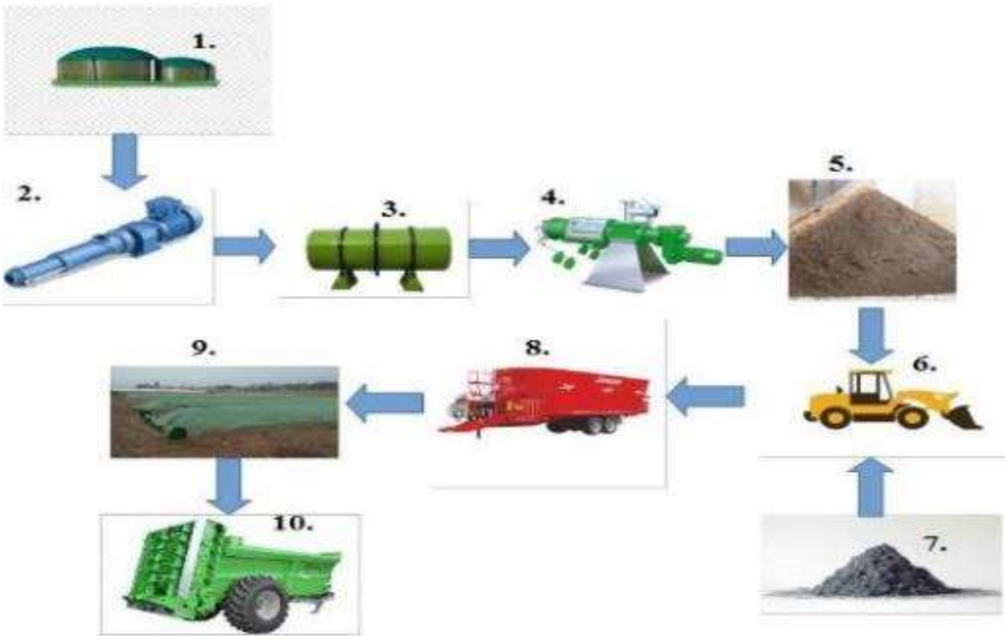

Figure 1. Scheme of the new fertilizer preparation technology

1. Biogas fermenters; 2. Digestate pump; 3. Digestate intermediate storage; 4. Separation of digestate into fractions; 5. Digestate solid fraction storage; 6. Digestate and ash loading; 7. Ash storage; 8. Mixing machine Trioliet with scales and tractor; 9. Application of mixed fertilizer in stirring and covering; 10. Spreading the new fertilizer on the field.

The post-fermentation digestate, which has a dry matter content of up to 7.5%, is pumped with a VANGEN screw-type pump. Pumping takes place through a 150 mm pipeline for optimal digestate flow. The digestate is pumped to the 10m^3 intermediate storage of the digestate separator. The buffer acts as a buffer to ensure a steady and continuous supply of digestate to the separator, as well as to prevent the siphon effect. The liquid manure separator EYS SP600 operates in continuous operation mode, as it is equipped with a liquid digestate intermediate storage in a volume of 10 m^3, which is always in level.
A single-stage sieve with a hole’s size of 0.75 mm was used in the separator. After separation, the liquid fraction of the digestate, in order not to consume additional energy, flows to the liquid digestate storage. In the liquid fraction, the dry matter content remains within 2%, because they are particles with a size smaller than 0.75 mm and the separator sieve cannot retain them. The solid fraction of the digestate from the separator falls into a warehouse created under the separator room, so that the digestate is sheltered and easily collected. This is necessary so that in the event of precipitation, another fraction of the digestate does not change the dry matter content, which after separation is 25.7%.
After separation, the solid fraction of the digestate was loaded into the Trioliet mixing machine with the bucket of the front loader. The mixing machine is equipped with electronic scales so that it is possible to observe the proportions of the mixture. The Trioliet mixing machine used is equipped with 3 augers, placed 1 meter apart from each other, which ensure uniform mixing of the ingredients throughout the volume. It is possible to prepare 8 t of mixture in each mixing portion of the new fertilizer. The agitator is driven by gimbal transmission from the tractor PTO. A 130 PS John Deere 6430 is used to drive the PTO. Digestate with wood ash should be added to the mixer alternately to make the mixture faster. After filling the entire preparation dose of fertilizer into the mixer, the mixing process takes place for 15 minutes. The operator carefully watches the process to ensure that the ingredients are thoroughly mixed. During the mixing process, the operator uses personal protective equipment to protect himself from physico-chemical damage.
After mixing, the new fertilizer is discharged from the mixer into the stirrups with the help of an integrated conveyor. The height and width of the stirrups are designed in such a way that the maximum bevel angle is formed. The stirrups are quickly covered with a gas-tight cover to delay ammonia emissions. The pH of the new fertilizer is 11.5, so nitrogen release is rapid. Due to the high pH, the resulting mixture neutralizes the microorganisms present in the digestate. Without a long wait, the mixed mixture is loaded into the JOSKIN manure spreader with the front loader. The spreader is equipped with accurate dosing of fertilizer from the tractor cab and also has its own scales. Spreading width is 24m (Figure 2).

Figure 2. Spreading of digestate and wood ash mixture

To prevent the fertilizer from compacting after transportation to the trial fields, the spreader has 2 auger conveyors and a conveyor belt. In the test fields, the new fertilizer was spread in rates of 15 t^{-ha} and 30 t^{-ha}.

Conclusions

Wood ash is a by-product from biomass cogeneration plants and boiler houses, while digestate is a by-product from biogas plants. Together, this is a valuable mixture of nutrients that add value to production by-products. Wood ash is produced by burning wood, while digestate is produced in the biogas fermentation process. The choice of such fertilizer raw materials is based on solving the problems related to reducing waste accumulation and more efficient use of existing resources. As a result, the benefit is not only from the environmental aspect, but also from the operation of farms in the circular circulation economy, or bringing them closer to working in accordance with the environment. The rational use of such by-products was realized by the application of the used technology

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GREEN LOGISTICS PRINCIPLES AND PRACTICES IN LOGISTICS COMPANIES IN LATVIA

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Abstract

Climate change is a major environmental problem, and the impacts of logistics on global atmospheric conditions have become an important research topic to identify the *impacts and efficiency of transport*. *Green logistics* principles are integrated into logistics systems and supply chains from raw material processing and extraction, production, packaging, transportation and storage to distribution to the final consumer and reverse logistics. A challenge for logistics managers is understanding, identifying and incorporating green logistics principles into their daily decision-making. This reasoning determined the *aim of the research*: to examine green logistics principles and the practices of implementing them by logistics companies in Latvia. To achieve this aim, a focus group survey and in-depth interviews were conducted with logistics companies in Latvia. *The survey results* showed that 84% of the companies surveyed had introduced and were implementing some green logistics practices in their operations, while 16% did not implement any. However, an in-depth assessment of the companies that had implemented a green logistics practice was performed through a recognition exercise. The most popular example of green logistics practice was route optimization, which meant finding the most efficient routes to move goods or services.

Keywords: *green logistics, supply chains, sustainability.*

Introduction

Several global strategic documents (Rio Declaration on Environment and Development, 1992; Agenda 21, 1992), which focus on the concept of sustainable development and include a global action plan outlining the requirements and mechanisms for practically implementing the assumptions of the concept, emphasize innovation and the use of technologies that have as little impacts on the environment as possible. However, in important analytical and strategic documents (OECD, 2021), road transport is referred to as one of the contributors to climate change and a significant source of carbon (CO_2) emissions. The dominant position of road transport concerning climate change is due to its dense logistics network oriented towards fast delivery and route flexibility. Climate change and carbon emissions are among the main environmental problems, and according to several scientists (McKinnon et al., 2015; Jayarathna et al., 2023; Fahimnia et al., 2015) the logistics impacts on global atmospheric conditions and sustainability have become the main research topic. At the same time, the European Commission released the document “Sustainable and Smart Mobility Strategy” (Sustainable and Smart Mobility Strategy, 2020) that stipulates that by 2030, at least 30 million zero-emission cars and 80000 zero-emission trucks drive on European roads, 100 European cities become climate-neutral, high-speed rail traffic doubles, regular collective journeys of up to 500 km within the EU should become carbon neutral, as well as automated mobility is available on a large scale. By 2050, however, almost all cars, vans, buses as well as new heavy-duty vehicles are expected to be emission-free, rail freight transport doubles, high-speed rail traffic triples, the multimodal Trans-European Transport Network (TEN-T) is comprehensive and equipped for sustainable and smart transport with high-speed

connectivity. To achieve these goals, the European Commission is committed to stimulating the demand for zero-emission vehicles through, for example, carbon pricing, taxation, road user charges and amendments to regulations governing the weights and dimensions of heavy-duty vehicles.
All the above-mentioned considerations show that the role of green logistics continues to increase and encourage all actors in the logistics system and supply chains to consider the impacts their activities have on the environment. The main goal of green logistics is to coordinate all activities in a way that the supply chains operate in the most efficient way, thereby minimizing the negative impact on the environment. However, the overall increase in public environmental awareness and the adoption of legal acts and global strategies concerning the management of environmental issues shape corporate social responsibility. Such reasoning determined the **aim of the research**: to examine green logistics principles and the practices of implementing them by logistics companies in Latvia. The research set the following specific tasks: 1) to summarise the green logistics principles and the reasoning behind the practices of implementing them in connection with the sustainable development goals; 2) to identify current green logistics practices by surveying logistics companies.

Materials and methods

The theoretical part is based on an analysis of strategic documents, plans and agendas and scientific findings on green logistics development by applying the monographic, induction and deduction methods. Statistical analysis and synthesis, comparison and surveying were employed to examine the logistics principles and practices applied and identify company opinions. To examine the logistics principles and practices, the research surveyed companies engaged in logistics representing their core economic activity, thus making a representative sample. The enterprises selected for the survey were those whose main activity, according to the classification of economic activities (NACE), is logistics. There are a total of 31 such enterprises, of which 84 % provided information and 16 % did not provide information about green logistics principles.

Results and discussion

Reasoning behind implementing green logistics in connection with the sustainable development goals

In scientific and practical discussions, some authors (Wu and Dunn, 1995; Rodrigue et al., 2001; Larsen-Skjott et al., 2007) suggest various assumptions and principles for the implementation of green logistics: 1) green logistics is oriented towards the principles of environmental awareness and sustainability; 2) green logistics is mainly focused on creating an environmentally friendly and efficient transport and distribution system; 3) green logistics represents principles integrated into logistics systems and supply chains that take care of nature and involve the logistics process from raw material acquisition and handling, production, packaging, transport and storage to distribution to the end consumer and reverse logistics. Therefore, we can state that green logistics represents several important aspects: economic, environmental and social, and we can define and determine the role of green logistics by associating these aspects with the sustainable development goals (Sustainable Development Goals, 2015).

Source: authors' construction based on Jedliński, 2014; Sustainable Development Goals, 2015.

Fig.1. Most important aspects of green logistics associated with the sustainable development goals

Based on the connections between green logistics and the sustainable development goals (Figure 1), one can identify the role of green logistics in achieving each sustainable development goal:

- ✓ zero hunger (SDG 2) – sustainable food supply chains, the creation and maintenance thereof following the green logistics principles;
- ✓ affordable and clean energy (SDG 7) – complying with the principles of energy efficiency and building cooperation for the efficiency of logistics chains and the development of green logistics infrastructure;
- ✓ decent work and economic growth (SDG 8) – green logistics contributes to sustainable economic growth based on introducing innovations and increasing economic productivity;
- ✓ industry, innovation and infrastructure (SDG 9) – green logistics is promoted to develop a sustainable and resilient transport infrastructure based on the diversification of logistics supply chains;
- ✓ sustainable cities and communities (SDG 11) – creating sustainable green logistics transport systems for supply chains to make a balanced impact of urbanization on supply chain intensity;
- ✓ responsible consumption and production (SDG 12) – green logistics is based on sustainable consumption and production patterns, which involves not only a sustainable supply chain but also less production and processing waste, with one of the solutions being the introduction of reverse logistics;
- ✓ partnerships for the goals (SDG 17) – actions and measures aimed at enhancing green logistics coordination and fostering sustainable development across borders at all stages of supply chains.

According to some research studies (Chin et al., 2015; Zhu & Sarkis, 2007), green logistics involves not only integrating sustainability principles into logistics operations to reduce the negative environmental impact of the movement of goods and information flows through the supply chain; an important role is also played by the development and coordination of green thinking at all stages of the supply chain. After summarizing the research studies by several authors, the following definition of green logistics could be created to be used at the next stage of the present research on the principles and practices of green logistics. **Green logistics** *represents the coordination of activities throughout the logistics supply chain to achieve the sustainable development goals by considering the environmental, economic and social aspects*.

In addition to the sustainable development goals, the authors emphasize other important trends that shape an understanding of the role of green logistics. The trends are as follows: an overall increase in public awareness of the need for environmental conservation and sustainability; legal acts governing transport pollution reduction; international standards for environmental pollution management; and corporate social responsibility.

Practices of implementing green logistics principles by logistics companies in Latvia

To examine the logistics principles and practices applied and identify company opinions about them, a sample of companies was selected – only the companies engaged in logistics representing their core economic activity and specialized in supplying logistics services to certain industries were included therein. Of the companies selected (n=31), 11% represented the transport and storage industry; 8% – trade (both wholesale and retail); 4% – agriculture; 4% – goods production; 3% manufacturing and 1% – woodworking. According to the survey data, 84% of the companies surveyed had introduced some of the green logistics practices in their business operations, while 16% did not implement any.

Next, only the companies that implemented any of the green logistics practices participated in the survey for an in-depth examination.

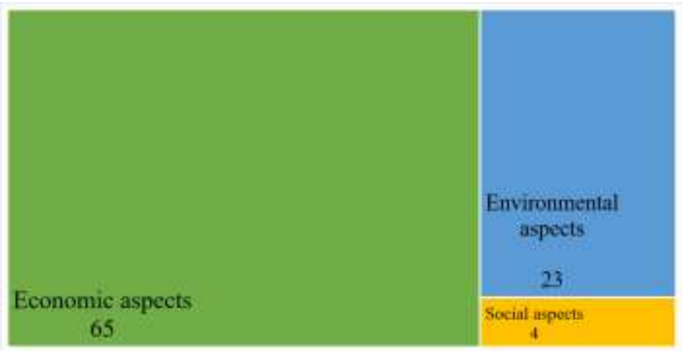
First of all, the survey identified opinions about the green logistics practices that had been in place at the companies, and the authors associated them with the sustainable development goals, see the results in Table 1.

Table 1. Green logistics practices associated with the sustainable development goals implemented by companies in Latvia

Green logistics practices	Replies, %	Achievement of a sustainable development goal	Aspects of green logistics impacts
Route optimization	19	Industry, innovation and infrastructure (SDG 9)	Economic aspects
Cargo optimization	15	Industry, innovation and infrastructure (SDG 9)	Economic aspects
Waste sorting and recycling	15	Responsible consumption and production (SDG 12)	Economic aspects
Development of a warehouse strategy considering a sustainable transport flow	9	Sustainable cities and communities (SDG 11)	Environmental aspects
Use of renewable energy in the logistics chain	8	Affordable and clean energy (SDG 7)	Environmental aspects
Use of environmentally friendly transport	6	Affordable and clean energy (SDG 7)	Environmental aspects
Compliance with the zero-waste principles	6	Responsible consumption and production (SDG 12)	Economic aspects
Implementation of the principles of reverse logistics	4	Responsible consumption and production (SDG 12)	Economic aspects
Intermodal transport for green logistics	3	Partnerships for the goals (SDG 17)	Social aspects
Sharing information on environmental objectives	1	Partnerships for the goals (SDG 17)	Social aspects
Green logistics principles are part of a company strategy	6	Decent work and economic growth (SDG 8)	Economic aspects

Source: authors' construction based on the survey results.

As shown in Table 1, 34% of the logistics companies optimized their routes and loads to follow the green logistics principles. However, 25% followed the principles of waste recycling, reverse logistics and zero waste in their business. It is positive that 19% of the companies understood the role of achieving the sustainable development goals and sought to achieve them in practice. After analysing the replies given by the companies concerning the sustainable development goals, one could conclude that 34% of the companies met the requirements of SDG 9 regarding sustainable and resilient transport infrastructure. Of the total, 25% met the requirements of SDG 12 regarding the implementation of sustainable consumption and production patterns, including the reduction of production and processing waste; 14% met the requirements of SDG 7 regarding compliance with the principles of energy efficiency for green logistics infrastructure. Relatively few, only 6% of the companies, could meet the requirements of SDG 8 contributing to sustainable economic growth through green logistics. Such a small number of replies could be due to the fact that the introduction and reorganization of green logistics require additional investments, which often create additional problems for companies concerning borrowing funds, setting the prices of logistics services and competing in the global logistics market.



Source: authors’ construction based on the survey results.

Fig.2. Distribution of company replies concerning the aspects of green logistics principles and practices, %

As shown in Figure 2, 65% of the companies applied green logistics practices to meet the sustainable development goals and focus on economic aspects, 23% tackled environmental aspects, while a relatively small number of companies, accounting for only 4%, introduced solutions related to social aspects.

Next, the researchers conducted an in-depth survey to identify activities that the companies were still preparing to perform in order to develop green logistics or to reorganize their supply chains following the green logistics principles; the activities were ranked according to the number of replies:

1. Restructuring the transport for the transition to an environmentally friendly transport fleet;
2. Introducing innovations and environmentally friendly technologies;
3. Using renewable sources for electricity generation in auxiliary processes and the supply chain;
4. Choosing cooperation partners that provide deliveries to customers with environmentally friendly transport;
5. The company plans to communicate with suppliers to further optimize the routes and use recyclable packaging;
6. Introducing reverse logistics practice;
7. Implementing an environmental policy by the company;
8. Distribution based on green orders and deliveries.

Overall, the survey participants emphasized that green logistics focused on responsible business, which could create trust in the company and support from customers and the public, while the introduction of green logistics requires additional investments that would lead to sustainable business.

Conclusions

The main goal of green logistics is to coordinate all activities in a way that the supply chains operate in the most efficient way, thereby minimizing the negative impact on the environment. The global sustainable development goals and their principles and operational aspects could be employed to introduce green logistics and assess the performance thereof.
The survey data revealed that 84% of the companies had introduced some of the green logistics practices in their business operations, while 16% did not implement any.
The survey revealed that 34% of the logistics companies optimized their routes and loads to follow the green logistics principles. However, 25% followed the principles of waste recycling, reverse logistics and zero waste in their business. It is positive that 19% of the companies understood the role of achieving the sustainable development goals and sought to achieve them in practice. Of the total companies, 65% applied green logistics practices to meet the sustainable development goals and focus on economic aspects, 23% tackled environmental aspects, while a relatively small number of companies, accounting for only 4%, introduced solutions related to social aspects.

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TOWARDS SUSTAINABLE CONSUMPTION: INSIGHTS FROM LATVIAN WASTE MANAGEMENT PRACTICES

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Abstract

Europe is grappling with resource limitations and environmental concerns, urging a transition towards waste minimization, reducing carbon footprints, and advocating for recycling. This shift towards sustainable practices aligns with the regional vision for a more environmentally conscious and resilient future. The focus of this study revolves around understanding the current behaviours and attitudes of Latvian residents, particularly in their food purchasing habits and resulting waste generation. Consequently, the aim of this study is to uncover the factors influencing consumer behaviour while promoting sustainable consumption and waste management practices in Latvia, with a specific emphasis on reducing organic food waste in Latvian households and encouraging waste sorting. To achieve these goals, the authors have analysed the current landscape of waste generation and analysed the EU waste treatment strategies and goals, supplemented by illustrative case studies. The research methodology encompasses a blend of survey and graphical interpretation of secondary data as well as analysis and synthesis methods for formulation of findings, conclusions and recommendations. These approaches provide a nuanced understanding of selected target groups of the survey, their preferences, behaviours, and prevailing attitudes towards waste management. The evaluation of waste generation in Latvia with a focus on biological and food waste aims to understand the unique challenges and opportunities within the Latvian context. The research findings highlight the importance of tailored interventions to promote sustainable consumption and waste sorting, considering negative side-effects, namely, greenwashing practices. Recommendations are provided for utilizing different marketing tools that resonate with specific segments of the Latvian population. By discerning the complex factors influencing consumer behaviour, stakeholders can develop effective strategies to cultivate a culture of sustainability and waste reduction in Latvia. This study provides new insights into the discourse on environmental sustainability in Latvia, offering practical guidance for policymakers, businesses, and communities as they strive towards a more environmentally friendly future.

Keywords: *sustainability; food waste; zero-waste; marketing communication.*

Introduction

Household consumption within the European Union significantly impacts the environment, often surpassing planetary boundaries. Approximately two-thirds of EU consumers acknowledge the detrimental environmental effects of their consumption habits. The most frequently suggested solution is altering consumption habits and production patterns. Nonetheless, research reveals a discrepancy between consumers' positive intentions and their actual behaviours. This discrepancy arises because consumers consider various factors besides sustainability when making purchasing decisions, such as price, availability, convenience, habits, values, social norms, peer pressure, emotional appeal, and the perceived impact of their choices. Additionally, consumption patterns serve as a means for individuals to express their identity both to themselves and others. Studies indicate that the environmental impacts of consumption are predominantly influenced by individuals' income levels.

The European Union has implemented several policies to encourage sustainable consumer choices. These policies include environmental product requirements, information and labelling standards, product guarantee regulations, climate legislation that incorporates CO2 emissions costs into production, and waste legislation that facilitates recycling. The European Commission is planning to introduce a legislative initiative aimed at empowering consumers for the green transition. The European Parliament has consistently supported efforts to enhance the sustainability of consumption in the EU. Recently, it has advocated for measures to ensure that consumers receive transparent, comparable, and standardized product information, particularly regarding product durability, reparability, and environmental footprint. According to Stankevich (2017), consumer behaviour in the context of marketing is a continuously researched topic, as understanding consumer reactions enables companies to enhance their marketing campaigns, making them more effective and achieving their objectives. The current challenge lies in influencing consumers in a manner that prompts them to purchase a specific product or undertake a desired action.

The responsiveness and willingness of consumers to involve themselves in waste minimization significantly vary among European countries. This is evident from several studies and surveys that highlight the differences in consumer attitudes and behaviours towards plastic waste and sustainability across various countries. A recent GfK study conducted in 25 countries, in collaboration with Europanel and Kantar, reveals that over 53 percent of European consumers identify plastic waste as one of their top three environmental concerns, followed by climate change at 44 percent. When asked about their single most pressing issue, 23 percent cited plastic waste compared to 15 percent globally. The highest levels of concern were observed in Slovakia, the Netherlands, and Germany. Although plastic waste is a prominent concern in Europe, it is closely linked to the broader issue of climate change, with an equal proportion of Europeans (23 percent) identifying it as their primary worry. Significant variations exist at the national level. In Hungary (31 percent) and Sweden (29 percent), climate change is a more significant concern than plastic waste. Conversely, in Russia, Slovakia, and the Czech Republic, plastic waste is perceived as a more substantial issue. Specifically, 17 percent of Russians, 20 percent of Czechs, and 27 percent of Slovaks regard plastic waste as their major environmental concern (Concerns about plastic…, 2019).

In Latvia, unsorted household waste constitutes the largest proportion of refuse generated (Central Statistical Bureau of Latvia, 2023). Consumer preferences and attitudes significantly influence the amount of waste produced at the household level (Nguyen and Johnson, 2020). Identifying the factors that affect these behaviours could enable policymakers to guide Latvian residents towards sustainable consumption. Moreover, research has established connections between consumption patterns and food waste generation (van der Werf and Gilliland, 2017). Adhering to the principles of the 3Rs of waste minimization—reduce, reuse, recycle—could lead to measurable improvements in Latvia's environmental footprint.

The aim of this article was to determine the deciding factors to minimise organic food waste within Latvian households and promote waste sorting behaviour.

Material and methods

The current article continues previous authors’ research direction dedicated to exploring of food industry enterprises and their motivation to involve is organic waste management (Grinberga-Zalite & Zvirbule, 2022; Grinberga et al., 2022). The authors have posed two tasks to reach the aim: 1) to evaluate the current state of waste generation in Latvia, with a specific emphasis on biological and food waste; 2) to analyse and synthesize data from customer survey to understand the preferences and behaviours of current target groups, and to develop incentives tailored to Latvia context. The research employed the following methods: systematic literature review, based on the latest Scopus database relevant publications and international waste management organizations’ publications; graphical methods; analysis and synthesis methods; survey method with open and closed type questions (n=93); deduction method for summarization of the overall research findings and logical construction of the conclusions and further recommendations. (Figure 1).

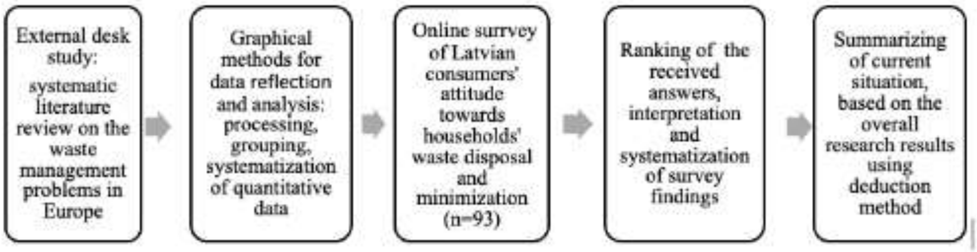
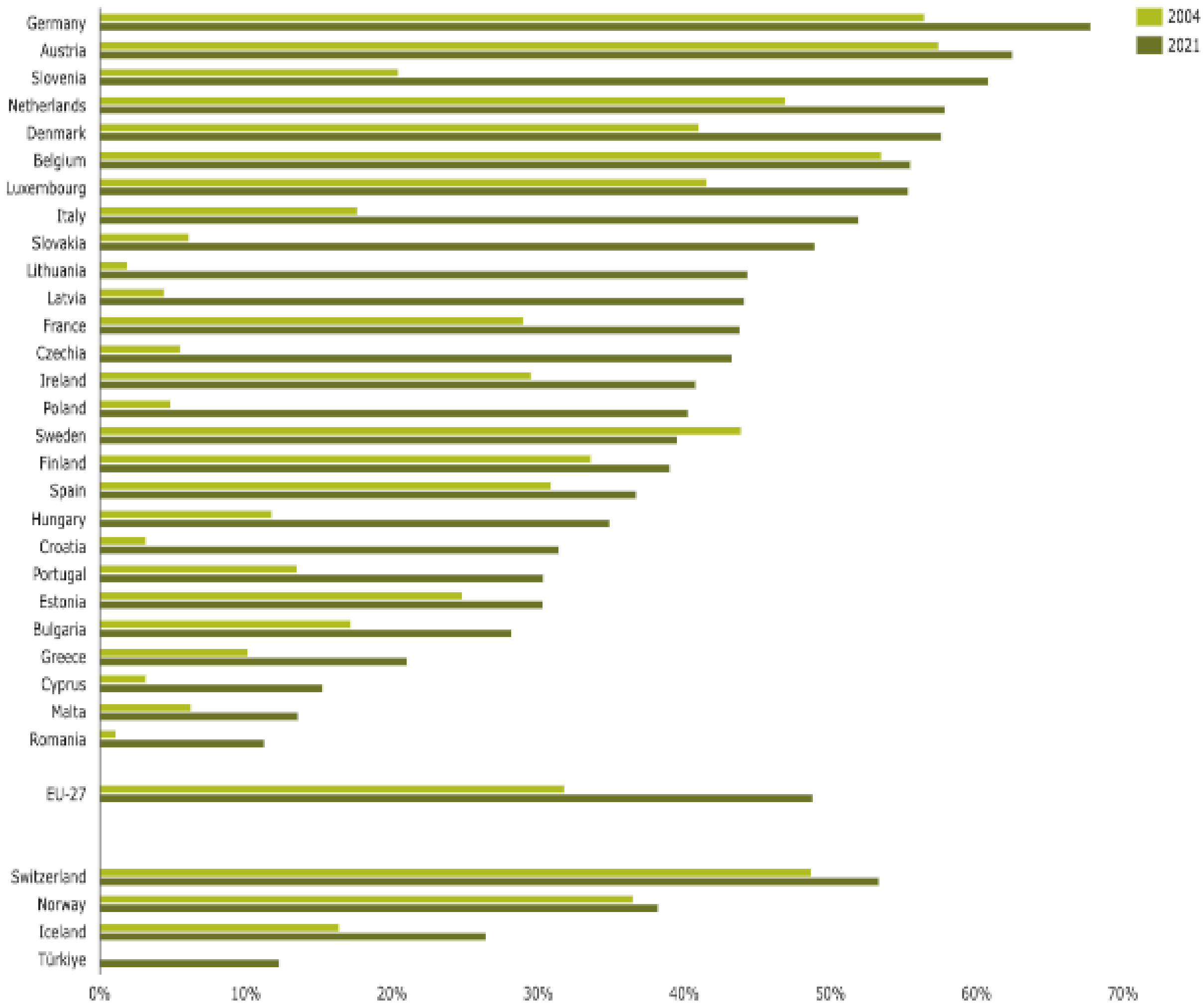


Figure 1. Research methodology steps

Results and discussion

According to European Environment Agency (2023), European countries, with the exception of Sweden, have raised their municipal waste recycling rates since 2004, demonstrating clear progress in waste management. Notably, some countries, including Slovakia, Lithuania, Slovenia, and Latvia, have achieved significant advancements, with increases exceeding 40 percentage points (Figure 2).



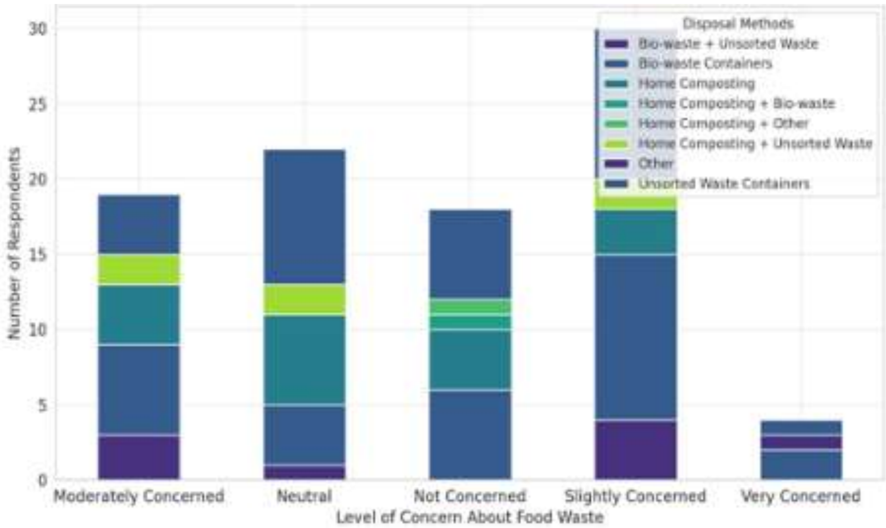
*Source: European Environment Agency, 2023

Figure 2. Municipal waste recycling rates in Europe in 2004 and 2024, %

Yet, since the EU has set a target of 60% for reuse and recycling of municipal waste by 2030, there are still significant steps to be taken due to the current situation, in which only 49.6% of all municipal waste in the EU currently being recycled or composted. Waste management practices vary across EU countries, with some countries like Belgium, the Netherlands, Denmark, Sweden, Germany, Austria, Luxembourg, Slovenia, and Finland having almost no landfilling and relying heavily on recycling and incineration. Landfilling is still prevalent in some eastern and southern European countries, e.g. in Latvia.

Waste management is a complex system encompassing institutional, technical, legal, and financial measures. In Latvia, the predominant types of waste generated by residents are packaging and organic waste. The current waste management plan for Latvia (2021-2028) underscores the importance of environmental protection, climate neutrality, and the prudent use of natural resources. This plan aligns with the EU Green Deal and the new Circular Economy Action Plan, and it supports the National Development Plan for 2021-2027 and the Action Plan for the Transition to a Circular Economy for 2020-2027. The plan prioritizes reducing overall waste generation, particularly for food waste and packaging, and steering the waste management system towards circular economy best practices. Key measures to achieve these goals include optimizing regional waste management by reducing the number of regions and restructuring the landfills within them, focusing on waste prevention and quantitative

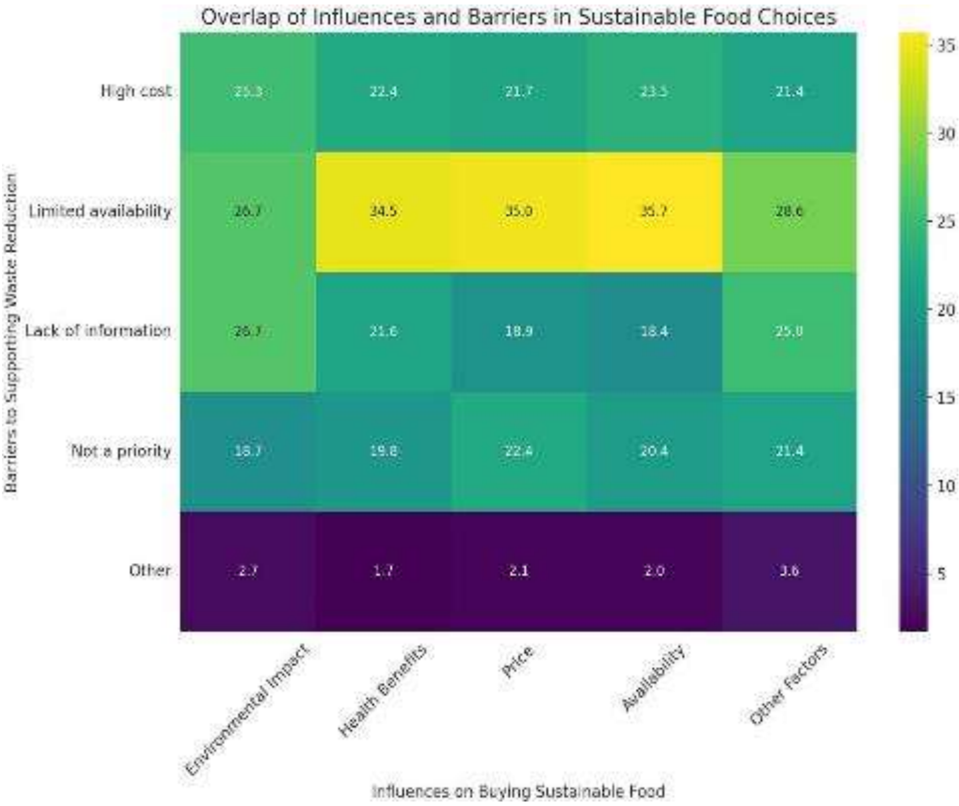
reduction, implementing separate waste collection at the source, and further developing waste infrastructure.
Despite national sustainability commitments, the present habits of Latvian residents cannot be considered very sustainable and insights can be gained from present disposal methods and the respondent’s level of concern about food waste within their household as people using only the unsorted waste containers do not report a lot of concern (Liepina, 2024). To provide a deeper insight into Latvians households’ habits, behaviours, biases towards sustainability etc., Liepina chose to collect data on the present habits and opinions of Latvian residents through a survey. The questions were designed to understand consumer behaviour and attitudes towards food waste reduction, as well as their future outlook regarding food waste reduction in Latvia through closed-ended questions with some allowing the participants to provide their opinion in a free-form. The respondents were chosen randomly through sharing the survey on social media and word-of-mouth within the author’s social circle. 93 responses have been collected in the period from April 1 - April 17, 2024.



*Source: Liepina, 2024

Figure 3. Level of concern about food waste within the households vs the disposal methods

The study also aimed at identifying the barriers that surveyed households encountered for uptalking new habits and criteria during shopping for food, e.g. choosing products with easier recyclable packaging, reusable products, products delivered through shorter supply chains etc. More than one third of respondents (34%) claim that they are not ready to pay higher costs to eliminate food; and food packaging waste, and significant part of respondents also mentioned practical inconvenience of new approaches often practiced by environmental activists. Lately, greenwashing as a negative side-effect of sustainability communication is expanding in Europe and the case studies about misleading marketing communication approaches, including in sustainable waste management, have an increasing trend (Nemes et al., 2022; Kirts, 2024; de Freitas Netto, 2020).
When comparing the reported barriers to purchasing sustainably sourced food with factors that could influence their decision to buy such food, the most commonly cited barriers - higher costs and practical inconveniences (lack of priority) - could potentially be mitigated by better prices, readily available options, and fair, understandable and evidence - based environmental benefits associated with these products.



*Source: Liepina, 2024.

Figure 4. Overlap of potential influences and barriers preventing support to waste reduction initiatives

Conclusions

The current research focused on sustainable consumption and waste management practices in Latvia, specifically looking at food purchasing habits and waste generation revealing the current waste generation trends, waste treatment strategies, and factors influencing consumer behaviour. The practical analysis emphasized the need for tailored interventions and using social marketing tools to promote sustainable consumption and waste sorting in Latvia. The current situation calls to action and urgent need for providing practical guidance for policymakers, businesses, and communities to conduct stricter monitoring of greenwashing cases alongside with educational campaigns how consumers and producers can be more environmentally friendly.

Recommendations

1. In order to enhance the awareness and reliability of sceptic consumers, policy makers in Latvia need to strengthen labelling regulations to help consumers identify and avoid food products with high waste potential.
2. It is necessary to educate consumers about the impact of food waste and the importance of sustainable practices by organizing social marketing campaigns involving food producers, HoReCa representatives, NGOs and other stakeholders that would have interest and ideas how to eliminate organic waste in production, consumption, waste management.

3. The Consumer Rights Protection Centre, in cooperation with IT and environmental specialists, should develop a separate website and mobile application for sustainable waste management ideas, compiling lists of certificates and labelling, various cases of greenwashing and tips on how to better understand of product complies with its description and check conformity with such product certificates by scanning the barcode of the product, thus reducing the implementation of unfair commercial practices and promoting the topic of greenwashing in Latvian society.
4. The Consumer Rights Protection Centre of the Republic of Latvia should conduct an in-depth study at the national level in a multidisciplinary cross-section in order to find out the prevalence of greenwashing in organic waste management system in Latvia and to explain the analysed results and situation to entrepreneurs, as well as to be able to compare Latvia’s specifics with the experience of other countries and conclude in which sectors companies need more assistance and guidance.

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FORECAST POST-2022 BARLEY PRODUCTION IN LIBYA USING TIME SERIES ANALYSIS TO ACHIEVING SELF -SUFFICIENCY

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Abstract

Barley is the second most important crop in Libya after wheat, Libya as many export oil countries, import more than 80% of basically requirement Food that is mean the country depends heavily on Global Market. with production levels subject to fluctuation based on factors like weather rainfall average. Etc. Libya is subject to variability influenced by factors such as supply and demand, import expenses, transportation... Etc. can also impact the pricing levels of barley. Barley plays a crucial role in Libya, serving as a vital crop for both human and livestock consumption. However, the country's agricultural sector heavily depends on seasonal rainfall. Since the rates of rainfall in Libya are very low, the estimated land area that receives 250 mm is 1% of the country’s area. 90% of Libya is desert. All of these factors have significantly led to increase in the quantity of barley imports. The study of the behavior of economic variables such as price of barley and price of wheat as alternative goods, quantity of barley imports and others, are useful in drawing up future policies. Particularly in light of the internal conflicts and political fragmentation in the country, for achieving self-sufficiency of barley has been a challenge by different factors contribute to this dependence on import the internal conflicts and political instability in the country. The main purpose of this study is to forecast the quantity of Production of barley in Libya using time series analysis from 2022 to 20235. Predicting of barley production in the future will greatly help in formulating agricultural policies for Libya.

Keywords: *Libya, Barley, production, Time series. Self sufficiency.*

Introduction

Barley holds significant importance as a key grain globally, ranking fourth in both production volume and cultivated area among cereal crops. In the late twentieth century, the annual global barley harvest reached around 140 million tons from approximately 55 million hectares. Known for its versatility and adaptability, barley has evolved to become the most adaptable cereal crop. With various economic applications, barley is primarily used as animal feed, with more than half of the barley produced in the United States dedicated to livestock feed. In Libya barley stands out as the primary rain-fed agricultural crop, emphasizing its crucial role in the country's agricultural landscape. (Kairia sebibk .el 2021). Barley cultivation in Libya is closely tied to annual precipitation patterns particularly during the autumn season when 80% of the cultivated areas rely on rainfall. The cultivated barley area has shown fluctuations over the years in 287 thousand hectares in 2000, before decreasing to 240 thousand hectares in 2002 and further to 200 thousand hectares in 2005. Despite these variations, barley production has seen an overall increase, from 71 thousand tons in 1980 to 264 thousand tons in 2000 and 299 thousand tons in 2003, followed by a slight decline to 260 thousand tons in 2005. Productivity per hectare has also shown improvement, rising from 0.253 tons per hectare in 1980 to 1.3 tons per hectare in 2005. The domestic production of barley in Libya is primarily utilized for human consumption and feed barley, with the local market relying on increased imports to meet demand. Any surplus barley is directly used for animal feed on local farms (Elbydi -2007). The collapse of two dams due to excessive rainfall in early September resulted in tragic loss of lives and extensive material damages. The heavy rainfall, exceeding 10 centimeters overnight from a cyclone in the Mediterranean Sea, caused flooding in settlements along the northeastern coast. Derna, a city with a population of approximately 90,000 people, was severely affected by the floods that led to the collapse of the dams. The disaster claimed the lives of over 20,000 individuals and caused widespread destruction. (FAO 2023) Barley production in country is severely constrained by insufficient rainfall, forcing reliance on imports. The majority of barley cultivation relies on rainfed agriculture, leaving it vulnerable to erratic rainfall patterns. Recent years have witnessed a significant decline in rainfall, rendering it inadequate for irrigating the crop and hindering its optimal growth.

Material and methods

Econometrics is used to estimate the economic relations and is interested in testing their conformity to reality and predict its path in the future. This involves collecting, recording, and tabulating data and views (AL fakari, 2016). A time series definition is a set of data for a given variable over a given period. For example, the time series (Ỵ^t……1……2), the data for the variable (□^{t}) are shown during the period (t ....), considering that (□^{t}) is a value of (□) when the value of t = 1. Time series is important in predicting the behavior of the variables in the future. The study of the behavior of economic variables such as price of barley and price of wheat as alternative goods, quantity of barley import and others, are useful in drawing up future policies. Most of the time series we deal with are non-static, and the reason is that one of the key elements of these strings is the arithmetic mean, the variance and the common variation may be unstable and change according to the time. This study is specifically dedicated to forecasting the quantity of barley in Libya, using time series This study forecasts barley production from 2022 to 2035 using EViews 10. It analyzes historical data from 2000 to 2022 and uses the ARIMA model to predict future production. Time series analysis helps understand patterns in economic variables like prices of Barley to improve forecasts analysis. Predicting of barley production in the future will greatly help in formulating agricultural policies for Libya. and extending the forecasts up to 2035 using EViews 10 software.

Testing for Stationarity We Have Two Hypotheses:
Null hypothesis **HO**: variable is not stationary or got unit root
Alternative **H1**: variable is stationary, the first method which can be used to check stationarity of the variables is to graph the series. The first method which can be used to check stationarity of the variables is to graph the series

Estimated of quantity of barley using ARDL Model.
R-squared is Hight 0.91 % probe (f-statistic) is 0.0 less than 5 %, this model should be free form (serial correlation, the residual should be normal distrusted, and heteroskedasticity).

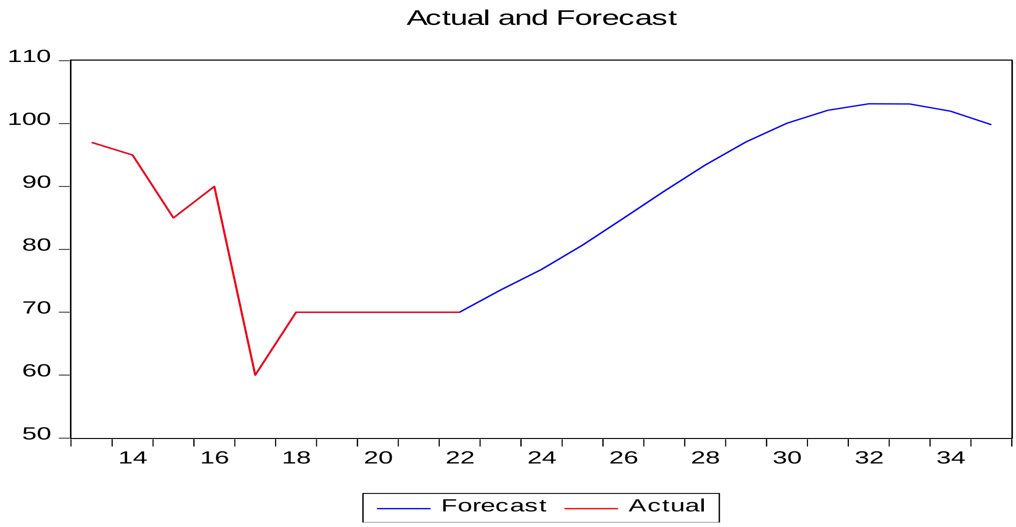
Table (1). The result of ARDL Model for Barley Production.

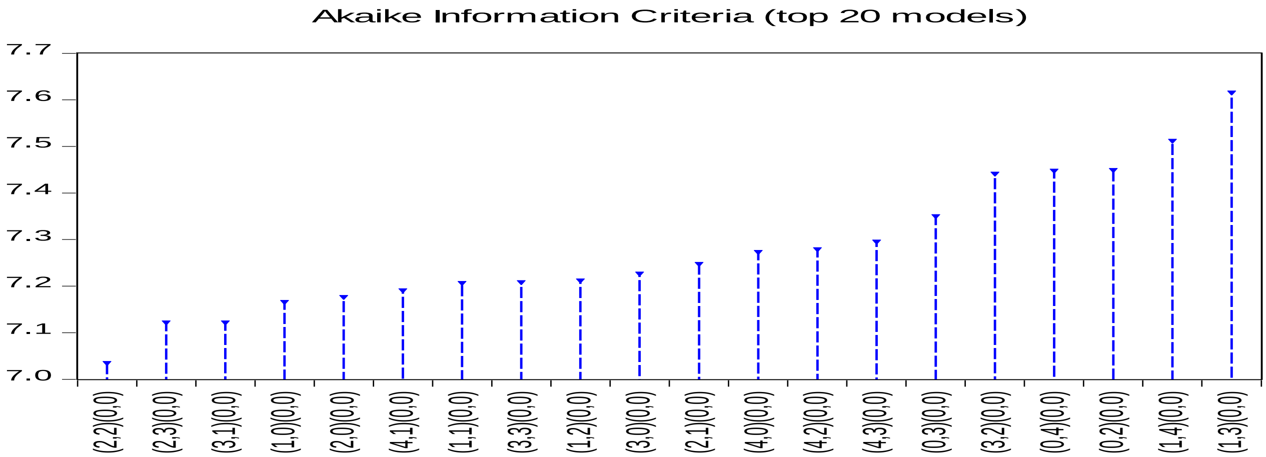
R^2	Serial correlation	Normal distribution test	Heteroskedasticity Breush_Godffry	ARDL Long Run and Bounds Test	Selected Model ARDL
0.91	0.53	0.59	0.49	F- Statistic 4.78	(1, 1, 0, 0, 0, 1, 0)

The first check in the model is serial correlation if the Probability Chi-Square is more than 5%then we cannot reject the null hypothesis, the null hypothesis is the variable not serial correlation, in the model probability. Chi-Square = 0.53 or more than 0.005, so we cannot reject the null hypothesis. So, we accept our null hypothesis; the model is free from serial correlation. The second step is to check the normal distribution using the Jarque-Bera test. If the probability more than 5% (Probability=0.59), that means the residuals series has normal distribution, and the last check is the heteroskedasticity

ARIMA Forecasting Models

Autoregressive Integrated Moving Average (ARIMA) models are estimated over the period 2000–2022 quantity of Barley (**the Production**). The ARIMA models provide a useful framework understand how the Barley production time series is generated. The ARAMA approach requires a Barley production time series to be tested for no stationarity prior to undertaking estimation and forecasting if a series is not stationary (that is, that series has a mean and variance that are not constant over time), the series has been to difference to transform it to stationary series, before generating forecasts .The Box-Jenkins approach to modeling Autoregressive Integrated Moving Average (ARIMA) processes was introduced in a seminal book by statisticians George Box and Gwilym Jenkins in 1970. An ARIMA process serves as a mathematical model utilized for forecasting, and the Box-Jenkins methodology entails the identification of a suitable ARIMA process, fitting it to the data, and subsequently utilizing the fitted model for forecasting purposes.





Results and discussion

The Results of Forecasting quantity of barley in Libya (2022–2035).

Libya's landscape is predominantly desert and semi-desert, covering over 90% of the country. This vast expanse experiences a continental arid/semi-arid climate, characterized by high temperatures, low rainfall, and limited soil fertility. The lack of perennial rivers means Libya heavily depends on groundwater for its water supply. However, this resource is unevenly distributed, with almost 80% of the population residing in the northern coastal plain. This concentration of people in one area creates further challenges for water management and agricultural production. Barley cultivation is particularly challenging in Libya due to these factors. While some rainfall agriculture occurs in the north, the low rainfall yields a small barley harvest. These limitations, coupled with the dependence on unreliable rainfall and scarce water resources, lead to fluctuating barley production levels. The quantity of barley produced is constantly impacted by the availability of water and the severity of the arid climate

The Results of Forecasting quantity of barley in Libya (2022–2035).

Years	***Production Of Barley***
2023	***73.5235***
2024	***76.7937***
2025	***80.6837***
2026	***84.9369***
2027	***89.2743***
2028	***93.4123***
2029	***97.0815***
2030	***100.044***
2031	***102.1100***
2032	***103.1475***
2033	***103.0932***
2034	***101.9550***
2035	***99.8116***

Conclusions and Recommendation

The forecasting of Barley Production in Libya helps Decision makers to formulate the correct agriculture policies and reach to self-sufficiency in this crop. Libya 90 % is desert the Barley crop grown in areas that depend on rain-fed agriculture and do not receive enough rainfall , percentage of rain or rainfall is very low, the biggest obstacle hindering to development of the agriculture sector in Libya is (WATER). The nation relies heavily on groundwater for its water sources. The distribution and use of water resources vary across regions, with about 80% of the population residing in urban areas on the northern coastal plain. Various climatic factors in the country, such as high temperatures, poor soil fertility, and low rainfall, hinder the cultivation of barley. After using the prediction model, it turns out that there is a development in the quantity production of this crop.bBarley production in Libya faces a critical challenge: a declining water supply coupled with increasing demand. From 2023 to 2035, barley production is projected to increase from 73.5235 to 99.8116, highlighting the growing need for this crop both for human consumption and animal feed. The majority of barley production relies on rain-fed agriculture in the north, but recent drastic drops in rainfall have made this approach unsustainable. The Water Problem: A Core Challenge Libya’s arid climate, with a predominantly desert landscape, makes water a precious resource. The majority of the population resides in the coastal plain, further straining water availability. This situation necessitates a multifaceted approach to secure a sustainable water future in Libya.

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THE ROLE OF WHEAT IN LIBYA AND THE IMPACT ON FOOD SECURITY: CHALLENGES AND OPPORTUNITIES

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Abstract

Cereals and their by-products continue to hold a significant position as the main source of food for both humans and animals. They play a crucial role in ensuring food security, particularly in countries that do not produce certain cereals, such as wheat. Consequently, some countries implement policies aimed at achieving self-sufficiency in cereal production. In Libya, wheat and barley are considered essential crops. These grains are typically cultivated through public projects that rely on irrigated agriculture Overall cereals, including wheat and barley, hold strategic importance in meeting the food requirements of the population in Libya. The country utilizes a combination of irrigated and rain-fed agriculture to ensure an adequate supply of grains, with the state playing a crucial role in supporting production efforts**.** Libya comprising of mostly dry and semi-dry. The cultivable area in Libya is estimated to be around 2.2 million hectares, accounting for only 1.2% of the total land area., there is a total area of 470,000 hectares of land available for irrigation., Cereals, specifically, importing 80% from Ukraine and 20% from the Russian Federation using the time series method. The main purpose of this study is to forecast the price of wheat from 2022 making forecasts up until 2035 using EViews software.

Keywords: *Libya, wheat, food security, forecast, price.*

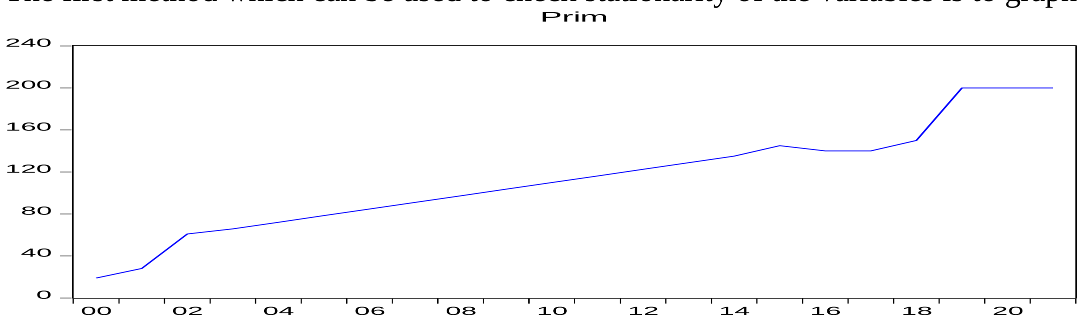
Introduction

Accordingly with FAO (2015): At the global level water resources will be enough to produce the food required in 2050, but many regions will face substantial water scarcity. In Libya, 90% of the land is desert, and the climate is arid. The country's population has tripled since the 1950s. It was 1,888,730 in the first census in Libya in 1954. It had grown to 5,657,632 in the last census 2006. the country depends on food imports, spending its valuable foreign exchange. This situation raises the questions about food security, and the attendant questions of political stability (LGAA, 2008). Until the early 1960s, the Libyan economy had a strong agricultural base. After the discovery of petroleum, Libya became a classic example of a dual economy (CW, 2001a, 2001b). By 2007 oil was contributing 71% of the GDP whereas agriculture's contribution was only 2%. Bit by bit, the Libyan economy had been transformed from a poor, largely agricultural, economy to one of Africa's strongest and wealthiest economies based on the oil and gas industries (European Commission, 2009) .The agricultural sector's importance to Libyan economy has declined steadily and contributed only 4.3% to Libya's GDP in 2002, By 2007, the Figure dropped to just 2% of the GDP. Out of a total of 1.8 million workers in Libya, just 135,700 were working in the agricultural sector (OBG, 2008). In Libya as in other oil-producing countries in the Near East and North African Region, agriculture has become a marginal sector (Casas, 1999). Libya currently exports only about 0.3% of its agricultural produce (European Commission, 2009). Agricultural imports have accounted for more than 25% of the total import bill (Casas, 1999). Domestically, however Libya is about 25% of the demand for wheat, barley, olives, dates, citrus fruits, vegetables, and peanuts. As the population grows, more food will be required to meet the domestic needs. keeping up with the growing demand presents a huge challenge for the local agricultural sector (OBG, 2008). An increasing reliance on oil revenues has seen agriculture decrease to only 2% of GDP by 2007and facing significant challenges, In the Arab countries, 90 % of the total area is desert, the agricultural sector consumes around 87% of the available quantity of water (UNDP, 2007) Libya one of them. This study aims to forecast wheat prices in Libya from 2022 to 2035 using the time series method and EViews software. This analysis will provide valuable insights into potential price fluctuations and their implications for Libyan food security and policy decisions

Material and methods

This study specifically focuses on forecast the price of wheat from 2022 making forecasts up until 2035 using EViews 10 software. a country that relies on importing over 80% of its food needs. By utilizing time series analysis to predict the Price of wheat import, policymakers can make informed decisions and develop effective agricultural policies for the country. using the time series method on EViews software from 2000 to 2021, as well as making forecasts up until 2035 using the ARIAM model. For example, the time series (Ỵ^t……1……2) represents the data for the variable (□^{t}) during the period (t ....), where (□^{t}) represents the value of (□) when t = 1. Time series analysis is important for predicting the future behavior of variables. By studying the patterns and trends in economic variables such as production, consumption, and Price of local wheat. Many time series data are non-stationary, meaning that the mean, variance, and covariance can change over time.

Testing for Stationarity We Have Two Hypotheses:
Null hypothesis HO: variable is not stationary or got unit root
Alternative H1: variable is stationary, the first method which can be used to check stationarity of the variables is to graph the series.
The first method which can be used to check stationarity of the variables is to graph the series.

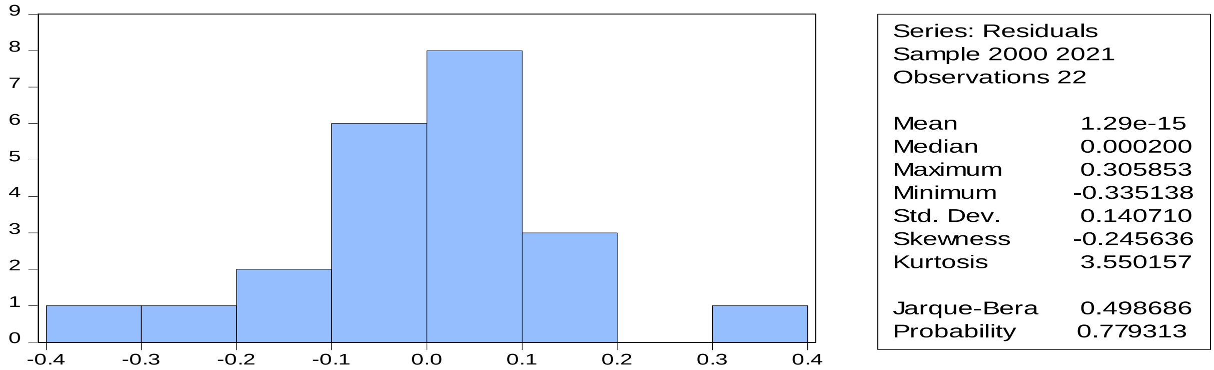


Using the augmented Dickey-Fuller (ADF) test for unit roots all variables are difference, the log A
 (**PRICE OF IMPORT WHEAT)** will be stationary at the level with (intercept)
difference Statistics= -4.85 more than -3.01 at 5% then **we can** reject the Null Hypothesis H0 and accept the alternative hypothesis.
Estimated PRICE OF IMPORT WHEAT USING LS least squares (NLS@ARMA)
R-squared is Hight 0.94 % probe (f-statistic) is 0.0 less than 5 %, this model should be free form (serial correlation, the residual should be normal distrusted, and heteroskedasticity).

Table 1. The result of (NLS@ARMA) Model for price of import wheat

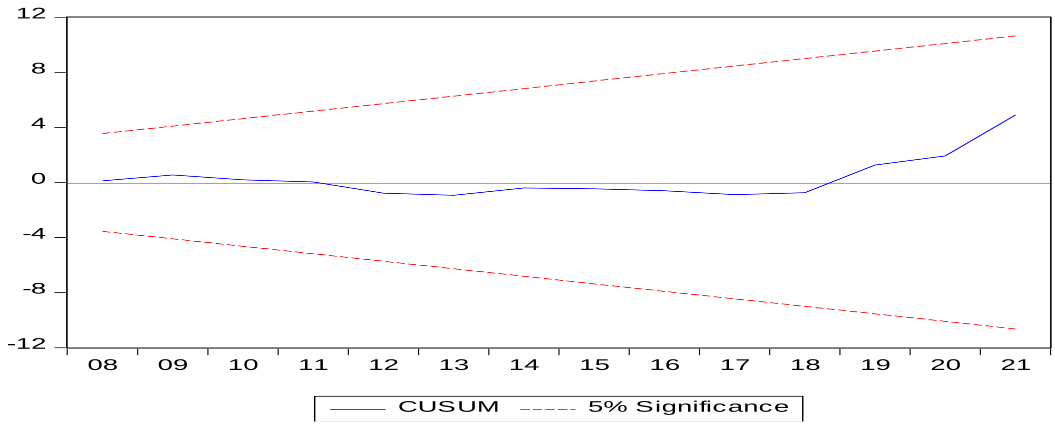
R^2	Breusch-Godfrey Serial Correlation LM Test:	Normal distribution test	Heteroskedasticity Test: ARCH
0.94	0.86	0.77	0.38

The first check in the model is serial correlation if the Probability Chi-Square is more than 5% then we cannot reject the null hypothesis, the null hypothesis is the variable not serial correlation, in our model probability. Chi-Square = 0.86 or more than 0.005, so, we cannot reject the null hypothesis. So, we accept our null hypothesis; the model is free from serial correlation. The second step is to check the normal distribution using the Jarque-Bera test. If the probability more than 5% (Probability=0.34), that means the residuals series has normal distribution, and the last check is the heteroskedasticity



Graph 1. Chi-Square

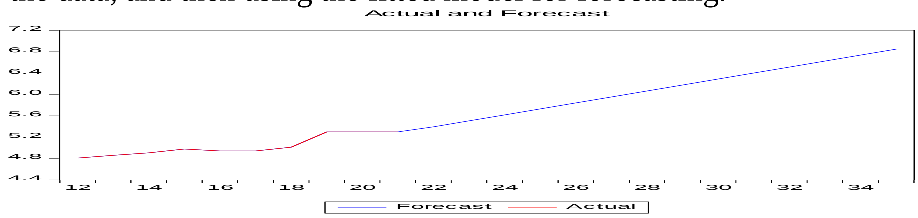
For checking the long relationship among the variables, that is whether variables have a relationship. if the F statistics exceeds the upper bound, we can reject the null hypothesis and accept the alternative. The hypothesis here F – statistic = 6.12, that is, more than the bound Test at 1%,5%, and 10%. Therefore, we can reject the null hypothesis and accept alternative hypothesis That means the variables have long relationship. Then using the cusum test to check the stability by observing the blue line between two red lines that mean the price of import wheat has relationship in the short time the blue.

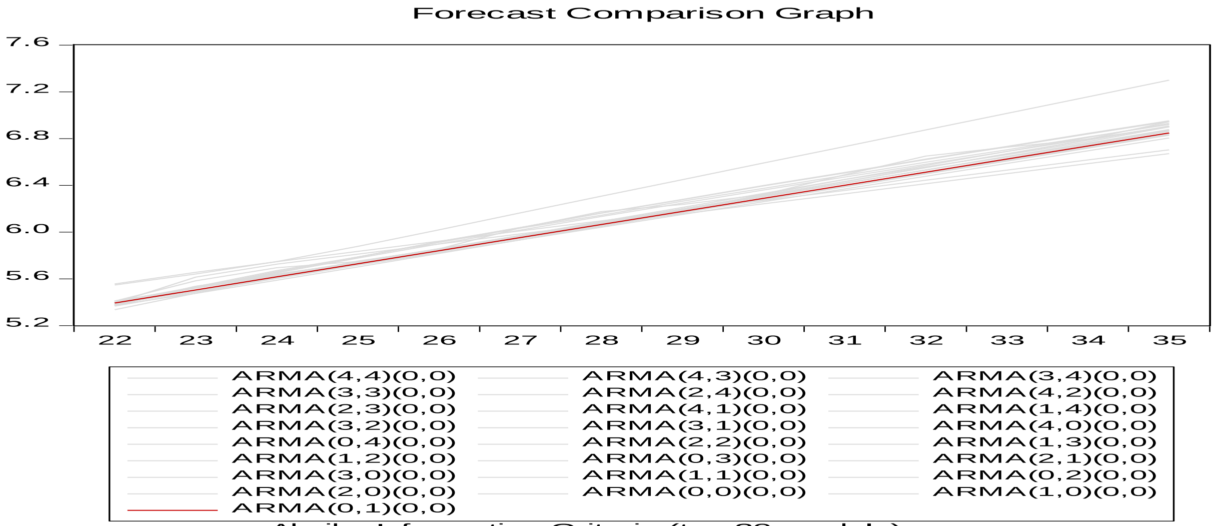


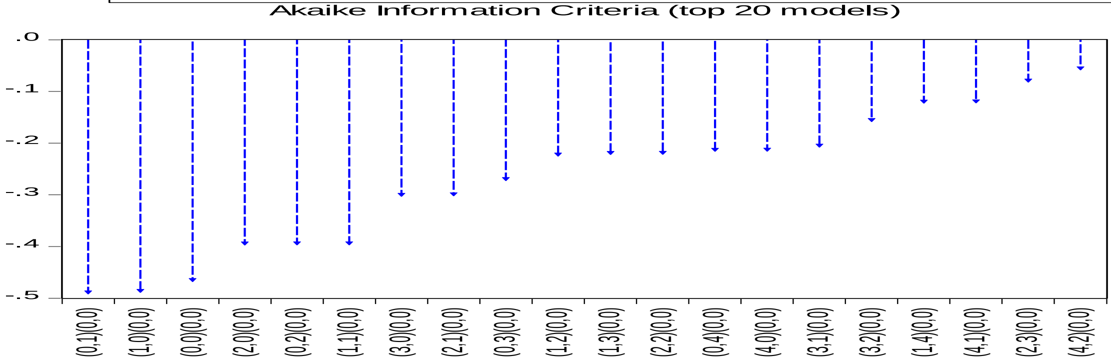
Graph 2. Cusum test

ARIMA Forecasting Models

The Box-Jenkins approach to modelling Autoregressive Integrated Moving Average (ARIMA) processes was described in a highly influential book by statisticians George Box and Gwilym Jenkins in 1970. An ARIMA process is a mathematical model used for forecasting Box -Jenkins modelling involves identifying an appropriate ARIMA process Fitting it to the data, and then using the fitted model for forecasting.







The Results of Forecasting price of import wheat (2022–2035).

A forecast is a conditional statement about the future. It is about what is expected to happen in the future. If various assumptions turn out to be valid, there are,j objective and subjective components involved in forecasting. The objective component consists of explaining past levels and patterns. While the subjective component is the application of the resultant knowledge the future (Bolan, 1984; Prasifka, 1988). There are several terms that are similar. However, they do not have exactly the same meaning as the words 'forecast' and 'prediction'. Prediction is used in a more general way than forecast. Predication is a statement about the future, whether or not conditional

Years	Price of import wheat
2022	208.6190
2023	217.2381
2024	225.8571
2025	234.4743
2026	243.0952
2027	251.7143
2028	260.333
2029	268.9524
2030	277.5714
2031	286.1905
2032	294.8095
2033	303.4286
2034	312.0476
2035	320.6667

Conclusions and Recommendation

Libya such as many Arabic countries import more than 80% of food requirement, WHEAT AND BARLEY for example are the basically items food. Libya importing 80% from Ukraine and 20% from the Russian Federation. Currently during the war between (Ukraine and RUSSIAN), WHAET PRICES RISE IN THE GLOBAL MARKET. because are the main source of wheat for the country and This rise in wheat prices will greatly affect the lives of Libyan citizens, which is accompanied by increase in the prices of wheat-derived materials AS (Bread and Sweet). Predicted of the price of import wheat so important for formulating agriculture policies in this country 90% is desert and the water resources so limited and population growth is so Hight The weather is very hot rainfall rates are very low , more people mean more demand of water and Food as well.in 2021 the price of import wheat was 200 Libya diner in 2022 208.7 Libyan diner , the price continues to rise year after year and the last forecast year in 2035 will be 320.666 Libyan diner .The country must take necessary measures to reduce its dependence on imports from abroad and encourage wheat cultivation within the country the problem of the water in Libya is the major problem in the country resources of water are so limited the climate of the country is not suitable for produce some crop and also many kinds of fruit because the temperature is Hight specially in summer season my suggestion is food for oil (Petroleum) because as country is so poor of water 90% is desert in the same time Libya is more than rich of oil with small size of population just 7.7 million of people.

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A SYSTEMATIC REVIEW OF THE IMPACT OF CLIMATE CHANGE ON AGRICULTURE AND FUTURE ADAPTATION STRATEGIES FOR NORTH MACEDONIA

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Abstract

Climate change poses significant challenges to agriculture worldwide, with implications for food security, livelihoods, and ecosystems. This review paper examines the impact of climate change on agriculture and explores adaptation strategies for North Macedonia. The paper synthesizes existing literature on the subject, drawing from peer-reviewed articles, academic papers, and reports. The introduction provides an overview of the interconnectedness between climate change and agriculture, emphasizing the vulnerability of the agricultural sector to changing climatic conditions. The materials and methods section outlines the methodology employed, including a systematic literature search, source selection criteria, data extraction, analysis, and synthesis. Results and discussion focus on five key areas: crop adaptation, water management, pests and diseases in crops, biodiversity, and agroforestry. Each subsection discusses the challenges posed by climate change in these areas and explores potential adaptation measures tailored to North Macedonian context. The conclusion highlights the urgency of addressing climate change impacts on agriculture and emphasizes the importance of adopting climate-smart agricultural practices. It underscores the need for collaborative efforts between stakeholders to promote knowledge exchange, capacity building, and policy interventions. This paper highlights the importance of adapting agricultural practices to mitigate the adverse effects of climate change on food production and rural livelihoods in North Macedonia. By embracing adaptation strategies, policymakers, farmers, and other stakeholders can enhance resilience and ensure sustainable agricultural development in the face of a changing climate.

Key words: *Climate change, Agriculture, Adaptation strategies, North Macedonia.*

Introduction

Climate change poses significant challenges to global ecosystems and agricultural systems worldwide. In the face of escalating environmental degradation and the proliferation of greenhouse gas emissions, agriculture stands as both a contributor to climate change and one of its most vulnerable sectors (Piguet, 2022). This intersection underscores the urgency of understanding the multifaceted impacts of climate change on agricultural productivity, food security, and rural livelihoods.

While agricultural systems have historically shaped landscapes and sustained human societies, modern food production practices have increasingly strained natural resources and exacerbated environmental degradation (Redden et al., 2014). With approximately 70% of the global population reliant on agriculture for their livelihoods, the repercussions of climate change reverberate across regions already grappling with distinct shifts in climatic patterns (IPCC, 2021).

The escalating atmospheric concentrations of greenhouse gases, including methane, carbon dioxide, and nitrous oxide, highlight the unprecedented nature of the current climate crisis

(Usman et al., 2021). The consequences of these changes manifest in extreme weather events and environmental disruptions, from devastating bushfires in Australia and the United States to the accelerated melting of polar ice sheets and rising sea levels (Canadell et al., 2021; Perkins, 2022).
Amidst these global challenges, North Macedonia stands particularly vulnerable to the impacts of climate change on its agricultural sector. Characterized by a dry climate, changing rainfall patterns, and rising temperatures, the country faces mounting pressures on its agricultural productivity and food security (MAFWE, 2022). Agriculture, comprising 7.9% of the nation's GDP, occupies a critical role in sustaining rural livelihoods and supporting approximately 193,000 family farms.
Projections from the World Bank indicate a looming future of heightened climatic instability in North Macedonia, with anticipated increases in temperature and precipitation deficits exacerbating existing challenges (MOEPP, 2020). In this context, it is imperative to explore adaptation strategies tailored to the unique socio-environmental landscape of North Macedonia.
This review aims to provide a comprehensive overview of the current state of climate change impacts on global ecosystems and elucidate potential adaptation strategies tailored to North Macedonia's agricultural sector. By focusing on adaptation measures aimed at mitigating climate change impacts, this review seeks to contribute to ongoing efforts to safeguard agricultural sustainability and rural livelihoods in North Macedonia.

Materials and Methods

Objective Statement:
The aim of this review paper was to comprehensively explore existing research and literature on the impact of climate change on agriculture, with a focus on North Macedonia. The objective was to outline the necessary adaptation measures and strategies to address these impacts.
Systematic Literature Search:
A systematic literature search was conducted in September-October 2023 to identify relevant peer-reviewed articles, academic papers, reports, and publications related to the impact of climate change on agriculture. Search terms included "climate change" and "agricultural adaptation measures," and databases searched included Google Scholar, Web of Science, Scopus, Emerald, Elsevier Science Direct, and the World Bank's Climate Change Information Portal. Additionally, official data published in The Annual Reports of the Ministry of Agriculture, Forestry and Water Economy were consulted.
Source Selection Criteria:
A total of 60 studies were identified and deemed appropriate for review based on their relevance to the subject matter. Selection criteria included the significance of the articles to the topic of climate change in agriculture and adaptation measures. Irrelevant or duplicated articles were excluded from the review.
Data Extraction and Analysis:
Essential insights were extracted and structured from the selected sources, focusing on challenges related to climate change impacts on agriculture and proposed adaptation solutions. This involved identifying recurring themes, emerging trends, and patterns in the literature. The selected articles were then analyzed to distill key findings, methodologies, and recommendations.
Synthesis of Information:
The synthesized information provides a structured overview of the challenges faced by agriculture due to climate change, along with corresponding adaptation actions. Insights from

the chosen articles were categorized into specific themes, including Crop Adaptation, Water Management, Pests and Diseases in Crops, Biodiversity, and Agroforestry. These themes serve as the basis for the discussion section of this paper.

Results and discussion

The impacts of climate change on agriculture in North Macedonia are multifaceted, affecting various aspects of crop production, water management, pest and disease control, biodiversity, and agroforestry. This section summarized in Table 1 discusses the key challenges identified in these areas and explores the potential adaptation measures tailored to the specific context of North Macedonia.

Table 1. Challenges and adaptation measures for agriculture in North Macedonia facing climate change

Key area	Challenges	Adaptation measures
Crop adaptation	Drought and heat stress	Crop rotation to optimize soil moisture retention and improve soil health
	Disruption of crucial stages of plant development	Adopting heat-resistant crop varieties
	Flower drop and reduced yield	Implementing precision agriculture techniques
Water management	Reduced water supplies due to climate change	Water-saving techniques like mulching, shading nets, and micro-irrigation systems
	Inefficient irrigation methods	Transition to modern irrigation systems like micro-irrigation and fertigation
	Soil erosion and yield loss	Extensive animal husbandry in water-scarce regions
Pests and diseases in crops	Heavy reliance on pesticides	Implementing integrated pest management (IPM) practices
	Misuse of pesticides	Using biological pest control methods
	Abiotic and biotic plant diseases	Developing disease-resistant crop varieties
Biodiversity	Decline in farmland biodiversity	Enhancing biodiversity monitoring and establishing protected areas that consider climate impacts
	Loss of ecosystem services	Promoting ex situ conservation efforts
	Impact on pollinators and soil biodiversity	Implementing sustainable land management practices
Agroforestry	Climate change threats to forests	Integration of trees or shrubs with crops to enhance resilience and offer biophysical and socioeconomic benefits
	Soil erosion and reduced soil health	Promoting awareness, capacity building, and policy support for agroforestry practices
	Loss of ecosystem services	Strategic tree planting and management to create microclimates and buffer crops against adverse climatic conditions

Crop Adaptation

Abiotic stressors, such as drought and heat stress, significantly impact crop development and morphology worldwide (Reidsma et al., 2009). Several studies indicate that these stressors, exacerbated by climate change, are likely to negatively affect crop yields in North Macedonia (Hristov, 2018). The country's diverse agro-ecological zones, including alpine, Mediterranean, and continental regions, exhibit variations in response to changing climatic conditions.

While higher temperatures may initially promote plant growth and shorten growing seasons, they can also disrupt crucial stages of plant development, leading to phenomena like flower drop and ultimately reducing yield (Demirevska et al., 2009; Jahanzad et al., 2020). This underscores the urgent need for adaptation strategies to mitigate the adverse effects of climate change on agricultural production.

Crop rotation emerges as a vital adaptation measure to optimize soil moisture retention and minimize water loss through evaporation and surface runoff (Yu et al., 2022). By enhancing soil structure, nutrient cycling, and resistance to erosion, crop rotation promotes soil health and resilience to extreme weather events such as floods and droughts. Additionally, it fosters microbial activity in the soil, contributing to disease suppression and overall plant health (Yu et al., 2022).

The planned strategy for agriculture and rural development in North Macedonia emphasizes the importance of ensuring consistent food production, stable farmer incomes, and sustainable rural development (MAFWE, 2022). This policy framework supports the cultivation of grain and vegetables, enabling continued production despite challenges posed by fluctuating rainfall and temperatures, as well as rising agricultural input costs.

Water Management

Water plays a pivotal role in agriculture, with factors like elevation and drought significantly influencing its quality and availability. Climate change-induced shifts, including reduced water supplies due to snowmelt, evaporation, and other climatic alterations, pose challenges for the agricultural sector and food security. Consequently, there is a pressing need for rational water management practices in agricultural production.

In North Macedonia, excessive irrigation practices can exacerbate water shortages for fellow farmers, compromise crop health, and increase susceptibility to diseases and pests. Moreover, inefficient irrigation methods contribute to soil erosion, yield loss, and elevated production costs. To address these challenges, farmers are increasingly adopting water-saving techniques such as mulching, shading nets, and micro-irrigation systems. These technologies help minimize water loss from soil evaporation and enhance water use efficiency by plants.

Transitioning from traditional irrigation practices to modern systems, such as micro-irrigation and fertigation, is crucial for optimizing water resources and mitigating climate change impacts. Proper management practices aim to improve the allocation and efficiency of irrigation water, taking into account factors like pricing, technology type, environmental conditions, and watering schedules (Hillel, 1997). Emphasizing the adoption of these advanced irrigation technologies and practices through farmer training programs is essential to enhance water conservation and resilience in agricultural systems.

In regions facing acute water scarcity, promoting extensive animal husbandry as an alternative to water-intensive crop cultivation can be economically viable and environmentally sustainable. By diversifying agricultural activities, farmers can reduce reliance on water-intensive crops and adapt to changing climate conditions more effectively.

Climate change poses significant challenges to water resources in North Macedonia, with smallholder farms being particularly vulnerable to increased heatwaves, severe droughts, and floods. Effective water management strategies are imperative to safeguard agricultural livelihoods and ensure sustainable food production amidst changing climatic conditions.

Pests and Diseases in Crops

Pesticides play a crucial role in modern agriculture, offering farmers labor savings and higher yields by effectively combating pests and diseases. While some small-scale farmers may opt for minimal pesticide use, large-scale crop producers often rely heavily on these chemicals to safeguard their crops. For instance, wheat farmers may apply weed killers to expedite drying and prevent losses from wet weather, while fruit and vegetable growers frequently employ pesticides to protect delicate varieties like strawberries.

However, the widespread use of pesticides comes with both economic benefits and environmental concerns. In the EU, pesticides account for approximately 7-8% of total agricultural production costs, highlighting their significant financial impact (Popp et al., 2013). Nonetheless, misuse of pesticides can lead to socio-economic and environmental repercussions, underscoring the importance of adopting alternative pest control methods, such as Organic Agriculture production, to reduce reliance on artificial fertilizers and pesticides.

Plant diseases pose a significant threat to crop production, necessitating effective preventive measures. Diseases can be categorized as abiotic (non-infectious) or biotic (infectious), with adverse environmental conditions often serving as catalysts for their development. Examples include extreme temperatures, moisture imbalances, air pollutants, soil contaminants, and toxins released by certain plants and fungi (Vicente and Holub, 2013; Koza et al., 2022).

Among the most common infectious agents in agriculture are bacteria, pathogenic fungi, viruses, and parasites. Preventive measures against bacterial infections include the use of pathogen-free seeds, seed treatment with hot water, soil solarization, and application of germicidal compounds (Vicente and Holub, 2013). Similarly, control strategies for fungi involve destroying infected plant matter, using healthy seeds, implementing regular crop rotation, and employing chemical and biological fungicides.

Viruses and viroids pose unique challenges due to their small size and ability to spread rapidly through the soil. Control measures include cultivating resistant crops, conducting indexing to determine virus presence, and implementing quarantine protocols to contain outbreaks (Fontdevila Pareta et al., 2023). Additionally, parasites are effectively managed through herbicide application on resistant crops, manual weeding, and crop rotation.

In cases of severe infection, farmers may need to resort to drastic measures such as destroying infected crops and implementing quarantine to salvage remaining yields (Almeida et al., 2019). Despite the challenges posed by pests and diseases, sustainable agriculture practices not only protect the environment but also contribute to long-term profitability in the agricultural sector.

Biodiversity

Biodiversity serves as a cornerstone of natural ecosystems and is essential for sustaining food resources worldwide. However, there is alarming evidence of global declines in farmland biodiversity, particularly observed in plants, pollinating birds, and insects, predominantly in regions like North America and Europe. Agricultural intensification processes, characterized by practices such as fertilization, tillage, and pesticide use, are recognized as the primary drivers of biodiversity loss and ecosystem degradation (ECA, 2020).

Over the past five decades, arable land has witnessed a significant decline in biodiversity, leading to biotic homogenization and species extinctions, particularly in Europe (Outhwaite et al., 2022; Maxwell et al., 2016). This trend is exacerbated by the intensification of agricultural practices and the removal of landscape features that support biodiversity (Czucz et al., 2022). The decline in biodiversity poses risks to essential ecosystem services vital for agriculture, including pollination and soil health.

Pollinators, such as bees and butterflies, play a crucial role in sustaining agricultural production by pollinating three-quarters of the world's most cultivated crops, thereby contributing to the production of fruits, vegetables, and seeds. Similarly, soil biodiversity, encompassing bacteria, fungi, and worms, enhances water and nitrogen use efficiency, while insects serve as biological control agents, regulating pest populations.

The intensification of agricultural activities and expansion of agricultural frontiers contribute to dwindling biodiversity, with pesticide use emerging as a key factor in altering farmland ecosystems, particularly impacting farmland bird populations. Excessive pesticide application can disrupt biological diversity and compromise habitat quality for diverse species.

As global demand for crops and food continues to rise, driven by population growth, biodiversity in both farmland and natural areas faces escalating risks. To address these challenges, adaptation strategies in North Macedonia must prioritize collecting climate impact data on biodiversity, especially in mountain ecosystems, enhancing biodiversity monitoring efforts, establishing functional systems of protected areas that consider climate impacts, and promoting ex situ conservation efforts (MOEPP, 2023).

Agroforestry

Recent Intergovernmental Panel on Climate Change (IPCC) report have underscored the significance of agroforestry in the context of climate change adaptation, prompting a growing recognition of its importance in national adaptation plans (IPCC, 2022; Meybeck et al., 2020). Agroforestry, characterized by the intentional integration of trees or shrubs with crops within plots, farms, or landscapes, emerges as a promising climate change adaptation strategy to enhance the resilience of farmers and agricultural systems, offering a multitude of biophysical and socioeconomic benefits (Rosenstock et al., 2019).

In response to climate change threats, the agricultural community must assess the long-term impacts on forests and devise restoration strategies to mitigate risks. Research highlights two key adaptation approaches: increasing tree diversity to accommodate heightened climate variability and implementing targeted interventions to address projected climate trends (Catacutan et al., 2017).

Agroforestry systems not only contribute to climate change mitigation by sequestering carbon dioxide but also offer multiple adaptation benefits, including improved soil health, enhanced water retention, and diversified income sources for farmers (Jose, 2009). By integrating trees or shrubs into agricultural landscapes, farmers can mitigate the impacts of extreme weather events, such as floods and droughts, while simultaneously enhancing biodiversity and ecosystem services (Rolo et al., 2023).

Furthermore, agroforestry practices promote sustainable land management by reducing soil erosion, conserving water resources, and enhancing agroecosystem resilience to climate variability (Ruiz et al. 2020). Through strategic tree planting and management, farmers can create microclimates that buffer crops against adverse climatic conditions, fostering greater agricultural productivity and food security in the face of changing climate patterns (Torquebiau, 2000).

To realize the full potential of agroforestry as a climate change adaptation strategy, concerted efforts are needed to promote awareness, capacity building, and policy support for its widespread adoption. Collaborative initiatives between governments, research institutions, and local communities can facilitate the implementation of agroforestry practices, fostering sustainable agricultural development and climate resilience in North Macedonia and beyond.

Conclusion

Climate change poses unprecedented challenges to agriculture worldwide, threatening food security, livelihoods, and ecosystems. This review paper has explored the impact of climate change on agriculture and outlined potential adaptation strategies for North Macedonia.
The findings highlight the urgency of addressing climate change impacts on agriculture, given its profound implications for global food production and rural livelihoods. From shifts in temperature and precipitation patterns to increased frequency of extreme weather events, climate change poses multifaceted risks to agricultural systems.
In North Macedonia, where agriculture plays a vital role in the economy and sustenance of rural communities, the effects of climate change are particularly pronounced. Changing rainfall patterns, rising temperatures, and water scarcity pose significant challenges to crop yields, soil health, and biodiversity.
However, amidst these challenges lie opportunities for adaptation and resilience-building. Through the adoption of innovative agricultural practices such as crop rotation, water management techniques, pest and disease control measures, agroforestry, and biodiversity conservation efforts, farmers can enhance their capacity to withstand climate variability and change.
Furthermore, strategic policy interventions and investments are crucial to support climate-smart agriculture initiatives and facilitate the transition towards more sustainable and resilient farming systems. Collaborative efforts involving government agencies, research institutions, civil society organizations, and local communities are essential to promote knowledge exchange, capacity building, and the adoption of climate-resilient agricultural practices.
In conclusion, addressing the impacts of climate change on agriculture requires concerted action at all levels. By embracing adaptation strategies tailored to local contexts and leveraging the potential of nature-based solutions, North Macedonia can mitigate the adverse effects of climate change on its agricultural sector and ensure food security, environmental sustainability, and prosperity for future generations.

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TRANSLOCATION OF ENDANGERED SPECIES *SERRATULA LYCOPIFOLIA* (VILL.) A. KERN. IN THE REPUBLIC OF MOLDOVA

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Abstract

In the Republic of Moldova, activities of translocation or reintroduction of rare species started recently, in 2020, and have been carried out by the researchers of the "Alexandru Ciubotaru" National Botanical Garden (Institute). The present study refers to an Asteraceae taxa – *Serratula lycopifolia* (Vill.) A. Kern. (=*Klasea lycopifolia* (Vill.) A. et D. Löve – species of community interest, protected by law, included in the Red Book of the Republic of Moldova, 3rd ed., the Habitats Directive, the Red List of endangered species developed by the IUCN. Translocation experiments of the *Serratula lycopifolia* species were carried out both in *ex situ* conditions, in the National Botanical Garden, and *in situ*, in the natural habitat. The population located near Gordinești village (Edinetz district), within the boundaries of "La Castel" Landscape Reserve, served as a source of planting material. In April 2021, 10 specimens were planted in the experimental plot of the National Botanical Garden, for conservation and study in *ex situ* conditions and as a seed source. *Ex situ*, the species developed well, and underwent all the ontogenetic stages. At the same time, as a result of the floristic research carried out, the natural habitat near Speia village, Anenii-Noi district, was selected for the creation of an *in situ* population. Subsequently, in May 2021, 20 plantlets of the *Serratula lycopifolia* were planted, and in the spring of 2022, additions were made with another 20 specimens. Monitoring of the newly created population was initiated. In the growing season of 2023, the population numbered 15 individuals, of which three plants flowered and produced seeds. As well as both, the translocated and the spontaneous populations were also monitored, providing an essential basis for evaluating the performance of the new population.

Keywords: *Serratula lycopifolia (Vill.) A. Kern., translocation, conservation, Republic of Moldova.*

Introduction

The reintroduction of rare and extinct plant species into natural habitats is particularly relevant in the context of the alarming decline in biological diversity. Experimental research related to the introduction of endangered species, threatened with extinction, represents an important direction, being part of a series of extensive measures taken to conserve biodiversity. In the Republic of Moldova, activities of reintroduction, translocation and repatriation of rare species, have not been carried out previously. Actions in this direction began in 2020, with the start of the project "Research and conservation of vascular flora and macromycobiota in the Republic of Moldova" (20.80009.7007.22), carried out by researchers of the Spontaneous Flora and Herbarium Department of the "Al. Ciubotaru" National Botanical Garden (Institute), within which 6 species of rare vascular plants were taken into study. Among them the subject of this study was *Serratula lycopifolia* (Vill.) A. Kerner, a species of community interest, protected by law, included in the Red Book of the Republic of Moldova, 3rd edition, in the *Habitats Directive*, cited in the *IUCN Red List of Endangered Species* as *Data Deficient* taxa (Bilz, 2011). The species requires effective conservation measures, so the aim of the study is to ensure its protection both in its natural habitat and *in* *situ* and *ex situ* conditions, while carrying out translocation actions and creating a new population with the purpose of conservation in a new biotope with similar conditions.

Material and Methods

The research was conducted during 2020-2023 according to the generally accepted method (Akeroyd and Jackson, 1995, IUCN Guidelines for Re-introductions, 1998, Gorbunov et al., 2008, Godefroid et al., 2011). The population of the landscape reserve "La Castel" (the protected area from the north of the republic – N 48º08'05", E 27º09'57") served as a source of planting material for translocation activities. In April 2021, 10 mature plants obtained from plantlets were planted in the experimental plot of the National Botanical Garden. The seed germination ratio was tested by placing on filter paper in Petri dishes at 18-20 °C and watered with distilled water and recording the proportion of germinated seeds. Manipulations and evaluation of germination were carried out at room temperature conditions. Subsequently, germinated seeds were transferred from Petri dishes to pots, which contained a peat and soil mixture. At the end of May, seedlings in the immature stage were transferred to pots of 10 cm diameter, for growth and acclimatization. They were placed outdoor in semi-shade conditions for 3 months, until they reached the vegetative stage, ready to be transferred to the natural habitat. Translocation experiences of the species *Serratula lycopifolia* were carried out in the natural habitat of similar conditions in the vicinity of Speia village, Anenii-Noi district (in the central part of the republic – N 47º00'36", E 29º18'58"). Plantlets were watered until accustomed to the new growing site. Monitoring of the newly created population was carried out once a month, and data on survival, growth, reproduction and plant health were recorded. Simultaneously, the spontaneous donor population was monitored.

Results and Discussion

Serratula lycopifolia (Vill.) A. Kerner (= *S. heterophylla* auct., non (L.) Desf. = *Klasea lycopifolia* (Vill.) A. et D. Löve (family Asteraceae) is a species of community interest, protected by law in the Republic of Moldova (category II – endangered) and in the Red Book of the Republic of Moldova, 3rd edition (Environmental legislation… 1999, Ionita, 2015). It is also included in the Red List of higher plants in Romania (Oltean et al., 1994), introduced in *Annex II* of the *Habitats Directive* (Convention …1979), cited in *The IUCN Red List of Threatened Species* (Bilz, 2011). The range of species distribution includes Central and Eastern Europe, the Western Balkans. In the Republic of Moldova, it grows on rocky, calcareous slopes, clay-sandy hills, in steppic glades, in white oak (*Quercus pubescens* Willd.) forests and thickets. Currently, 3 localities of distribution of the species are known in the vicinity of the villages: Gordinești, Fetești (new point identified by authors in May 2024), Edinetz district and Colosova, Grigoropol district.

The assessment of the threat status of *Serratula lycopifolia* for the territory of Republic of Moldova was made according to the IUCN Red List Categories and Criteria (2012) – [CR B2ab(iii,iv,v)].

In *in-situ* conditions, *Serratula lycopifolia* is protected in the Landscape Reservations "La Castel" and "Fetești", Edinetz district, within Euro-Siberian steppe woods with *Quercus* spp. – 91I0*, the European priority habitat, according to NATURA 2000 (Interpretation…2013). The plant is a perennial Ponto-Pannonian hemicryptophyte. Xeromesophillous species. It blooms in May-June, the fruits ripen in July. It multiplies by seeds and vegetatively. It is pollinated by insects. It is a melliferous and decorative plant during the flowering period.

In this study, the population of the species *Serratula lycopifolia* that grows near Gordinesti, was evaluated, which also served as a genetic source for the translocation experiment. It grows in small groups of 3-15 individuals or larger, forming extensive clumps of about 30-50 m^2, with an abundance of 2-3 and height of 70-100 cm. The total number of individuals has been estimated approximately, but certainly exceeds 500 individuals. The vegetal carpet of the glades is dominated by *Festuca valesiaca* Gaudin, *Salvia nemorosa* L., *Elytrigia repens* (L.) Nevski. Among the accompanying species are: *Poa angustifolia* L., *Stipa pulcherrima* C.Koch, *Adonis vernalis* L., *Asparagus tenuifolius* Lam., *Marrubium peregrinum* L., *Teucrium chamaedrys* L., *Euphorbia cyparissias* L., *Artemisia pontica* L., *Achillea stepposa* Klok., *Astragalus pseudoglaucus* Klok., *Galium verum* L., *Salvia pratensis* L., etc.

In the spring of 2020, trips were made to the natural habitat of the species under study, aiming both to carry out the floristic research and to collect the botanical material, necessary for translocation. Being a very dry year, *Serratula lycopifolia* did not develop floriferous stems, so immature plantlets were taken to be planted in pots of 10 cm in diameter.

In April 2021, 10 specimens were planted in the experimental plot of the National Botanical Garden (Figure 1), for conservation and study in *ex situ* conditions and as a seed source. The use of an *ex situ* source can reduce the impact of seed collection from the wild populations by managing for the viability of these populations (Cochrane et al., 2007). *Ex situ*, the species developed well, and underwent all the ontogenetic stages (Figure 1), and vegetative propagation by plantlets from renewal buds on rhizomes was high and ranged from 5 to 16 vegetative specimens emitted annually by a mother plant.

Figure 1. *Serratula lycopifolia* under *ex situ* conditions in the experimental plot of the "Al. Ciubotaru" National Botanical Garden (Institute)

At the same time, as a result of the floristic research carried out, the natural habitat near Speia village, Anenii-Noi district, was selected for the creation of an *in situ* population. This sector represents a glade with primary steppe vegetation at the edge of the forest of *Quercus pubescens* Willd. with *Cotinus coggigria* Scop. and *Acer tataricum* L., on a South-West facing calcareous slope with an inclination between 25°-40°. The vegetal carpet of the glades is dominated by *Festuca valesiaca* Gaudin, *Stipa pulcherrima* C.Koch, *Achillea ochroleuca* Ehrh., *Teucrium chamaedrys* L., *Elytrigia intermedia* (Host) Nevski, *Centaurea marschalliana* Spreng., *Salvia nutans* L., *Helichrysum arenarium* (L.) Moench, *Asparagus verticillatus* L., *Scorzonera mollis* M. Bieb., *Valerianella brachystephana* (Ten.) Bertol., *Crepis tectorum* L., etc.

Subsequently, in May 2021, 20 plantlets of the *Serratula lycopifolia*, vegetatively obtained, were planted (Figure 2, A) and in the spring of 2022, additions were made with another 20 specimens, obtained from seeds (Figure 2, B).

A B

Figure 2. Seedlings of *Serratula lycopifolia*

Afterwards, monitoring of the newly created population was carried out, assessed the survival rate and basic morphometric parameters (number of leaves, length and width of leaf blade, etc.) were measured. In the growing season of 2023, the population numbered 15 individuals out of 40, of which three plants flowered and produced seeds (Figure 3).

Figure 3. Serratula lycopifolia in situ conditions in the Speia site

In this study we tested seed germination of Serratula lycopifolia (Vill.) A. Kern. under laboratory conditions. For testing, 50 seeds were placed in Petri dishes on filter paper in three repetitions moistened with distilled water (Figure 4). On the fifth day from the beginning of the imbibition, the first seeds germinated, and germination was recorded over 16 days, until new germinations were not observed. Based on the obtained data, the germination rate was calculated, which constituted 64-68%. The high germination rate is important for rare plant species as seed availability can be limited (Guerrant et Kaye 2007).

A B

Figure 4. *Serratula lycopifolia*: A – seed germination; B – seedling

At the same time, the germination of seeds embedded in the substrate (in greenhouse conditions) at a temperature of 18-22ºC, was tested to determine the growth power – an index representing the germination capacity of seeds (Sfeclă, 2022), which constituted 32%. This experiment showed that the seed growth power of *Serratula lycopifolia* is only half the germination rate tested in Petri dishes, so pre-sprouting seeds before incorporation into the soil would be recommended.

Conclusions

To understand the biological and ecological peculiarities of the critically endangered species *Serratula lycopifolia*, as well as the limiting factors, the natural habitat around Gordinesti village, Edinetz district was studied, its condition assessed, the structure and ecology of the existing donor-population studied. At the same time, several habitats with characteristics close to those in which *Serratula lycopifolia* grows were investigated. As a result of this, the most appropriate location was identified, according to the requirements of the species, where it would be translocated – the steppe glade in the vicinity of Speia village, Anenii-Noi district.

In the growing season of 2020, vegetative material (seedlings) of the species *Serratula lycopifolia* was taken from the taxon's natural-donor habitat for conservation under *ex situ* and multiplication conditions.

In April 2021, 10 specimens were planted in the experimental plot of the National Botanical Garden, for conservation and study in *ex situ* conditions and as a seed source. Subsequently, in May 2021, 20 plantlets of the *Serratula lycopifolia*, vegetatively obtained plantlets were planted and in the spring of 2022, additions were made with another 20 specimens, obtained from seeds. Subsequently, from May to September, the survival rate of individuals of the newly created population was monitored, the success evaluated and basic morphometric parameters were measured. As a result, in the growing season of 2023, the population numbered 15 individuals, of which three plants flowered and produced seeds. We plan to monitor the translocated population over ten years to be able to correctly assess the success of the activities to create a new population of an endangered species.

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LEVERAGING BLOCKCHAIN TECHNOLOGY TO ENHANCE SUSTAINABILITY AND TRACEABILITY IN GLOBAL AGRI-FOOD VALUE CHAINS

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Abstract

This study investigates the transformative potential of Blockchain technology in enhancing sustainability and traceability within the global agri-food value chain (GVC). Employing a qualitative research methodology, we conducted an in-depth analysis of six diverse case studies encompassing various segments of the agri-food sector across different geographical regions. The analysis explores how Blockchain can address existing challenges related to transparency, efficiency, and accountability in supply chains. Our findings reveal that Blockchain implementation significantly improves product traceability by providing immutable and real-time records, thereby enhancing food safety and quality assurance. Additionally, the technology facilitates greater operational efficiency by streamlining processes and reducing transaction costs, while also enabling more accessible and secure trade finance solutions for stakeholders. Furthermore, Blockchain contributes to social and environmental sustainability by ensuring ethical sourcing and promoting responsible production practices. Despite these promising benefits, the study identifies several obstacles to widespread adoption, including issues of interoperability between different systems, the need for standardized protocols, high implementation and maintenance costs, and complex regulatory landscapes. To overcome these challenges, the research underscores the necessity for collaborative efforts among public institutions, private enterprises, and technological innovators. Such collaborations should focus on developing unified standards, supportive regulatory frameworks, and scalable solutions tailored to the agri-food sector's specific needs. The study concludes by recommending further longitudinal research to assess the long-term impacts of Blockchain integration and to explore strategies for mitigating the identified limitations, thereby fully leveraging the technology's potential to revolutionize the global agri-food value chain.

Keywords: *Blockchain, global value chain, agri-food, traceability, transparency.*

Introduction

Food is one of the most fundamental human necessities. Despite significant advancements, even in a pre-COVID-19 and pre-Ukraine war world, over 135 million people globally experienced acute hunger. This food shortage can be directly linked to unsustainable human activities such as excessive deforestation, pollution, and economic downturns (United Nations, 2022). With the recent global pandemic and ongoing war, it is feared that by 2030, more than 840 million people will be unable to meet their basic daily food needs (Nature Editorial, 2022). The problem is exacerbated by the fact that food value chains are often very long, spanning multiple countries, making it challenging to reliably trace food products from 'farm to fork.' Additionally, more than a third of the food produced globally is wasted each year due to inefficiencies along the food supply chain (Yadav et al., 2021).

In 2015, world leaders took a significant step towards global cooperation and multilateralism by joining forces to achieve the 2030 Sustainable Development Agenda (SDGs). Among these, SDG 2 aims to ensure 'food for all' by 2030. Healthy and nutritious food can also positively contribute to better health. Thus, the interrelationship between SDG 2 (food for all) and SDG 3 (health for all) should not be overlooked. These goals are not only spatially proximate but also share a closely linked foundation based on evidence. Healthy and nutritious food can prevent many lifestyle-related diseases. Scientific evidence establishes that obesity is the primary cause of many severe diseases, such as hypertension, diabetes, and heart diseases (Ali, 2021).

As our resources remain limited and the global population continues to grow at a geometric rate, expected to surpass 8 billion in 2022, the pressing question is how can we, despite all constraints and challenges, ensure the timely achievement of SDGs 2 and 3? The question is pertinent as more than 3 billion people worldwide cannot enjoy and afford regular, healthy food (World Bank, 2020). In other words, while over 840 million people are unable to have regular daily access to food (i.e., SDG 2), the number rises to over 3 billion people globally when the discussion shifts from 'access to food' to 'access to healthy food' (i.e., SDGs 2 and 3 collectively).

Interestingly, SDG 12, namely 'sustainable consumption and production patterns,' with its focus on the supply chain, can be a crucial factor in achieving SDGs 2 and 3. This can be explained by the fact that while we cannot indefinitely increase the resources deployed to produce more food, we can certainly improve efficiencies along the food chain to increase total production (Coelli et al., 2005). Increased efficiency, both dynamic and static, contributes to higher productivity. More efficient resource use and better resource allocation, through improved production and allocative efficiency, respectively, can help obtain more output from the same limited production factors. Greater innovation, also known as dynamic innovation in industrial policy, can enhance both the quality and quantity of production. This, in turn, leads to an upward shift in the production possibility frontier (PPF) (Kokkinou, 2013).

Among the emerging technological innovations that could address these challenges is Blockchain technology. Blockchain, with its decentralized and immutable ledger system, offers a novel approach to enhancing traceability, transparency, and trust across global agri-food value chains (GAVCs). By enabling real-time tracking of food products from farm to fork, Blockchain can significantly reduce inefficiencies and food waste while ensuring that consumers receive safe and high-quality food. Moreover, the integration of Blockchain in GAVCs can help achieve SDG 12 by promoting sustainable consumption and production patterns, ultimately supporting the goals of SDGs 2 and 3.

This article delves into the potential deployment of Blockchain technology in global value chains to enhance trust in global trade and achieve health and food for all (SDGs 2 and 3). It addresses the issue from the perspective of GAVCs, which are long and complex, often extending across many countries. The industrialization of food means that GAVCs are more globally dispersed than ever. Tracing and tracking food along the global value chain is a challenging and costly task. The research question this article seeks to answer is: How can Blockchain technology be sustainably deployed across the entire global agri-food value chain, and can it help effectively track and trace food products from farm to fork, thereby enhancing consumer trust in global trade?

To systematically address this research question, the article is organized as follows. The "Introduction" section examines the interrelationships between SDGs 2, 3, and 12. The "Literature Review" section provides a review of the literature and identifies the gap in the current literature that this study seeks to fill. It also highlights the methodology followed in this research.

Material and methods

This research investigates how Blockchain technology can be integrated into global agri-food value chains (GVCs) to improve traceability and transparency. To achieve this, a multiple case study approach was adopted, following the guidelines of Yin (2003). This approach is particularly suited for exploring contemporary phenomena within real-life contexts, allowing for an in-depth understanding of the complexities involved in implementing Blockchain in GVCs.

Data Collection:

The study employed a qualitative research design, utilizing both primary and secondary data sources. Primary data were collected through semi-structured interviews with key stakeholders involved in the selected Blockchain pilot projects. These stakeholders included project managers, supply chain analysts, technology providers, and end-users. The interviews aimed to gather detailed insights into the implementation processes, challenges faced, and perceived benefits of Blockchain in enhancing traceability and transparency within agri-food GVCs.

Secondary data were gathered from a comprehensive review of project documentation, industry reports, and relevant academic literature. This included technical reports, white papers, and case study descriptions provided by the companies involved in the pilot projects. The combination of these data sources allowed for triangulation, enhancing the reliability and validity of the findings.

Case Study Selection:

The research focused on six prominent Blockchain pilot projects, selected for their relevance and diversity in application within the agri-food sector. These projects were:

- Walmart: A project focusing on enhancing food traceability and safety by implementing Blockchain across its supply chain.
- TradeLens (Maersk/IBM): A collaboration aimed at improving transparency and efficiency in global trade logistics.
- Barclays:A financial services project exploring the use of Blockchain for trade finance in agri-food GVCs.
- CORE Project: A European Union initiative to enhance supply chain security and efficiency using Blockchain technology.
- NAFTA/CAFTA: A project aimed at improving cross-border trade transparency and compliance within the North American and Central American Free Trade Agreements.
- Walmart's Customer Satisfaction Project: A second Walmart initiative focusing on leveraging Blockchain to enhance customer satisfaction through better product transparency.

Methodological Approach:

An inductive approach was employed, whereby data collection and analysis were conducted in a bottom-up manner. This approach allowed for the mapping of supply chain operations and the generation of insights directly from the data, rather than testing predefined hypotheses. The analysis involved coding and categorizing the data to identify emerging themes related to the implementation of Blockchain technology in GVCs. These themes were then used to develop a conceptual framework outlining the potential benefits and challenges associated with Blockchain integration in the agri-food sector.

Overall, the combination of multiple case studies, diverse data sources, and an inductive analytical approach provided a robust methodological framework for exploring the real-world applications of Blockchain technology in global agri-food value chains.

Results and discussions

Exploring blockchain applications across various segments of GVCs revealed promising opportunities alongside significant challenges. Here, we discuss the findings from six notable blockchain pilot projects, highlighting their impact on enhancing traceability and transparency at different stages of the supply chain (refer to Fig. 1: From Farm to Fork in a Global Agri-food Value Chain).

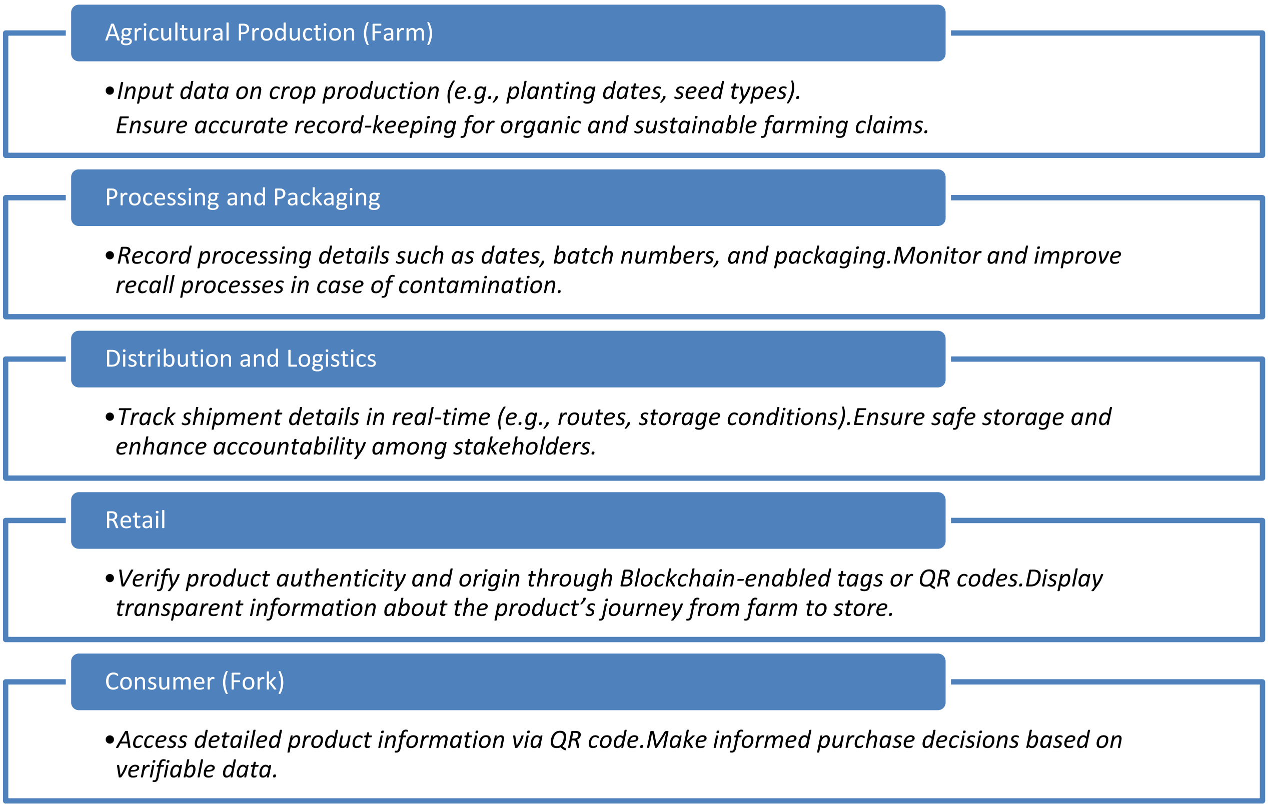

Figure 1: Integration of Blockchain Technology in the Global Agri-Food Value Chain (From Farm to Fork)
Source: Created by the authors.

Enhancing traceability and transparency

- **Improved traceability:** Projects like Walmart and TradeLens demonstrated how Blockchain can effectively track product origins, processing data, and shipment details. This level of traceability is critical for ensuring food safety, as it allows for faster and more precise recalls when food safety issues arise, ultimately strengthening consumer trust. The immutability of Blockchain records ensures that the data cannot be tampered with, providing a reliable source of truth for all supply chain stakeholders.
- **Increased transparency:** The use of Blockchain by TradeLens and Walmart provided real-time shipment information and transparent access to critical data for all involved stakeholders. This transparency empowered informed decision-making, enhanced collaboration among supply chain participants, and increased overall accountability. Blockchain's decentralized nature means that no single entity controls the data, reducing the likelihood of information asymmetry and fostering a more equitable distribution of information.

Improved efficiency and customer satisfaction

- **Reduced costs:** Blockchain technology streamlined trade finance transactions for Barclays and simplified customs processes for the CORE Project. These improvements led to reduced transaction times and lower import costs, demonstrating Blockchain's potential to enhance efficiency in GVCs. The automation of processes through smart contracts also contributed to cost reductions by minimizing the need for intermediaries and reducing the likelihood of human error.
- **Enhanced customer satisfaction:** Walmart's implementation of Blockchain not only improved traceability but also boosted customer satisfaction by ensuring that products were safe, high-quality, and had verifiable origins. The ability of customers to access product information directly through Blockchain-enabled platforms provided them with greater confidence in their purchasing decisions, reinforcing brand loyalty.

Challenges and facilitators

- **Barriers to adoption:** The analysis identified several common challenges to Blockchain adoption, including resistance to change, complex regulatory environments, managing large volumes of data, ensuring data security, and integrating Blockchain with existing systems. These challenges underscore the need for a comprehensive understanding of both the technical and organizational aspects of Blockchain implementation.
- **Facilitators for successful implementation:** Strategic collaborations, such as the partnership between Walmart and IBM, played a crucial role in overcoming some of these challenges. These collaborations provided the necessary technical expertise and resources to support successful implementation. Additionally, the success of early adopters like Walmart and Maersk/IBM has inspired other companies to explore Blockchain, highlighting the importance of demonstrated use cases in driving broader adoption.

The bigger picture

Integrating Blockchain into agri-food GVCs can contribute significantly to achieving Sustainable Development Goal No. 2, "zero hunger," by promoting complete transparency throughout the value chain. By providing a clear and unalterable record of food production and distribution, Blockchain can also streamline customs procedures, potentially reducing non-tariff trade barriers and facilitating sustainable trade.However, widespread adoption faces hurdles:

- **High initial costs:** Implementation costs can be significant.
- **Data security concerns:** Data security and privacy require careful consideration.
- **Standards and regulations:** Clear standards and regulations are necessary for interoperability and compliance.

Blockchain technology holds immense potential to revolutionize agri-food GVCs by enhancing transparency, traceability, and efficiency. To achieve successful implementation, a strategic and collaborative approach is required, along with overcoming technical, organizational, and regulatory challenges. Through collaboration, stakeholders can unlock the full potential of blockchain to build sustainable and resilient agri-food GVCs, contributing to sustainable development goals and fostering a more transparent, equitable, and efficient global trade system.

Conclusion

In conclusion, integrating Blockchain technology into supply chain management presents a promising opportunity to enhance transparency, traceability, and operational efficiency throughout the entire value chain. Successful implementation of Blockchain solutions in the agri-food sector requires the development of robust traceability systems, supported by complementary technologies such as RFID tagging, smart weighing systems, and onboard inspection cameras. Leveraging Blockchain in conjunction with these technologies and the Internet of Things (IoT) enables businesses to streamline processes, mitigate risks, and foster trust among stakeholders.

Blockchain technology offers significant benefits in terms of traceability by providing an immutable and transparent record of each stage in the supply chain, from farm to fork. This capability allows for precise tracking of product origins, processing, and movement, which is critical for ensuring food safety and quality assurance. Additionally, Blockchain enhances transparency by providing real-time access to data for all stakeholders, which fosters greater accountability and informed decision-making. This level of transparency can address issues such as food fraud and reduce inefficiencies in the supply chain.

Operational efficiency is also improved through the use of Blockchain, as it automates processes via smart contracts and integrates with technologies like RFID and IoT. This integration reduces transaction times, cuts costs, and streamlines overall operations.

However, the adoption of Blockchain in supply chains is not without challenges. Key considerations include ensuring data privacy while maintaining transparency, addressing scalability issues as transaction volumes and data grow, and navigating regulatory compliance. Effective implementation requires careful design to protect sensitive information and to establish clear standards and regulations for Blockchain technology.

Moving forward, further research and development are necessary to refine Blockchain solutions, adapt them to specific supply chain contexts, and overcome existing barriers to adoption. Strategic planning, collaboration among industry players, and innovation will be crucial in addressing these challenges. With these efforts, Blockchain technology has the potential to revolutionize agri-food supply chains, aligning with sustainable development goals and fostering a more transparent, equitable, and efficient global food trade ecosystem.

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CLIMATE CHANGE IMPACT ON VEGETABLE CROPS AND POTENTIAL FOR ADAPTATION

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Abstract

Climate change is emerging as one of the major constraints for global food security and will become more prevalent in the coming years. Agriculture is one of the leading sectors affected by climate change. This review is concerned with climate change impacts on the production and quality of vegetables and the crucial need for adaptation. Since many physiological, biochemical, and metabolic activities of plants are temperature-dependent, fluctuations in daily mean, minimum, and maximum temperature are the principal effect of climate change negatively affect vegetable production. Here is discussion of case studies involving the impact of increased temperature on the production of key vegetable crops. Climate change could improve some quality attributes resulting in the improvement of some nutritional traits, however, negative effects could be observed on product appearance. In addition, climate change variables may have indirect effects through the incidence of diseases and insect pests. The agricultural industry may be impacted by climate change, which calls for adaptation and mitigation of the negative effects on agricultural productivity, especially on the yield and quality of vegetable crops. Improved vegetable production systems, greater biodiversity utilization, the use of biotechnology and genomic strategies, the genetic engineering of various stress tolerances, and finally the development of climate-resilient vegetables are some possible adaptation and mitigation measures. To combat the effects of climate change on vegetable crops, a comprehensive strategy is required rather than a single strategy.

Key words: *Climate change, Biodiversity conservation, Food and Nutrition security, Biotechnology.*

Introduction

Natural greenhouse gases (GHGs), mainly carbon dioxide (CO_2) and methane, are essential for life on earth and are controlled by a variety of factors (cycle of carbon).
The earth's temperature would be 35°C colder on average if these GHGs weren't storing heat (Maslin, 2004). This vital cycle has been disrupted by the industrial age, which uses the carbon stored in living things to fuel industries and advance growth. Evidence relating rising GHGs to increasing global average temperatures has been accumulating since the 1960s (IPCC, 2014; Keller, 2007). Each month's average land and ocean surface temperature since the turn of the century has been higher than average temperatures for the 20th century (*Kokic et al.*, 2014), and current patterns of climate change are diverging from earlier cycles.
Numerous studies have conclusively shown that manmade GHG emissions are the primary cause of global warming since the middle of the 20th century (Maslin, 2004; IPCC, 2014). Societies, economies, and the state of the world's health would all be significantly impacted by the repercussions of global climate change (Parise, 2018; Bhattacharyya, 2019).
Since the late 19th century, the average global surface temperature has increased by around 1.62°F (0.9°C), primarily as a result of increased carbon dioxide levels and other anthropogenic emissions into the atmosphere. The majority of the warming took place over the last 35 years, with the five warmest years on record occurring after 2010, with 2016 being

the warmest year ever. In the middle of the 19th century, it was established that gases like carbon dioxide trap heat. There is no doubt that increased concentrations of greenhouse gases must result in the Earth warming.
As a result of climate change, which is now occurring and will get worse in the future decades, it is anticipated that billions of people, especially those in developing nations, would experience food shortages. Agriculture is one of the first industries to be researched in terms of potential climate change effects due to its significance to humans (Gornall et al., 2010). The consequences of climate changes on major and stable crops gained a substantial interest; however, vegetable crops did not receive a similar concern.

Physiological reactions of vegetables to climate change:

Elevated carbon dioxide (CO_2)
The main components of the Earth's atmosphere are nitrogen (78.1%), oxygen (20.9%), and a trace quantity of carbon dioxide (0.031%). Water vapor, CO_2, and trace amounts of other gases (methane, nitrous oxide, and ozone) that absorb radiation exiting the Earth's surface collectively produce what is known as the greenhouse effect. (IPCC, 2001).
The Earth's infrared light is absorbed by CO_2 and other gases, which traps heat and accounts for the warming effect. Since a large portion of all the Infrared radiation from the Earth is the source of energy; when CO_2 concentrations rise, more of this energy will be retained in the atmosphere and contribute to global warming (Lloyd and Farquhar, 2008). From pre-industrial periods to 2005, the atmospheric carbon dioxide concentrations grew by about 35% (to 0.0417%) (Lindsey, 2020; IPCC, 2007). Agriculture, in addition to industrial activity, also contributes to the emission of greenhouse gases.
Changes in the atmospheric CO_2 concentration can affect the growth and physiological behavior of plant tissues many impacts for several vegetable crops, have been thoroughly investigated (Dong et al., 2018; Cure and Acock, 1986; Idso and Idso, 1994). These investigations came to the conclusion that elevated atmospheric CO_2 affects plant water potential, stomatal conductance, firmness, seed yield, net photosynthesis, biomass output, proteins, sugars, and organic acid contents, as well as light, water, and nutrient usage efficiency.
Elevated temperatures:
A higher need for water can result from warmer temperatures because they can make it easier for air to absorb water vapor. The water reservoir in agricultural soils may be reduced or suppressed by higher evapotranspiration, which would cause water stress in plants. It is widely known that water stress tends to speed up fruit ripening in addition to reducing crop productivity (Henson, 2008). Higher temperatures can disrupt the growth and development of different plant organs by altering the morphology, anatomy, physiology, and finally the biochemistry of plant tissues. Extreme marketable yield declines may result from these occurrences. Several environmental conditions have an impact on the growth and development of vegetables.
High temperatures can have an impact on a plant's development during the stages of photosynthesis, respiration, aqueous interactions, membrane stability, and hormone, primary, and secondary metabolite levels. Depending on the species and degree of stress, high temperatures can hinder or even prevent seed germination (Motsa et al., 2015; Carter and Vavrina, 2001; Bewley, 1997). The majority of a plant's sensitivity to temperature is mediated by the plant's biochemistry. the majority of physiological activities proceed regularly in between zero and forty degrees Celsius. However, the minimum temperatures for the growth of vegetable crops are substantially smaller and, depending on the species and ecological origin, they can even be pushed below zero degrees Celsius for temperate species from cold

climates like lettuce and carrots. On the other hand, species from tropical climates, including several cucurbits, can withstand temperatures as high as 40°C (Went, 1953).
The ratio of photosynthesis to respiration has a general temperature influence on plants. For a high yield, not only should photosynthesis be high, but the ratio of photosynthesis to respiration should be significantly higher than one. The ratio described here is typically higher than ten at temperatures around 15 °C (Went, 1953). Temperatures above normal modify the activity of enzymes and the electron transport chain, which has an impact on the photosynthetic process (Sage and Kubien, 2007). According to (Moore et al., 2021; Lloyd and Farquhar, 2008) the photosynthetic process can also be indirectly impacted by higher temperatures by increasing leaf temperatures and affecting stomatal conductance.
Photosynthetic activity is proportional to temperature variations:
The rate of several enzyme-catalyzed biological reactions can be accelerated by high temperatures. But over a particular temperature many enzymes stop working at a certain temperature, potentially altering the capacity of plant tissue to withstand heat stressors. When creating a harvest index, temperature is of utmost significance. The crop will mature faster when the temperature is greater during the growing season. Exposure to high temperatures, increased levels of carbon dioxide and ozone, and other environmental factors can have a direct or indirect impact on the yield and quality of vegetable crops (Mattos et al., 2014). According to Wurr et al. (1996), crops like lettuce, celery, and cauliflower planted in higher temperatures reached maturity sooner than those produced in lower temperatures.
Vegetable crops clearly change as a result of the aforementioned climate changes. Below are prospective effects of climate change that affects vegetable production.
Climate change impacts on vegetable production:
Temperature, atmospheric CO2 concentration, and drought are the three main climatic factors that influence the production of vegetable crops.
Temperature
Since many physiological, biochemical, and metabolic activities of plants are temperature-dependent, fluctuations in daily mean, minimum, and maximum temperature are the principal effect of climate change that negatively affects vegetable production. High temperatures can cause physiological problems in a variety of vegetable crops.

Case study of different vegetable crops:

a.) Root crops

Potato
Due to its rigorous temperature and day length requirements for tuber formation, potato is one of the vegetable crops that is most susceptible to climate change. By extending the crop growth season in high altitude and temperate regions of the world, temperature rise benefits potato farming whereas, it disfavors the potato production by shortening the growing period in subtropical regions. (Sandhu et al., 2018; Ayyogari et al., 2014). The optimum tuber formation takes place at 20°C and an increase in temperature of above 21°C causes severe reduction in the potato tuber yield while at 30°C complete inhibition of tuber formation occurs (Sekhawat, 2001). A moderate harvest index is recorded at 20°C night temperatures indicating that temperature stress is limiting the partitioning of photosynthates to the tubers while a low harvest index is recorded at more than 20°C night temperatures (Pandey et al., 2009).
The growing season is shortened in subtropical regions, which is detrimental to potato output (Sandhu et al. (2018); Ayyogari et al. (2014)). When temperatures rise over 21°C, the yield of potato tubers is severely reduced, and above 30°C, tuber formation is completely inhibited (Sekhawat, 2001). Temperatures between 20 and 30°C are optimal for tuber formation. While a low harvest index is recorded at nighttime temperatures greater than 20°C, this indicates that

temperature stress is restricting the partitioning of photosynthates to the tubers (Pandey et al., 2009).

b.) Fruit crops

i. Tomato

Temperature, either alone or in conjunction with other environmental conditions, has a significant impact on tomato vegetative and reproductive processes. Sadashiva et al. (2016). As a result of reduced fruit set and poorer fruit quality, elevated temperature can result in significant productivity losses for tomatoes.

High temperatures cause aberrant floral development viability, bud drop, and reduced glucose availability, which all impair overall productivity (Hazra et al., 2007). Sunburn, lycopene synthesis disruption, the emergence of yellow regions in the damaged tissues, poor fruit set, and a delay in ripening, yellow-shouldered fruit, a white core, and blossom - end rot are signs of high temperature stress on tomatoes (Trinklein, 2012; Kader et al., 1974).

Fruit color is a crucial factor in determining whether a tomato is marketable. 25–30°C is the ideal temperature for the lycopene pigment to form in tomatoes. Lycopene begins to degrade above 27°C, and above 40°C, it is fully destroyed. Similar high temperatures over 25°C have an impact on tomato pollination and fruit set (Kalloo et al., 2001). The poor reproductive performance of tomatoes at high temperatures is caused by improper pollen synthesis, abnormal growth of the female reproductive tissues, hormonal imbalances, decreased levels of carbohydrates, and lack of pollination (Peet et al., 1997). According to Lurie et al. (1996), high temperature prevents ripening by preventing the accumulation of mRNAs associated to ripening, which prevents continuous protein synthesis, including the formation of ethylene, the buildup of lycopene, and cell-wall disintegration.

ii. Cucumber

In cucumber, a rise in temperature has a negative impact on sex expression, blooming, pollination, and fruit setting. Long days and high temperatures tend to retain vines in their male phases whereas short days and low temperatures tend to stimulate more female blossoms. In high temperatures, cucumber fruit yield reduced (Meng et al., 2004). Cucumber flowers drop off early due to extremely high temperatures (Kumar et al., 2011). Fruits become bitter when cucumber plants are subjected to heat stress during the fruit development stage (Kumar et al., 2011).

iii. Cucurbits

Temperature changes cause melons to be less sweet and cause fruits to take longer to ripen. In cucurbitaceous plants like bottle gourds and pumpkin, a warm, humid climate produces increased vegetative development and results in inadequate formation of female flowers, which affects yield (Singh, 2010).

iv. Okra

High temperatures in okra induce poor seed germination. While floral abscission and ovule abortion, flower drop in okra is observed at high temperatures above 42°C (Dhankhar and Mishra, 2001).

v. Legumes

Increased abscission of flower buds, blooms, and immature pods, as well as decreased pod output, mature pod size, and seed production, are all effects of high temperatures on Fabaceae family. High nighttime temperatures are most problematic when anthesis begins and pod development is advanced. In the presence of high nighttime temperatures, pods larger than 3 cm typically abort and shrivel rather than abscise (Konsens et al., 1991).

c. Herbs and species:

Chilli pepper: Pepper plants are unable to develop fruit when exposed to high temperatures at the post-pollination stage. High temperatures both cause and are influenced by the development of red color in ripe chili fruits (Erickson and Markhart (2002). According to

Saha et al. (2010) and Arora et al. (1987), chili plants exhibit flower drop, ovule abortion, poor fruit set, poor fruit size, and fruit drop at high temperature. According to Thuy and Kenji (2015), during temperature stress, the weight and quantity of seeds per sweet pepper fruit decreased.

Effect of atmospheric CO_2:

The development and growth of plants are directly impacted by rising atmospheric CO_2. Affected by exposure to higher CO_2 levels are physiological factors in vegetable crops.

The photosynthetic rates of potato plants growing in high CO_2 environments may initially be higher, but as the CO_2 concentration rises, the photosynthetic rates will eventually decrease (Burke et al., 2001). According to Rothan et al. (1997), the high atmospheric CO_2 content prevents tomato fruit from ripening by suppressing the expression of genes related to fruit ripening. This impact is likely owing to the stress that high CO_2 has on plants.

According to studies, plants cultivated in high CO_2 environments may exhibit faster leaf and stem growth, which could lead to denser canopies with greater humidity levels that are more conducive to disease proliferation. Lower rates of plant decomposition seen in high CO_2 environments may increase the amount of crop residue that disease organisms may overwinter on, leading to higher inoculum levels at the start of the growing season and earlier and faster disease epidemics. Higher CO_2 levels, which increase the generation of fungal spores, can have an impact on pathogen proliferation. The host plant may experience physiological changes as a result of elevated CO_2, which may strengthen the host's defenses against infections (Coakley et al., 1999).

Effect of Drought:

As average temperatures have risen due to climate change, an increase in the rate of evaporation from soil and transpiration from plants causing a drought condition has been recorded (EPA, 2021). The prevalence of drought conditions adversely affects the germination of seeds in vegetable crops like onion and okra and sprouting of tubers in potato (Arora et al., 1987). Potato is highly sensitive to drought. A moderate level of water stress can also cause reductions in tuber yield owing to an increase in transpiration from plants and soil due to climate change, drought conditions have been reported as average temperatures have climbed (EPA, 2021). The sprouting of potato tubers and the germination of seeds in vegetable crops like onion and okra are both negatively impacted by the frequency of drought conditions (Arora et al., 1987). Potato is quite drought-sensitive. Reduced tuber yield can also result from moderate water stress. (Jefferies and Mackerron, 1993; Romero et al., 2017) The drought is a problem since leafy vegetables like spinach have commercially viable succulent leaves. Their quality is decreased as a result of situations that decrease their water content (Ors et al., 2017). Increased soil salinity and water loss from plant cells due to reverse osmosis are two effects of drought. The production of most vegetables is decreased as a result of increased water loss in plant cells and suppression of various physiological and biochemical activities, including photosynthesis and respiration (Pena and Hughes, 2007).

Climate change impacts on vegetable pollination

According to Espadola (2021) and Kearns et al. (1998), pollination is an essential step in the reproduction of the majority of flowering plants, including vegetable crops. According to Memmott et al. (2007) and Hegland et al. (2009), pollinating agents' changing behavior may make climate change a hazard to pollination operations. Schweiger et al(2010). The most detrimental impact on pollinator interactions among all environmental conditions is a rise in temperature.

Lower seed output as a result of pollinator activity being negatively impacted by temperature rise. It is necessary to develop better assessments that can be used to evaluate environmental threats due to climatic change, especially global warming assessing the potential risks associated with current and emerging methods for managing pollinators and determining how these methods will affect Environment, output, and profit (Lee et al., 2009). According to Willmer and Stone (1997), several bee species have developed physiological and behavioral mechanisms that allow them to regulate the temperatures in their flight muscles before, during, and after flight. According to Corbet et al. (1993), pollination services could be significantly reduced as a result of pollinator behavior changes to avoid excessive temperatures in the future due to global warming.

Climate change impacts on vegetable quality:

Effect of Temperature:

According to Wolf and Ferguson (2000), sunburn is the most frequent temperature-related disease seen in vegetable crops. Even at relatively modest temperatures (between 25 and 30 °C), tissues exposed to direct sunlight might experience the symptoms of sunburn. Climate change-related temperature increases may cause a decline in certain vegetable harvests, the length of their reproductive cycle, which leads to a decline in the quality of the marketable product's appearance and size. In comparison to beans grown at 21/16°C, common beans cultivated at temperatures above 27/22°C (day/night) during seed development generated smaller seeds (Abdus Siddique and Goodwin, 1980). According to Bisbis et al. (2018), pea plants have been shown to mature more quickly and produce smaller seeds when exposed to brief fluctuations in temperature. Temperatures over 17-28/3-12°C (day/night) in head lettuce increased the percentage of loose and puffy heads, tipburn, and leaf chlorosis with an accumulation of bitter compounds (Wien, 1997). Onions were reported to develop more slowly when cultivated at 32°C as opposed to 27°C (Coolong and Randle, 2003). During leafy and Brassica vegetables have been linked to an increase in the tipburn disease at high nighttime temperatures (Saure, 1998). According to research by Rosales et al. (2011), high temperatures in tomato plants have been proven to reduce the concentration of carotene, lycopene, and antioxidants as well as macronutrients. According to Pett and Wolfe (2000), quick head opening, purple discoloration, and unattractive fibers in asparagus spears are all related to the detrimental effects of elevated air temperature on asparagus's apparent quality.

Increased temperatures brought on by climate change may, in some cases, improve the visual and nutritive qualities of vegetable crops. Antioxidants, which are parts of essential oils linked to flavorful characteristics, build in carrots at high temperatures (Ibrahim et al., 2006). In compared to tomatoes harvested at lower temperature periods, the carotene and antioxidant content in tomatoes has been reported to rise at higher temperatures (Rosales et al., 2011), while in lettuce, heat stress increased the tocopherol and antioxidant content (Oh et al., 2009).

Effect of Carbon dioxide ($CO_{2)}$

Reports on the effects of elevated CO_2 concentrations on the apparent and nutritional quality of vegetable crops are relatively limited.

a.) Leafy vegetables

Several studies, mostly with leafy vegetables, on the enriched CO_2 effects on product quality indicate a possible nutritional improvement resulting in enrichment in sugars, ascorbic acid, phenols, flavonoids, and antioxidants content (Bisbis et al., 2018).

Numerous researches, particularly involving leafy vegetables, on the effects of enhanced CO_2 on product quality suggest a potential improvement in nutrition due to an enrichment in the concentration of sugars, ascorbic acid, phenols, flavonoids, and antioxidants (Bisbis et al., 2018).

According to Becker Klaring (2016), red leaf lettuce cultivated at 1000 ppm CO_2 had higher levels of sugars, flavonoids, and caffeic acid derivatives. According to Jin et al. (2009), lettuce, celery, and cabbage cultivated at 800-1000 ppm CO_2 had higher vitamin C contents.

Increased total phenolic content and antioxidant capacity, as well as an increase in lettuce's total chlorophyll content, were the results of growing lettuce and spinach under elevated CO_2 conditions of 700 ppm, but several macro and micronutrients were significantly reduced in the edible parts of both species (Giri et al., 2016).

b.) Root crops

In root crops (carrot, radish, and turnip), high CO_2 (1000 ppm) resulted in the deterioration of several key nutritional indices, such as protein, vitamin C, minerals, vital fatty acids, and amino acids, which were reduced (Azam et al., 2013).
According to Hogy Fangmeier (2009), high CO_2 levels (550–680 ppm) in potato plants altered the quality of the tubers. Regarding appearance, elevated CO_2 levels enhanced common scab and tuber deformity while decreasing tuber greening but increased dry matter, starch, and vitamin C content, as well as decreased nitrate content, which negatively affects potato tuber taste and toxicological potential, were all positive effects of the high CO_2 (Hogy Fangmeier, 2009; Kumari Agrawal, 2014; Vorne et al., 2002).

c.) Fruit vegetables

Most studies on tomatoes found that CO_2 at 700–1000 ppm enhanced the amount of sugar and carbohydrates in tomato fruits, which are factors that affect flavor (Behboudian and Tod, 1995; Khan et al., 2013; Moretti et al., 2010; Wei et al., 2018).
Improvements in colour and the sugar to acid ratio were also noted (Wei et al., 2018). Although elevated CO_2 did not affect tomato fruit firmness (Wei et al., 2018), it was found that tomato fruits had decreased levels of protein, organic acids, vitamin C content and macronutrients during growth (Behboudian and Tod, 1995; Khan et al., 2013).
Effect of drought and salinity

a.) Fruit vegetables:

i. Cucumber

High salinity exposure and drought had a significant negative impact on development, photosynthesis, biochemistry, and cucumber fruit quality and texture (Ouzounidou et al., 2016). Cucumber cultivars experienced significant growth reductions after being exposed to high salinity of 150 mM NaCl (Ouzounidou et al., 2014).

ii. Tomato

According to Conesa et al. (2014), tomatoes grown under water stress had a longer shelf life and experienced less weight loss.

b.) Leafy vegetables

Furthermore, dryness has been linked to the preservation of quality during vegetable storage, although different reactions have been seen for "other crops."

c.) Root vegetables

In contrast, carrots that experienced drought stress during their growth lost more water during storage and were more susceptible to chilling damage. (Toivonen Hodges, 2011).

Climate change adaptation measures:

The ability to adapt to the changes will play a role in any potential effects of climate change on the agricultural sector (FAO, 2001). There's a demand for ways to adjust to and lessen the negative effects of climate change on agricultural output, especially on the growth, quality, and yield of vegetable crops. Advanced breeding lines are being created, and the primary vegetable crops' germplasm has been found to be drought and high temperature tolerant.

Additionally, the current research is focused on the creation of production systems with higher water-use efficiency that are anticipated to reduce the effects of hot and dry circumstances in vegetable production systems.

1.) Enhancing Vegetable Production Systems:

It is possible to increase the production of vegetable crops cultivated in hot conditions using a variety of management techniques. Among the tactics include modifying fertilizer application, precise and direct water supply to the root zone, grafting to promote disease tolerance, and the use of soil amendments.

Grafting has mostly been used to combat soil-borne illnesses that impact the growth of fruit and vegetables such solanaceous plants and cucurbits (Edelstein, 2004). However, if suitable tolerant rootstocks have been chosen, it can provide tolerance to soil-related environmental challenges such drought, salinity, and low soil temperature. Low soil temperatures were easier to tolerate for grafted plants. In contrast, eggplants grafted onto S. integrifolium x S. melongena rootstocks grew better at lower temperatures (18°C to 21°C) than non-grafted plants (Okimura et al., 1986). Solanum lycopersicum x S. habrochaites rootstocks allow their grafted tomato scions to tolerate low soil temperatures (10°C to 13°C). According to Mohammed et al. (2018), grafted watermelon plants were more tolerant of below-optimal temperatures than ungrafted ones, which allowed for production under stressful conditions.Several papers (Rouphael et al., 2018; Kumar et al., 2018; Penella and Calatayud, 2018; Schwarz et al., 2010; Agnello, 2018) have already reviewed the benefits of grafting in strengthening vegetable resistance to abiotic stressors.

2.) Biodiversity

The urgent need to develop new vegetable types for increased resilience to abiotic and biotic challenges was made clear by climate change. Breeding programs for vegetables that can withstand climate change can benefit greatly from local and traditional variety as well as the genetic diversity in the wild relatives of domesticated vegetables. Wild relatives are important sources for coping with climate change because they give plant breeders the genes and features needed to create plants that withstand biotic and abiotic challenges (Lane and Jarvis, 2007).

Agrobiodiversity—which includes wild relatives as well as the gene pool that may already reflect species reactions to climate change—is found in agricultural fields and bioreserves. Many genetically diverse plant collections, improved crop varieties, traditional landraces, and wild crop species are stored in the gene banks around the world.

3.) The Role of Biotechnology and Genomics:

Innovative technologies will be required as a supplement to conventional breeding techniques to increase agricultural productivity in unfavorable settings. Plant breeders now have a variety of techniques to improve phenotypic screening, thanks to recent developments in the biotechnology industry, including in vitro screening, molecular markers, marker-assisted selection, and genetic engineering. These methods are currently being used in research to create improved stress tolerant plants that effectively fight climate change (Arora et al., 2011; Collard et al., 2005; Mtui, 2011).

4.) Develop climate-resilient vegetables:

The capacity to foresee, get ready for, and react to dangerous climatic occurrences, trends, or disturbances is known as climate resilience. Assessing how climate change may affect existing or future climate-related risks and taking action to better manage those risks are all part of improving climate resilience.

Reduced poverty and hunger in the face of climate change are the main goals of climate-resilient agriculture. Climate-resilient agriculture places a strong emphasis on changing the

existing agricultural systems in a way that goes beyond merely maximizing output. It supports locally, regionally, and globally sustainable food production systems from an economic, social, and environmental perspective.
The development of heat, drought, and disease-tolerant vegetable varieties, soil health, irrigation, and water management in vegetable production systems are some of the strategies being used to help vegetable growers adapt to climate change and increase vegetable production. The adaptation of vegetable cultivars to rising temperatures, drought, and the occurrence of pests and diseases is another consideration.
In order to lessen losses brought on by the effects of climate change, vegetable breeders must immediately focus on the introduction of climate-resilient vegetables. Additionally, future improved vegetable types should be suited to low-input farming without the use of expensive and environmentally harmful chemicals or scarce inputs like water.

Conclusion and Future prospects

In the future years, the impact of climate change on food security will increase and is already one of the main obstacles. Among all the effects of climate change, the effects of temperature induced by global warming on field crops and vegetables are the most significant. Other pressures like drought, salt, and others are also brought on by climate change.
To combat the effects of climate change on vegetable crops, a multifaceted strategy is required rather than just one technique. The most efficient and sustainable course of action in a drastically changing climate will be a unified strategy that takes into account all viable options. Current vegetable production systems must be adjusted to the potential effects of climate change in order to reduce the impact of climate change on vegetable crops.
The focus should be on creating production systems with better water use efficiency that can be used in hot climates and during droughts. Additionally, agronomic strategies that shield vegetable crops from less-than-ideal environmental conditions must be continuously improved and used, especially in developing nations.
Vegetable breeders should concentrate on assessing the vast genetic resources in gene banks and the wild that have the capacity to adapt to a changing environment. It is advisable to use the abundant genetic diversity found in wild species and landraces as sources of selection in vegetable breeding programs.

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GROUNDWATER QUALITY FOR IRRIGATION AND DOMESTIC USES OF AIR MOUNTAINS IN SEMI ARID REGION (AGADEZ, NIGER)

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Abstract

In the arid Aïr Massif, most of groundwater resource is located in the fractured aquifers of the Precambrian basement and in alluvial aquifers. The water of fractured aquifers of the basement is generally highly mineralized and this resource is poorly characterized regarding their suitability for different uses. This groundwater supplies the population with drinking water and supports irrigation, which is the main activity of the population. Some water sources have a high content of mineral salts and other elements unsuitable for consumption and irrigation. The aim of this study is to demonstrate the suitability of water for human consumption and irrigation. The methodology, based on WHO and European Union water portability standards and SAR (Sodium Adsorption Ratio), has shown that 24% of the groundwater is suitable for human consmption as well as domestic uses and these water be from alluvial aquifers. Moreover, 79% of the groundwater sampled have electrical conductivities (EC) that exceed the WHO accepted value (250 µS/cm). These water of basement aquifers have a high levels in certain elements including SO_4^{2-}, Na^+, Mg^{2+}, F^- exceeding the WHO and EU standards are not suitable for human consumption. Nevertheless, they are used for therapeutic virtues. As for the suitability of groundwater for irrigation, it is clear that these are excellent qualities to good for irrigation in their large majorities. However, the springs water presented very high risks of soil salinization. This study made it possible to characterize the suitability of Timia groundwater for human consumption and irrigation.

Keywords: *Water quality, Basement aquifers, SAR, Soils salinization,WHO.*

Introduction

Water resources in Aïr massif, are currently affected by a high pressure, and water is essential component of life. Water resources are mainly obtained from two sources such surface and ground water (Khan and Qureshi, 2018). Quality of water is function of chemical elements which is in) water and these concentrations. This concentration define the quality of water and these different uses in water supply and in agricultural activities (Akinyemi et *al.*, 2005; Chitmanat and traichaiyaporn, 2010; Haldar et *al.*, 2020; Zainurin et *al.*,2023;Cabrera et *al.*,2023). Irrigated agriculture is important as a response to food security and economic development (Allali, 2005).

The demand for water in the Aïr area for the water consumption of the population of irrigation and livestock, seems to be very important, and will experience considerable growth due to the increase in the population rate and the development of irrigated agriculture to cope with food insecurity.

As a result, groundwater will be increasingly challenged to meet these multiple demands. Hence the need to make a thorough study on the quality of these waters for consumption and their suitability for irrigation in this context of climate variability.
In a context of climate variability whose effects are quite sensitive in the study area, characterized by a depletion of the alluvial aquifer which is the main source of water (Alhassane et *al.,* 2019). Thus, knowledge and monitoring of groundwater quality is essential for their domestic and agricultural use. In addition, in this zone considered favorable to irriagtion because of its oasian character, and the quantities produced in 2009 are of the order of 7070 tonnes of all crops combined, of which 80% of this production represents vegetables, 13% of citrus fruits and 7% of cereals (Alhassane et *al.*, 2019).
Irrigated cultivation is the main activity of the populations of the rural of Timia area and water needs are increasing because of the demographic weight of the population, the increase in farms and the cultivated areas. The implementation of water management techniques, its rational use and its qualility because some aquifers shows a high mineralized water from the aquifers of alterites(Alhassane et *al.*, 2019) are necessary to be known for divers uses.
It is in this context that the present study aims to determine the suitability of groundwater showing high salinity with salt deposits on the ground. This will help to define the different uses of groundwater in the study area.

Material and Methods

These aquifers are contained in granite and cracked gneiss, their flow varies from 1 to 8 m^3/h, the depths of water inflows are between 16 and 24 m, while static levels are between 9 and 25 m. These waters are very loaded with mineral salts.
During the sampling campaign it was sampled 40 water points composed of: 9 sources and 1 boreholes (basement aquifer), 13 wells (alteration layer) and 17 wells (alluvial layer). The sampling sites were selected on the basis of the hydrogeological characteristics of the basin, and in order to sample all aquifers in the area. In addition, the number of samples taken by slick depends on the extent of the slick, therefore the slick Since the sand is the most extensive, it has the highest number of samples.
The various structures that were sampled were chosen according to their good spatial distribution in the basin. This allowed sampling of the majority of the water tables in the study area. The alluvial aquifer of the Timia Valley, which constitutes the main water resources of the area, is the most sampled compared to other aquifers (alterites and basement). Water sources closest to the fractures were also sampled.
All sites are geo-referenced, which allowed their carry-over on the cartographic funds. On each site, the physicochemical parameters (conductivity, temperature, pH and alkalinity) are measured in situ, before rinsing and filling (2) plastic bottles in 500 ml polyethylene, respectively for the laboratory of SOMAÏR Arlit for the analysis of chemical elements.
The area of Timia, is well known for its important vegetable activity from irrigation, which is practiced on the edges of the koris. Thus, the characterization of the quality of the water available for irrigation is essential, the methods are as follows:
• Sodium Adsorption Ratio (SAR)
According to the US Salinity Laboratory Staff (1954) the physical characteristics of soils are possibly modified by the SAR. The latter is given by the following equation:

$$\mathbf{SAR} = \frac{\mathbf{Na}}{\sqrt{\mathbf{Ca} + \frac{\mathbf{Mg}}{\mathbf{2}}}}$$

With: SAR risk index; Na, Ca and Mg ions concentrations of water in meq/l.

SAR indices are used to classify waters according to their sodium risks. Thus, four (4) classes of water are distinguished, according to their suitability for irrigation. This classification is supplemented by total water salinity, expressed as EC electrical conductivity, (in four classes), (table 1).

Table 1. Classification of irrigation water according to SAR values and electrical conductivity (US Salinity Laboratory Staff, 1954).

SAR Class	**SAR Value**	**Suitability of water for irrigation**
S1	0 to 10	Water with low risk of salinization, and suitable for all soils
S2	10 to 18	Water with medium risk of salinization, can only be used for plants tolerant of salinity and for aerated soils (coarse texture) and permeable
S3	18 to 26	Water with high risk of alkalization, can be used only for plants very tolerant of salinity and for soils well drained and better with addition of organic matter
S4	>18	Water with very high risk of alkalization, not usable for irrigation
Elelectrical Conductivity (EC) Class	Value of EC (µS/cm	Suitability of water for irrigation
C1	Inferiors of 250 µS/cm	Low conductivity, water, without risk of salinization
C2	250 to 750 µS/cm	Medium conductivity, water suitable for plants with salt tolerance
C3	750 to 2250 µS/cm	High conductivity, water with high risk of salinization, can only be used for plants with high tolerance to salinity and for well-drained soils
C4	> 2250 µS/cm	Very high conductivity, water not indicated for irrigation

The second method is the Wicox method which based on the calculation of the percentage of sodium in all major cations contained in water from the following relationship:

$$Na\,(\%) = \frac{Na}{Ca + Mg + Na + K} * 100$$

The Wilcox diagram is based on the total mineralization in mg/l (x-axis) and the percent sodium value (y-axis). The water intended for irrigation is divided into five (5) groups according to its qualities which are: excellent-good, good-possible, possible doubtful, doubtful-unsuitable (poor), and improper or bad.

Results and Discussion

- Suitability of waters for human consumption

The comparison of the physico-chemical parameters of the groundwater of the Timia basin with the accepted limit values (WHO, 2011;Schulhof, 1997; Larocque,2022), (WHO standards 2011 and of the European Union 1998 (Schulhof, 1997) (Table 2) shows that for the Electrical parameters (EC), 79% of the groundwater sampled has electrical conductivities that exceed the WHO (WHO,2011) accepted value (250 µS/cm). Among these, 55% have values: 288 ≤ EC ≤ 990 µS/cm µS/cm and 24% have conductivities 1671 ≤ CE ≤ 5480 µS/cm (springs). Thus, it should be noted that only the waters of the alluvial aquifer are suitable for human consumption.

About major elements 3% of the points (springs) record SO_4^{2-} (280 mg/L) contents higher than the maximum value allowed (250 mg/L) by the European Union standard (WHO,2011). Nevertheless, this value is acceptable for the WHO standard. 5% of the works (2 wells) show values in NO_3^- exceeding the permissible value by the WHO (2011).

In basement groundwater, 24% of the springs either (9 springs) have Na^+ levels above the WHO (2011) standard (200 mg/L) while 16% (6 springs) are characterized by Mg^{2+} concentrations above the WHO limits (50 mg/L). These are mainly highly mineralized springs. Morever, for the undesirable elements, 29% of the samples collecting in the basement aquifers and alterites show fluoride contents higher than WHO standards (WHO, 2011). On the other hand, for the alluvial aquifer, the fluoride content is relatively low.

These results show that the water of the alluvial aquifer and some of the alteration aquifer are in the WHO and European Union standards (Larocque,2022;Schulhof, 1997). Thus, in the Timia basin, the water is of acceptable quality for human consumption as well as for domestic use. On the other hand, the waters of the base have high levels of certain elements (EC, SO_4^{2-}, Na^+, Mg^{2+}, F^-) far exceeding the WHO (2011) and EU standard are not suitable for human consumption. Nevertheless, they are used for therapeutic virtues.

- Suitability of groundwater for irrigation (SAR, Sodium Adsorption Ratio)

The Richards diagram (1954) (fig.1a) shows that groundwater is divided into the following classes:

- S1C1: water with low risk of sodium and low salinity, they are of excellent quality for irrigation;
- S1C2: water with a low risk of sodium and relatively medium salinity, they can be used in irrigation for plants with a salt tolerance;
- S1C3: these are waters with a high risk of salinization belonging to (2) two structures that capture the alteration reservoir (Assligh and Timia_2);
- S2C3, S3C4 and S4C4 they correspond to waters with a very high risk of salinization, and belong in majority to the springs and to a structure that captures the alterites aquifers (Abarak_Abda).

Such types of groundwater from bedrock and alterites with a high risk of soil salinization were highlighted in Liptako and Zinder (Yahouza et *al*., 2018; Souleymane et *al*., 2020; Nazoumou et *al*., 2016; Babaye et *al*., 2016). However, even in sedimentary zones these types of water are frequent and defois with very high soduim contents (Rouabhia and Djabri,2010).

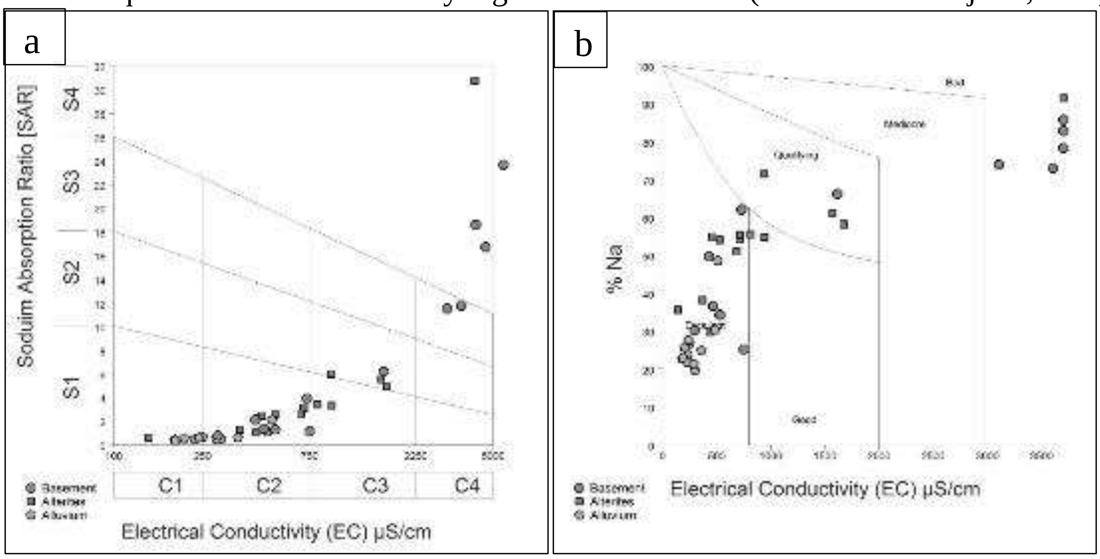

Figure 1. a) Richards diagram, for Timia Basin groundwater classification et b). Wilcox diagram for Timia Basin groundwater

- Wilcox Diagram (1948)

The diagram by Wilcox (1948) (fig.1b) shows that the groundwater of the Timia basin, in their vast majority are of excellent quality for irrigation. However, the waters of some works that capture alterites (puits_Houdou; Abarak; Kirip_Ecole) and of the basement (CE ≤ 2000 µS/ cm) are eligible for irrigation. Finally, wells (Abarak_Abda) and water from all springs with electrical conductivity (EC) above 3000 µS/cm except Tchit_in _Wazgar are mediocre to worst quality for irrigation.
In basement zones, and mainly in mountain massifs, mineralized waters with high chlorures and sulfates are evidenced (Dazy and Razack, 2010; Stober and Bucher, 1999; Zuppi et al., 2004; Muralt et Vuataz, 1993).

Conclusion

According to WHO drinking standards groundwater of Timia basin is acceptable quality for human consumption as well as for domestic use. On the other hand, the waters of the basement have high levels of certain chemical elements (EC, SO_4^{2-}, Na^+, Mg^{2+}, F^-) far exceeding the WHO and EU standard are not suitable for human consumption. From the results of the study of the suitability of groundwater for irrigation, it appears that these are of excellent to good quality for irrigation in their great majority. However, some waters, particularly basement aquifers water and alterates aquifers water, are poor, presenting very high risks of soil salinization. However, it would be good to conduct work in the future on the origin of high mineralization of spring waters because some of them are used in the theurapy of certain gastric diseases.

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BIOPHYSICAL CHARACTERIZATION OF THE IRRIGATION ALONG THE TELWA VALLEY, IN THE DEPARTEMENT OF TCHIROZERINE, AGADEZ

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Abstract

This study highlights the biophysical characteristics of Telwa valley stream crossing three municipalities in the department of Tchirozerine, Region of Agadez (Niger). The study involved a sample of 48 producers chosen from 12 villages in the three communes then quantitative data from questionnaire results was analyzed and interpreted by using descriptive statistic. For the purpose of the study, Sentinel-2 Imagery were used to produce a supervised classification map, also the area and extent of Telwa stream were determined from google earth via disitalization with ArcGIS software. The Telwa stream passes through the 3 municipalities over 102 Kilometers and largely crossing the commune of Dabaga over 63 Kilometers. It's observed more rocky environment with few and scarce vegetation in Dabaga whereas there are more bare land in Tchirozerine and Agadez. The findings revealed that major biotic constraints to irrigation include : pest attacks, weed infestations and diseases. The insects especially aphids (81.3%), birds (62.5%), parasitic weeds dominated by two most invasive species (Cyperus rotendus (L.) and Cynodon dactylon (L.)) (81.3%) and rodents (83.3%) are among the most common biological stresses to irrigation farming in the area. For abiotic constraints, they relate mainly to early drying up of the wells as experienced by 75% farmers, to water erosion causing the silting up and leaching of soil, which in return results in decrease of cropland and destruction of crops during flood events remarked respectively by more than 50% and 60% of farmers.

Keywords: *Irrigation, Contraints, Characteristics, Telwa valley, Agadez.*

Introduction

Niger Republic is a sahelian country whose climate characteristics are low rainfall, variable in time and space, high temperatures and high winds, tending to accentuate its aridity (Aliou *et al*., 2014). Despite these negative constraints, agriculture is the most source of income in Niger's economy. More than 80% of the population is active in agriculture, while the sector accounted for about 45% of Gross Domestic Product (GDP) (INS, 2012).
The sector is dominated by food crops, particularly rainfed cereals such as millet, cowpea, sorghum, maize, and rice.
Nowadays, approximately 6.5 million ha are cultivated in the rainy season and a further 73,000 ha are used for intensive horticulture production in the dry season (Ministry of Agricultural Development and Ministry of Animals Resources 2017). The irrigable land potential of Niger is estimated at 270,000 ha on the basis of exploitable waterand soil resources (FAO-AQUASTAT, 2015 and Moussa, 2016).
In the Agadez region, where the climate is much more arid with very low average rainfall, sometimes less than 200 mm per year, only irrigated cultivation is possible, the populations living there have managed to develop a vegetable and arboreal agriculture at the level of the oases established along *wadis*. The water supply is provided from superficial water table

which is fed by the stream runoff in the rainy season (Moumouni, 2014), the water catchment being done through wells whose depth generally varies from 4 to 10 meters, dug near the *kori* (Ghali et al., 2016).
In this region and other parts of the country, farming is practiced on the small land lot by using non mechanized tools where its traditional character (issaka *et al.,* 2021). The main irrigated crops in the area are vegetables; wheat and maize are practiced as cereals. This irrigation farming faces many constraints associated with biophysical factors which unfortunately hinder its development. The paper aims to identify the biophysical aspects of the irrigation area exploitations in the municipalities of Agadez, Dabaga and Thirozerine of Agadez region area in Niger. It’s specifically about to:

- ✓ describe the biophysical characteristics of the of the study area
- ✓ identify the main biophysical constraints to irrigation farming in the

Materials and Methods

The main materials used in this study consists of list of all irrigation sites in the region, a GPS for the geo-referencing of sites and surface measurements; individual and focus group discussions questionnaires for the field survey and a smart phone for taking pictures. The study relies on the use of primary and secondary data. Also, the use of GPS made it possible to collect the geographical coordinates of all the villages and gardens sampled in order to measure the total surface area of all the gardens representing the samples to highlight the irrigated land potential of the area of 'study. Google Earth and ArcGIS softwares were also used for mapping.

Sample size

The study carried out along Telwa Valley stream crossing three communes (Agadez, Dabaga and Tchirozerine) of Agadez region in Niger. In each commune, four (4) villages were selected on the basis of the importance of market gardening activity and the size of the cultivated areas. Then, four (4) producers were surveyed in each village, which resulted in a sample of twelve (12) villages and forty-eight (48) farmers/producers questioned.

Data Analysis

The study involves both qualitative and quantitative techniques of analysis. Quantitative data from questionnaire results was analyzed and interpreted by using descriptive statistic techniques including percentage, ratio, mean, and the analysis presented in tables and charts. A supervise classification map was produced to determine the biophysical characteristics of the study area and extent of Telwa valley stream.

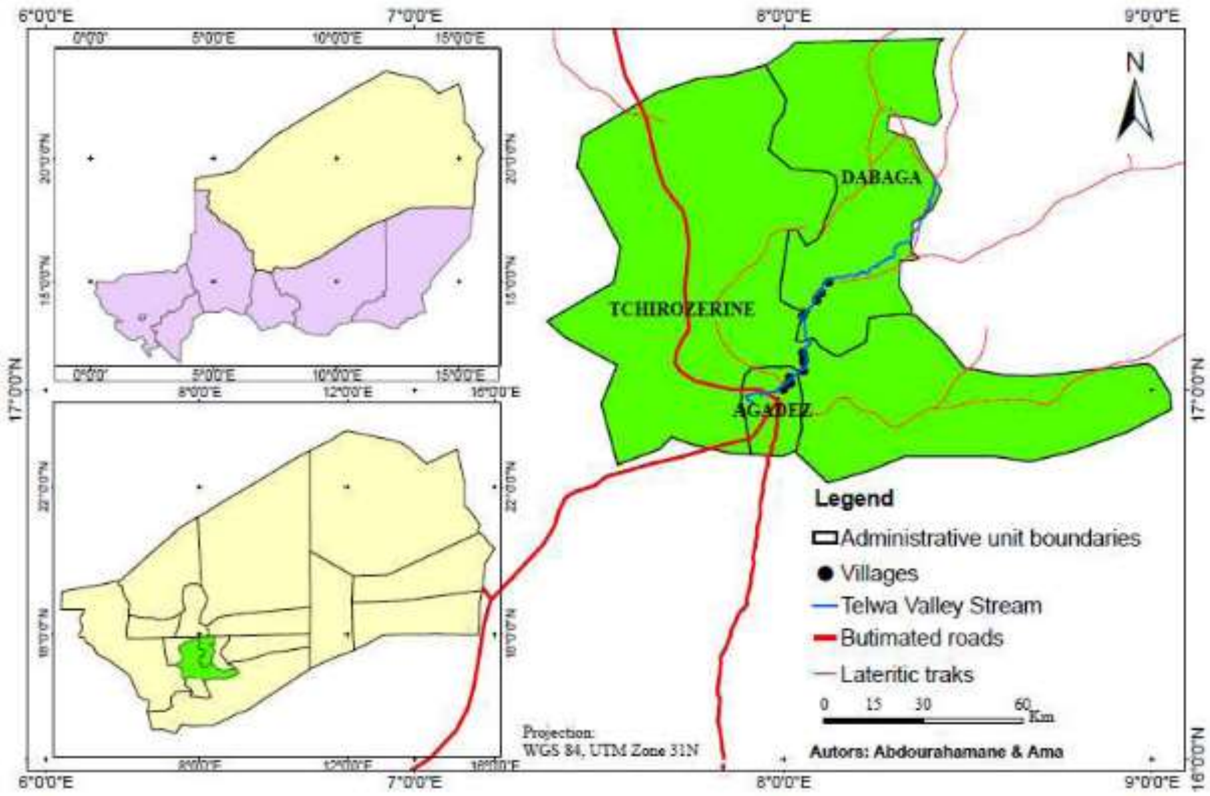

Figure 1. The Study location, the three municipalities and the selected villages

Results and discussion

Characteristics of the Study Area
Irrigated areas along the stream in the three municipalities

The Telwa valley stream passes through three communes over 102 Km from Dabaga to Agadez. The stream length for the sampled study area which extends from the village of Babaré (Dabaga Municipality) to the one of Tchiguefen (Agadez Municipality) is estimated at 45 Km long. The map below shows the irrigated land of the 3 municipalities accounting together about 3584.9531 Ha. It can be also noticed from the map, more covered irrigated land in Dabaga having alone 79% of irrigated area, Tchirozerine with 14% and Agadez municipality with only 7 %.

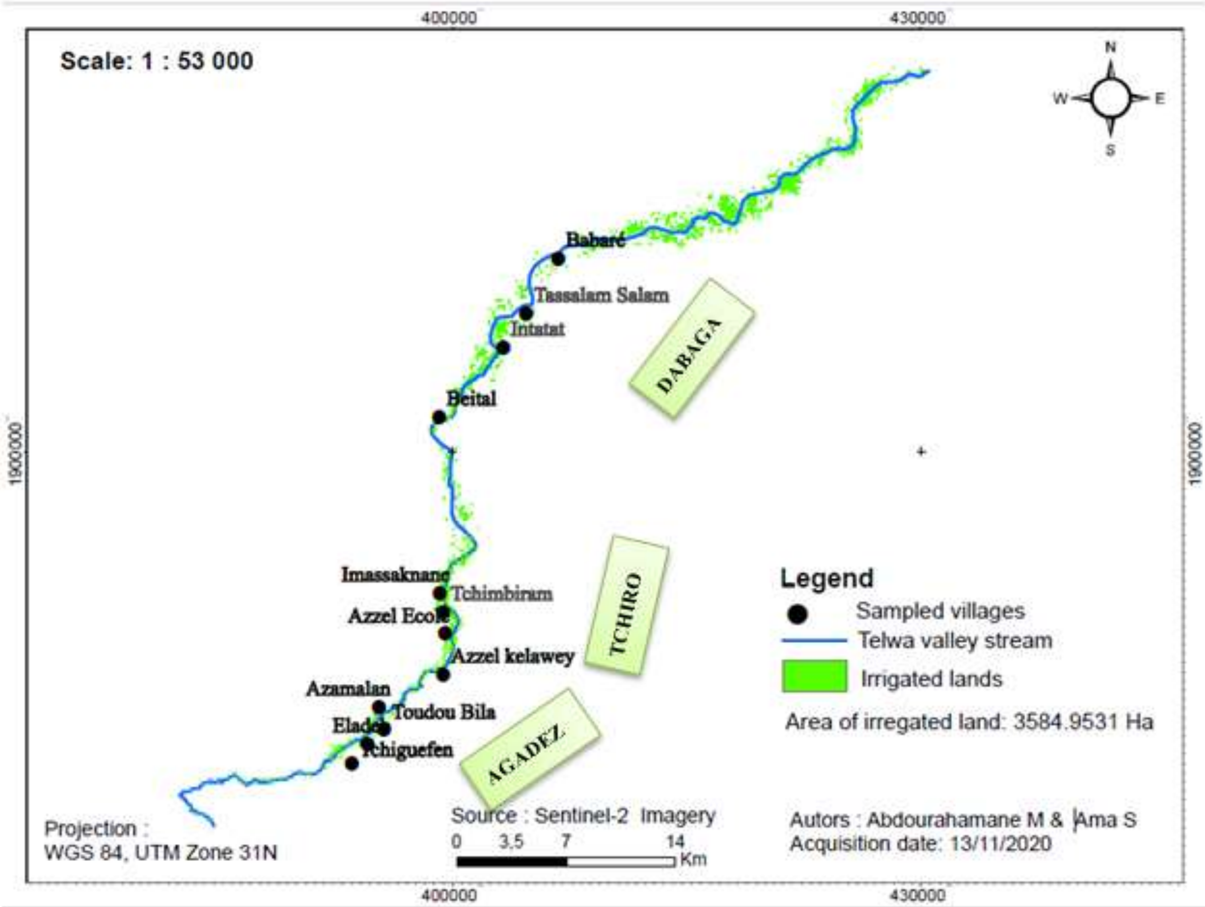

Figure 2: Irrigated Land and Villages in the 3 Municipalities
Source: sentinel-2 imagery

Biophysical characteristics of the Study Area

Using supervised classification, this study adopted seven classes which are vegetation, irrigated land, bare land, rocks, habitations and Telwa valley stream. It's important to note that there are many other small streams throughout the three municipalities where it's observed scattered irrigated lands across these communes. Also for habitations, only Agadez town was considered as it encompasses the most important build up across the Telwa stream. Furthermore, from table 1 and figure 3, it's observed more rocky environment concentrated in Dabaga, with few and scarce vegetation in the area whereas there are more bare lands in Tchirozerine and Agadez. The irrigated aera is only observed across Telwa stream.

Table 1. Area coverage of classes (in hectare: ha)

Municipality	Végetation	Rocks	Bare lands	Build up	Irrigation
Tchiro	81066.6	377005.5	446700.3	-	501.1
Dabaga	17296.1	250973.4	52021.9	-	2825.6
Agadez	3552.6	14033.8	20544.7	2622	258.3

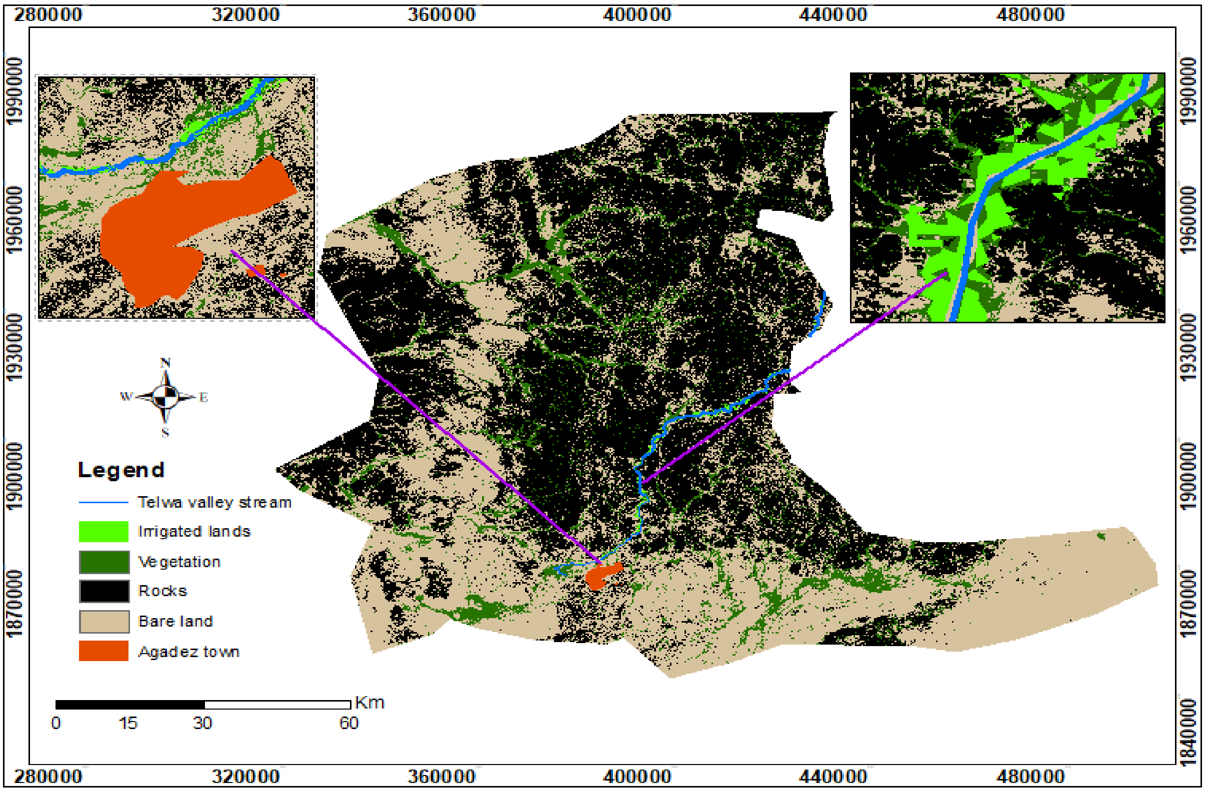

Figure 3. Map Showing the Characteristics of Study Area

Source: sentinel-2 imagery

The main constraints to irrigation farming along Telwa valley

The study identified the most common biotic and abiotic constraints in the three municipalities.

Biotic constraints

The figure 4 illustrates the main biotic constraints experienced in the area concerned by the study. Indeed, the invasive insects, rodents, weeds, birds, diseases, mites and other pest are the most important constraints with proportions of farmers’ responses.

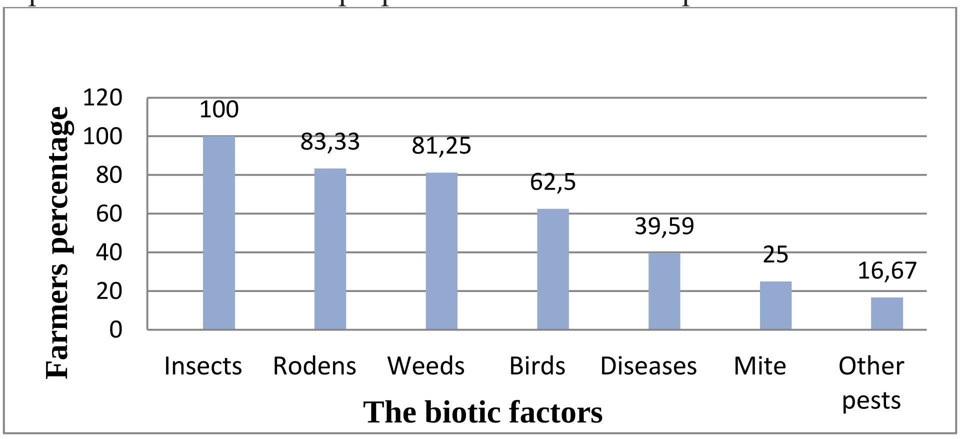

Figure 4. Main Biotic Constraints to Vegetable Production in the 3 Municipalities

Table 2. Relative Incidence of the Main Biotic-Constraints According to the Production Area.

Bio-aggressors	**Percentage frequency (%)**				**Main affected crops**
	Agadez	Tchirozerine	Dabaga	Total	
caterpillars	27.1	27.1	12.5	66.7	Cabbage, tomato, maize, moringa, onion
Aphids	25.0	29.2	27.1	**81.3**	Squash, cucumber, onion and nightshades
cucurbit fly	0.0	16.7	6.3	22.9	All cucurbit crops
Mylabris	0.0	4.2	8.3	12.5	Cucurbit crops (mostly the Squash)
Locusts	16.7	2.1	0.0	18.8	Almost all leaf crops
Mite	16.7	6.3	2.1	25.0	Nightshed crops and moringa
Birds	22.9	22.9	16.7	62.5	Wheat, tomato, pepper and newly sown seeds
parasitic weeds	22.9	33.3	25.0	**81.3**	Cyperusrotundus and Cynodondactylon infeste all crops
Rodents	27.1	31.3	25.0	**83.3**	Young plants (seedlings) and fruits
Diseases	16.7	12.5	10.4	39.6	Tomato and onion
Jackal & Camel	6.3	4.2	6.3	16.7	Jackal targets melon & water melon and camel all crops
N = 48					

The table above summarizes all biological constraints encountered by peasants in their farming practices in percentage frequency within the three municipalities. As reported by farmers each type of crops is specifically most vulnerable to one or more bio-aggressors attacking a specific part of the crop.

Thereby, aphids, birds and rodents have the most significant occurrences and relatively well distributed across all communes, whereas, constraints related to mylabris, locusts, jackals and camels are less experienced in the area. However, locust and red spiders attacks are more perceived in Agadez and occur less in Dabaga.

Abiotic Constraints

The table 2 shows the major physical constraints affecting the development of irrigated crop production in the Telwa valley area.

Table 3. Relative Incidences of Abiotic Constraints in the 3 Municipalities

Related Abiotic constraints	**Percentage frequency (%)**			
	Agadez	Tchirozerine	Dabaga	Total
Waterlogged gardens	16.7	8.3	18.8	43.8
Decrease in arable land (size)	18.7	12.5	29.2	**52.1**
Leaching of land (soil)	8.3	12.5	18.8	39.6
Bank erosion	12.5	8.3	8.3	29.2
Destruction of crops	12.5	27.1	25.0	**64.6**
Early drying up of wells	27.1	31.3	16.7	**75.0**
Waterproof bedrock	4.2	20.8	10.4	35.4
Extreme temperatures	16.7	16.7	4.2	37.5
N=48				

The early drying up of wells is the most physical constraint mentioned by farmers. About 75% of gardens are no longer operational from the month of April because of water shortage. Also, violent water flow of Telwa stream is related to many other constraints. The heavy rains combined with rocky environment and relief slopes in the area result in overflow of stream causing substantial damage on crops and cropland. These constraints include crop destruction as stated by 64.6% farmers, water logging 43.75%, decrease in arable land 52.1%, and leaching of land 39.6% which contribute to soil fertility loss. Other problems are related to waterproof bedrock and extreme temperatures.

Conclusion

This study allowed identifying the biophysical characteristics of irrigation area along Telwa valley. From the produced maps it can be noticed both the most and less irrigated areas along this valley and this can be served as a guide to Public and NGOs’ farmers partners interventions in the area. The provision of reliable water supply all year long and protection of banks along Telwa stream are the most important factors to expend irrigation farming and increase its productivity in the area. The incidence rise of some pests, diseases and weed infestations represents also another constraint which could affect significantly the irrigation productivity in the area, where urgent and adequate response is needed to tackle or mitigate these issues.

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OPTIMIZING PHOTOSYNTHETIC MICROBIAL FUEL CELLS: EFFECTS OF ELECTRIC STIMULATION ON BIOFILM GROWTH

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Abstract

Photosynthetic microbial fuel cells (pMFCs) are a developing technology for sustainable energy production, harnessing the combined effects of biological and electrochemical processes. In a typical pMFC, microorganisms form a biofilm on the anode and oxidize organic substrates to produce electrons, which are transported through an external circuit to the cathode, generating electricity. In the cathode compartment there are photosynthetic organisms, which make the difference comparing to typical microbial fuel cell (MFC). In this case these organisms are *Chlorella vulgaris* and *Arthrospira platensis*. Photosynthetic microbial fuel cells can be used for applications such as renewable energy generation and wastewater treatment. This study explores the influence of electric stimulation on biofilm growth and the overall functionality of pMFCs. Various stimulation voltages were applied to determine their effect on biofilm formation and microbial activity, with the most effective voltage identified as 1 V. Different stimulation periods were also tested, and the best results were obtained with constant 4-day stimulation at the start of the experiment. Additionally, the effect of electric stimulation was examined in the presence of *Chlorella vulgaris* and *Arthrospira platensis* in the cathode chamber, yielding some interesting results. These findings contribute to the optimization of pMFCs through bioelectrical regulation, offering potential advancements in renewable energy technologies and wastewater treatment applications.

Keywords: *microbial fuel cells, wastewater treatment, electric stimulation, renewable energy, biofuels.*

Introduction

Photosynthetic microbial fuel cells (pMFCs) are an innovative technology for sustainable energy production. By combining microbial fuel cells with photosynthetic organisms, pMFCs harness the combined power of biological and electrochemical processes to create electricity (Niju et al., 2021). In a pMFC, microorganisms form biofilms on the anode surface, where they break down organic materials, releasing electrons that are transported through an external circuit to the cathode, generating electricity (Obileke et al., 2021). This technology has a wide range of potential applications, including renewable energy generation and wastewater treatment, as it can use organic waste as a fuel source while producing clean electric energy (Khandaker et al., 2021).
In recent years, there have been significant advancements in microbial fuel cell technology, particularly in the development of electrode materials and configurations (Zhou et al., 2011). These developments have led to improved electron transfer mechanisms and overall system efficiency. Researchers have been exploring the potential of MFCs for wastewater treatment and electricity generation, which has sparked increased interest in this technology (Schäfer et al., 2020). Additionally, MFCs have been used for resource recovery of nutrients, critical metals, desalination, and biosensing (Paucar and Sato, 2021). Notable large-scale projects for

wastewater treatment (e.g., Electrogenic BioReactor) and electricity generation (e.g., Plant-e, light-emitting diodes) have been undertaken (Kalathil et al., 2018). Despite these advancements and applications, further research is needed to fully unlock the potential of MFCs and pMFCs. The choice of electrode materials, especially the anode, plays a crucial role in achieving the highest efficiencies of this technology (Yaqoob et al., 2020). Upgrades in the cathode chamber, such as microalgae and cyanobacteria cultivation, open new possibilities in the field of microbial fuel cells, leading to the development of photosynthetic microbial fuel cells due to the photosynthesis process conducted by algae and cyanobacteria (Elshobary et al., 2021).

Microbial fuel cells utilize bacteria to convert organic substrates into electrical energy. The performance of MFCs can be significantly enhanced by stimulating the bacteria involved in the process. Various methods are employed to stimulate these bacteria, focusing on optimizing the conditions for their growth and activity, which in turn boosts the efficiency of electron transfer and power output (Pandya et al., 2024). Stimulating bacteria in MFC is a multifaceted approach involving genetic, bio-, and electrochemical stimulation and engineering strategies. By optimizing biofilm formation, electrode materials, environmental conditions, and using mediators, the activity of bacteria can be significantly enhanced, leading to improved efficiency of MFCs in energy production (Angelaalincy et al., 2018; Varbanov et al., 2019).

The main goal of this research is to explore the impact of electric current on cell adhesion on the anode surface. Specifically, the study aims to understand how electric stimulation affects biofilm formation on the anode. The underlying hypothesis is that specific levels of electrical current stimulation can accelerate bacterial growth and enhance the electrochemical activity of the anode, thereby improving the overall efficiency of pMFCs. Additionally, it is assumed that introducing microalgae and cyanobacteria into the cathode space can significantly increase the efficiency of electric current and photosynthetic organisms’ growth.

Materials and methods

An experimental setup was designed to study the effects of electric current on biofilm growth and pMFC functionality. The setup included four glass reaction kits, each with two 1000 ml chambers. These chambers were connected using a *Nafion* perfluorinated ion exchange membrane, secured with a metal clamp to prevent leaks. The anode chamber was continuously mixed with a magnetic stirrer, while the cathode chamber at the first stage was filled with Phosphate-buffered saline (PBS, 1X) and aerated to maintain proper oxygen levels. The anode, which is a crucial part of the setup, was created using a 10x12 cm carbon felt with a high surface area and favorable properties for biofilm formation. The cathode was a 15 cm long graphite rod chosen for its stability and conductivity. In order to provide electric stimulation, an additional 6 cm long graphite rod electrode was introduced into the system. These electrodes were connected using external copper wires, and both voltage and current were accurately monitored using meters from the SCAME brand. Various voltages ranging from 0.5V to 4V were applied to the system, with stimulation periods varying from sequences of 15 minutes (four times a day over 24 hours) to continuous stimulation for up to four days.

Microbial strains necessary for biofilm development were sourced from the local wastewater treatment plant in Olsztyn, specifically from the anaerobic chamber. These strains were cultivated using a nutrient solution prepared according to the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) 826 medium for *Geobacter sulfurreducens*, which included components such as NH_4Cl (1.50g), Na_2HPO_4 (0.6g), KCl (0.10g), Na-acetate (0.82g), $NaHCO_3$ (2.50g), Na_2-fumarate (8.00g), along with essential vitamins and minerals to support optimal microbial growth and activity.

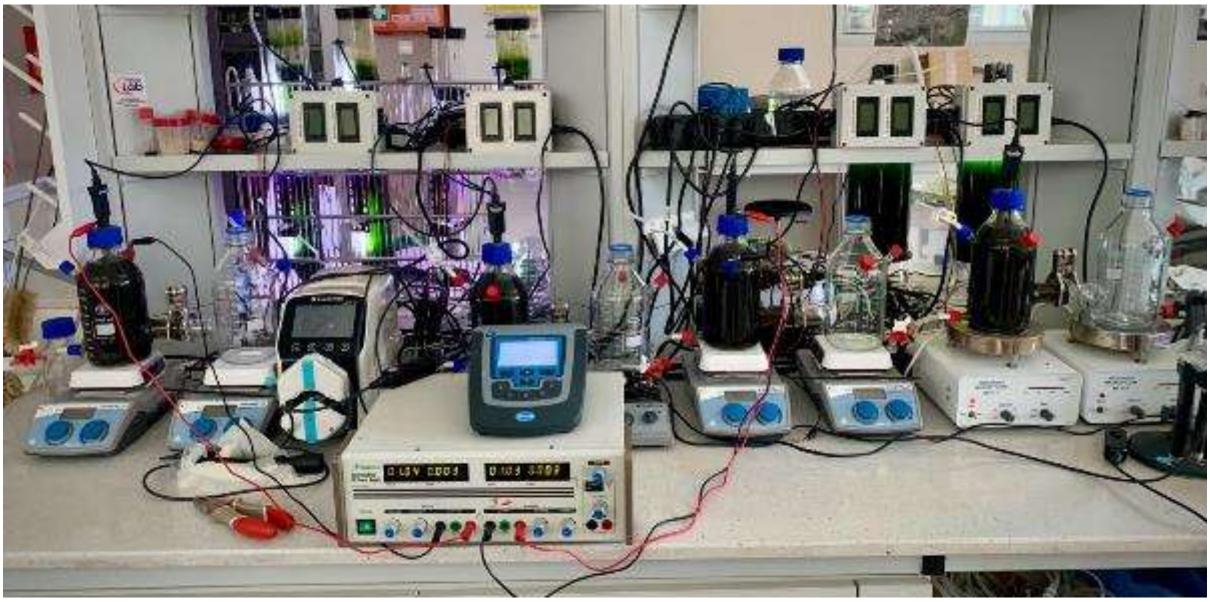

Figure 2 pMFC reactors laboratory setup with electric stimulation (R1 and R3).

In the next stage, *Chlorella vulgaris* and *Arthrospira platensis* were introduced into the cathode chamber, instead of the PBS. A volume of 50 ml of each type of microalgae and cyanobacteria was added, with the remaining reactor volume filled with a nutrient solution based on industrial fertilizers *Azofoska* and *Polifoska*, prepared at the Department of Environmental Engineering of the University of Warmia and Mazury in Olsztyn, Poland. The algae and cyanobacteria, sourced from ongoing cultivations at the department, were provided with adequate lighting through an *Aquael Leddy LED* lamp with a color temperature of 9000 K. To ensure sufficient carbon dioxide supply for the growth of *Chlorella vulgaris*, aeration was employed, utilizing atmospheric air with a carbon dioxide (CO_2) concentration of 0.04%.

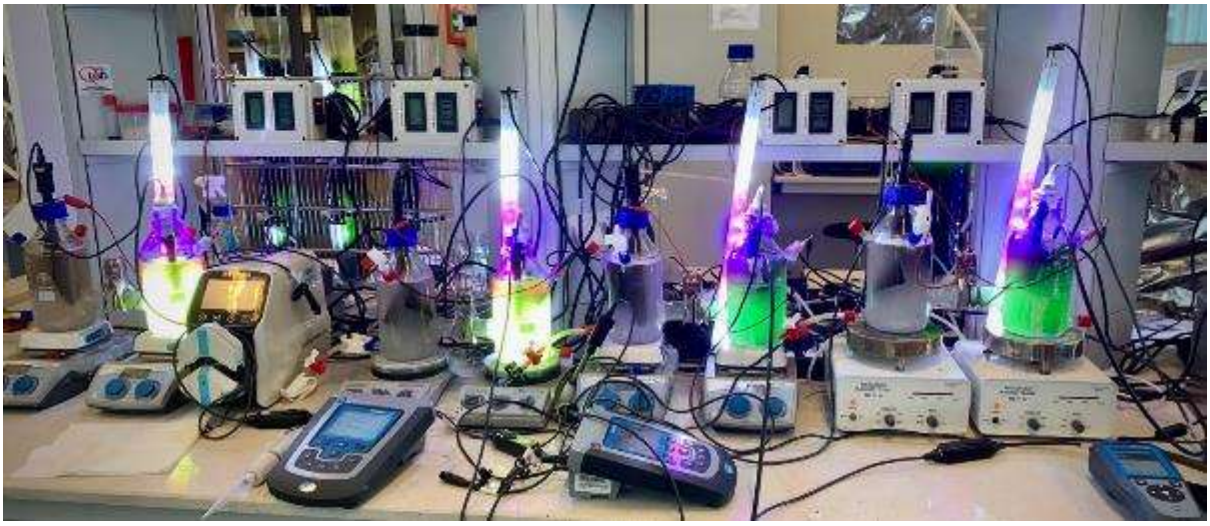

Figure 3 pMFC reactors laboratory setup with Chlorella vulgaris (R1 and R2), and Arthrospira platensis (R3 and R4) cultivation in cathode chamber

Biofilm growth and pMFC performance were rigorously assessed using a variety of methods. Dry mass tests were conducted to quantify biofilm growth, while continuous monitoring of voltage output provided insights into the electrical performance of the system. The efficiency of the wastewater treatment process was evaluated through measurements of Chemical Oxygen Demand (COD), Total Nitrogen (T_N), Total Phosphorus (T_P), Ammonium (NH_4), Orthophosphates (PO_4), Potassium (K), and Iron (Fe) content. These tests were conducted using a HACH Lange UV-VIS spectrophotometer DR5000 and HACH HT200S Nitrogen High Temperature Thermostat with dedicated cuvette tests. Additionally, Total Organic Carbon (TOC) was analyzed using a Shimadzu TOC-L Total Organic Carbon Analyzer with SHIMADZU ASI-L Auto-sampler. Algal growth was monitored with a BBE Moldaenke AlgaeOnlineAnalyser, and pH and potential differences were measured with a HACH Lange HQ440d multi-meter.

Results and discussion

While investigating the influence of electrical stimulation on biofilm growth and the overall performance of photosynthetic microbial fuel cells, the experiments varied in stimulation voltages and durations and also incorporated microalgae *Chlorella vulgaris* and cyanobacteria *Arthrospira platensis* as described above to evaluate their effects on system efficiency.

Different stimulation voltages ranging from 0.5V to 4V were applied to the pMFCs. It was observed that a voltage of 1V yielded the most favorable conditions for biofilm growth, resulting in a denser and more uniform structure compared to other voltages. The biofilm formed under this condition exhibited enhanced electrochemical activity of the anode. Optimal stimulation was achieved by applying 1V continuously for four days at the beginning of the experiment, significantly accelerating bacterial growth. In contrast, higher voltages, particularly those exceeding 4V, proved detrimental, causing bacterial cell death and reducing biofilm density and pMFC performance.

Continuous monitoring of the voltage output throughout the experiments revealed that reactors stimulated with 1V displayed a rapid increase in voltage output, reaching up to 200 mV. These reactors achieved a shorter start-up time and maintained higher voltage outputs over extended periods, indicating improved longevity and stability. Conversely, reactors exposed to higher voltages exhibited delayed start-up times and lower overall voltage outputs, underscoring the adverse effects of excessive electrical stimulation on microbial activity.

The wastewater treatment efficiency was evaluated by measuring the reduction in Chemical Oxygen Demand, Total Nitrogen, Total Phosphorus, NH_4, PO_4, and Iron levels. Reactors stimulated with 1V for four days demonstrated the highest reduction in these parameters, signifying effective wastewater treatment. This suggests that optimal electrical stimulation not only enhances electricity production but also improves the biodegradation and removal of contaminants in wastewater.

The introduction of *Chlorella vulgaris* into the cathode chamber had a positive impact on both electricity generation and algal growth. Reactors that had undergone prior stimulation of 1V for four days and subsequent addition of *Chlorella vulgaris* showed increased voltage outputs and enhanced algal biomass. This symbiotic relationship between the algae or cyanobacteria and the bacteria in the anode chamber likely contributed to the improved performance. However, the addition of *Arthrospira platensis* did not yield significant benefits, possibly due to the specific reactor configuration, which might have caused *Arthrospira platensis* to accumulate in the constriction between the anode and cathode spaces, thereby limiting its effectiveness.

The results of this study underscore the importance of controlled electrical stimulation in enhancing pMFC performance. Moderate electrical stimulation, particularly at an optimal voltage of 1V for a continuous four-day period, was found to boost biofilm growth and electrochemical activity significantly. These findings align with previous research suggesting that electrical stimulation can accelerate microbial metabolism and biofilm formation. However, it is crucial to optimize stimulation parameters as excessive stimulation can harm microbial communities.

Moreover, the integration of *Chlorella vulgaris* into the system further amplified these benefits, highlighting the potential of combining microbial and algal cultures in pMFCs. This dual system not only enhances electricity generation but also offers additional advantages in biomass production and wastewater treatment. Future research should focus on optimizing reactor design to better accommodate different types of microalgae, and further explore the interactions between bacterial and algal communities in pMFCs.

Conclusion

The study has demonstrated the significant impact of electrical stimulation on the growth of biofilm and the overall functionality of photosynthetic microbial fuel cells. Through a series of experiments varying in voltage and duration, it was found that a stimulation voltage of 1V, applied continuously for four days, optimally enhances biofilm formation and pMFC performance. This optimal stimulation condition accelerated bacterial growth and increased the electrochemical activity of the anode, resulting in higher and more stable voltage outputs.

Additionally, the integration of *Chlorella vulgaris* into the pMFC system was shown to further improve both electricity generation and biomass production. This symbiotic relationship between bacteria and algae enhances the overall efficiency of the pMFCs, providing a dual benefit of energy production and effective wastewater treatment. However, the addition of *Arthrospira platensis* did not yield the same benefits, indicating the need for further reactor design optimization to accommodate different microalgae and cyanobacteria types.

The findings underscore the potential of controlled electrical stimulation in improving the performance of pMFCs. By optimizing stimulation parameters, it is possible to significantly enhance microbial metabolism and biofilm formation, leading to better energy and wastewater treatment efficiencies. Future research should focus on refining reactor configurations to maximize the benefits of combining microbial and algal cultures and further exploring the interactions between these communities within pMFC systems.

In conclusion, the study provided valuable insights into the role of electrical stimulation in pMFCs and highlighted the potential of integrating microalgae and cyanobacteria to create more efficient and sustainable bioelectrochemical systems. These advancements could lead to more effective applications in generating renewable energy and environmental bioremediation.

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ENSEMBLE EVALUATION AND MEMBER SELECTION OF REGIONAL CLIMATE MODELS FOR CROP MODEL IMPACT ASSESSMENT IN CENTRAL GREECE

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Abstract

Climate change poses a significant challenge to Greek agriculture, impacting crop productivity and sustainability. Wheat is a crucial crop, primarily grown in regions with favorable climatic conditions and fertile soils. Climate change negatively affects wheat production through increased temperatures, extreme weather events, and changes in precipitation, leading to reduced yield and seed quality. The impact varies by region, with high-altitude areas experiencing temperature fluctuations and reduced snow cover, while coastal areas face sea-level rise and coastal erosion. Practices such as cultivating resilient wheat varieties, adjusting sowing dates, and improving water management are essential. Climate change impacts increasingly rely on ensembles, which combine multiple climate models to balance individual model errors. Careful selection and evaluation of ensemble members is essential for accurate predictions. In this study, a region in central Greece was selected for its diverse elevation and climate conditions. Data from eight meteorological stations for the period 2006 to 2023 and 11 EURO-CORDEX regional climate model simulations for emission scenario RCP4.5 were used. The selection methodology ranked models based on the sRPI index, which evaluates temperature deviation performance. Preliminary results from the calibrated CERES-Wheat model in Greek conditions showed that selecting only the best simulation scenarios provided more reliable predictions for flowering, maturity, and potential yield compared to using the full ensemble. Careful selection of ensemble members based on climate is expected to ensure more reliable agronomic model predictions, helping farmers better adapt to climate change and maintain crop sustainability and productivity.

Keywords: *ensemble model evaluation, climate change, wheat production, CERES-Wheat, simulation scenarios, Greece.*

Introduction

Climate change significantly impacts crop productivity and sustainability, posing a challenge for Greek agriculture (IPCC, 2023). Among the various crops cultivated in Greece, wheat holds particular importance. It is mainly grown in Thessaly, Macedonia, Thrace, and central Greece, areas known for their good climate and fertile soil (Koutsika et al., 2010). However, the effects of climate change (rising temperatures, extreme weather events, and alterations in precipitation patterns, etc.) are increasingly threatening wheat production, leading to reduced yields and compromised seed quality (Farooq et al., 2023; Jägermeyr et al., 2021).

The impact of climate change on wheat production differs among various regions in Greece. High-altitude areas experience significant variations in temperature and reduced snow cover, while coastal areas face the difficulties posed by rising sea levels and coastal erosion. To mitigate these adverse effects, modern agricultural practices, such as cultivating resilient wheat varieties, adjusting sowing dates, and enhancing water management strategies, are crucial (Collins & Chenu, 2021).

Climate data analysis indicated a general trend of increasing temperatures and changing precipitation patterns across Central Greece. Average temperatures are projected to rise by 1.5°C to 2.5°C by mid-century, with more pronounced increases during the summer months (Georgoulias et al., 2022). Precipitation patterns are also expected to vary, with some models predicting drier conditions and others indicating increased winter rainfall. These changes are expected to impact the wheat growing season, affecting crop development stages and yields.

To mitigate the adverse effects of climate change, several adaptation strategies are recommended. These include cultivating resilient wheat varieties with enhanced tolerance to heat and drought, adjusting sowing dates to avoid peak temperature periods and align with favorable climatic windows, and improving water management through efficient irrigation practices and water conservation techniques (Collins & Chenu, 2021). The adaptation strategies proposed here are essential for sustaining wheat production in central Greece amid climate change, supporting farmers in making informed decisions to enhance crop resilience and ensure food security.

Accurate climate analysis is fundamental for developing effective adaptation measures. Such analysis often relies on ensembles, which combine multiple climate models to mitigate individual model errors and provide a comprehensive picture of future climate scenarios. Reliable predictions necessitate thorough selection and evaluation of ensemble members. The targeted selection of ensemble members, based on climate variables and geomorphological characteristics, is anticipated to ensure more accurate agronomic model predictions (Minaei et al., 2022; Ruane & McDermid, 2017; Wada et al., 2023). These advances help farmers adapt to climate change, thereby ensuring the sustainability of Greece's agricultural sector and maintaining crop productivity (Brunner et al., 2020; Knutti et al., 2017; Merrifield et al., 2020). In this study, a region in central Greece was chosen due to its varying elevation and climatic conditions. The research utilized data from eight meteorological stations for the period 2006 to 2023 and 11 EURO-CORDEX regional climate model (RCM) simulations across emission scenario RCP4.5. Models were ranked using the sRPI index, which assesses temperature deviation performance of RCMs.

Materials and methods

The study focuses on a region in central Greece, due to its diverse elevation and climatic conditions, providing a comprehensive landscape for studying the impacts of climate change on wheat production. Data were collected from eight meteorological stations within this region (Fig. 1). These stations provided daily observations of precipitation (mm), maximum and minimum temperatures (^{o}C), while solar radiation data were provided from the Copernicus Atmosphere Monitoring Service (CAMS) (Guevara et al., 2021) for the period from December to June (i.e., the growing season) over 17 years (2006-2023). These stations are located at altitudes ranging from 98 to 760 meters above sea level.

Additionally, simulated climate data were obtained from 11 Regional Climate Models (RCMs) of the EURO-CORDEX project, with a spatial resolution of approximately 12.5 km. The RCMs simulated climate conditions under emission scenario RCP4.5 (Georgoulias et al., 2022). The consistency between the simulated and observed data was guaranteed by utilizing data from the grid cells that contained the meteorological stations (Figure 1).

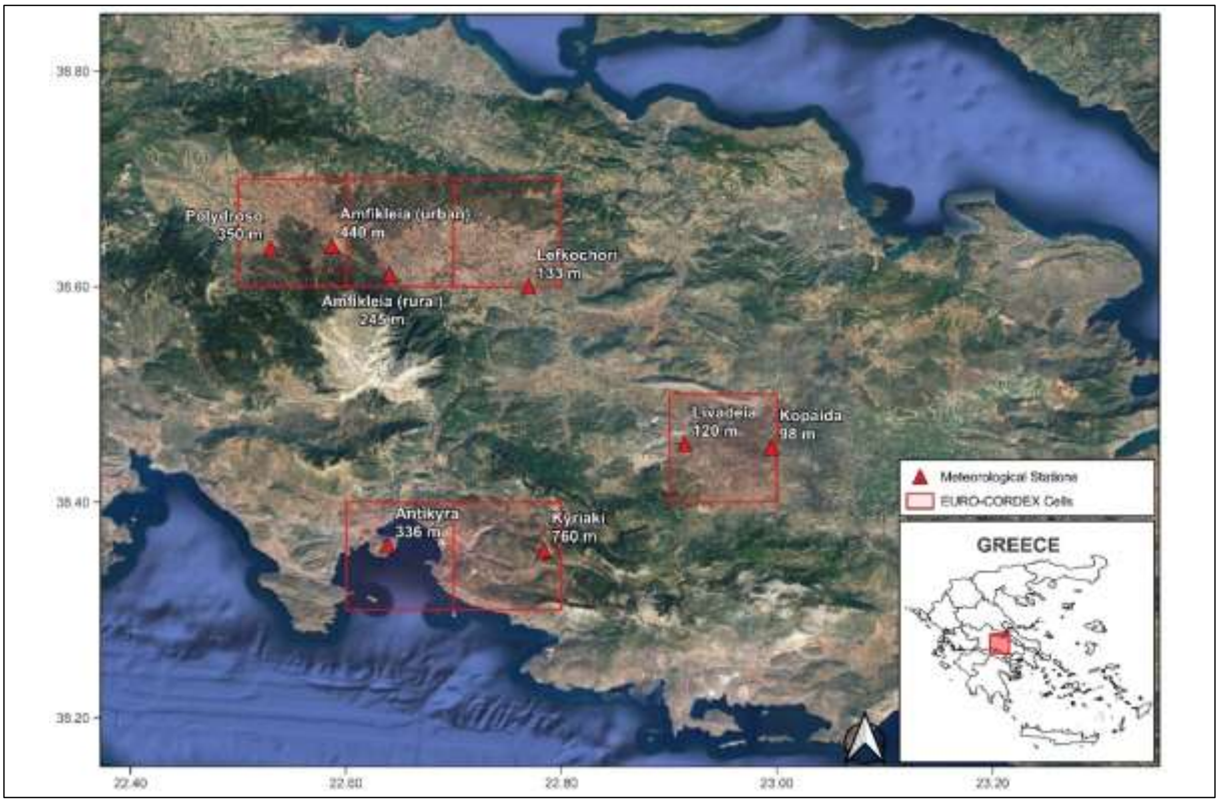

Figure 1. Study area with meteorological stations (red triangles). Their elevations and the respective EURO-CORDEX cells (red rectangles) are also shown

To ensure the selection of suitable climate model ensemble members, the methodology proposed by Aschonitis et al. (2019) was adopted. This method involves evaluating each of the 11 climate models using the standardized Root Mean Square Error index (sRPI) for each emission scenario. The sRPI index assesses model performance based on 12 statistical criteria that measure the deviation of temperature simulations from observations (Table 1). Models were ranked accordingly, with the models being categorized into top-performing models (sRPI > 50%) and low-performing models (sRPI < 50%) (Table 2).

Table 1. Statistical Criteria Used to Evaluate Climate Models

Abbreviation	Statistical Criterion
d	Index of Agreement
NSE	Coefficient of Efficiency (Nash-Sutcliffe efficiency)
KGE	Kling-Gupta efficiency
VE	Volumetric Efficiency
AME	Absolute Maximum Error
MAE	Mean Absolute Error
MBE	Mean Bias Error
RMSE	Root Mean Squared Error
R4MS4E	Fourth Root of the Mean Quadrupled Error
RAE	Relative Absolute Error
RVE	Relative Volume Error
SSE	Sum square error

Regional agronomic data from greek conditions were used to calibrate the CERES-Wheat model (Nikou and Mavromatis 2023), a crop model of the Decision Support System for

Agrotechnology Transfer (DSSAT) (Hoogenboom et al. 2019). Crop simulated flowering dates, maturity dates, and potential yield were compared between the top-performing models, low-performing models, and the full ensemble to determine if there are any advantages of selective model inclusion. The performance of climate models and the CERES-Wheat model was assessed using common statistical metrics.
Based on crop model predictions, three sowing dates (November 15, December 1, and December 16) were tested to evaluate climate models and to determine if changing the sowing date could effectively mitigate the adverse impacts of climate change on wheat production. Accurate climate predictions combined with agronomic models are intended to provide practical insights that could assist Greek farmers adjust to changing climate conditions and maintain sustainable wheat production and food security in the region.

Results and discussion

The evaluation of the 11 climate models using the standardized Root Mean Square Error index (sRPI) revealed significant variability in their performance. Models, which had the highest sRPI scores, consistently showed lower deviations from observed temperature data, indicating their suitability for simulating local climate conditions. Conversely, models with lower sRPI scores exhibited higher errors and were deemed less reliable (not shown).

Table 2. Performance of Regional Climate Model Simulations

Category	Model Simulation
Top-Performing	CCLM4-8-17_ICHEC-EC-EARTH
	HIRHAM5_ICHEC-EC-EARTH
	RCA4_MPI-M-MPI-ESM-LR
	REMO2009_MPI-M-MPI-ESM-LR_r1
	REMO2009_MPI-M-MPI-ESM-LR_r2
Low-Performing	ALADIN63_CNRM-CERFACS-CNRM-CM5
	RACMO22E_CNRM-CERFACS-CNRM-CM5
	RACMO22E_ICHEC-EC-EARTH
	RACMO22E_MOHC-HadGEM2-ES
	RCA4_ICHEC-EC-EARTH
	RCA4_MOHC-HadGEM2-ES

The CERES-Wheat model simulations provided insights into how different climate scenarios would impact wheat phenology and yield. The results for the three sowing dates revealed several key findings. For anthesis dates, the best-performing models predicted dates within a range of 1-3 days (based on means and medians of Fig 2) earlier than observations, while the low-performing models 8-12 days later and the full ensemble within a range of 4-6 days later. There is agreement between the three emission scenarios.
For maturity dates, the best simulation runs provided more accurate predictions (within 0-1 days earlier than observations), whereas low-performing models and the full ensemble predictions resulted in later (11 to 14 days and 6 to 8 days accordingly) predictions. An agreement was also found between the three emission scenarios.
Yield predictions from the top-performing models showed a variability in deviation ranging from -1% to +2% depending on the emission scenario compared to the low-performing models that showed an increase in the range of 21% to 25% and the full ensemble indicating

also an increase by 16% to 22%. These results demonstrate the value of evaluating the climate model simulations before its use in impact assessment studies.

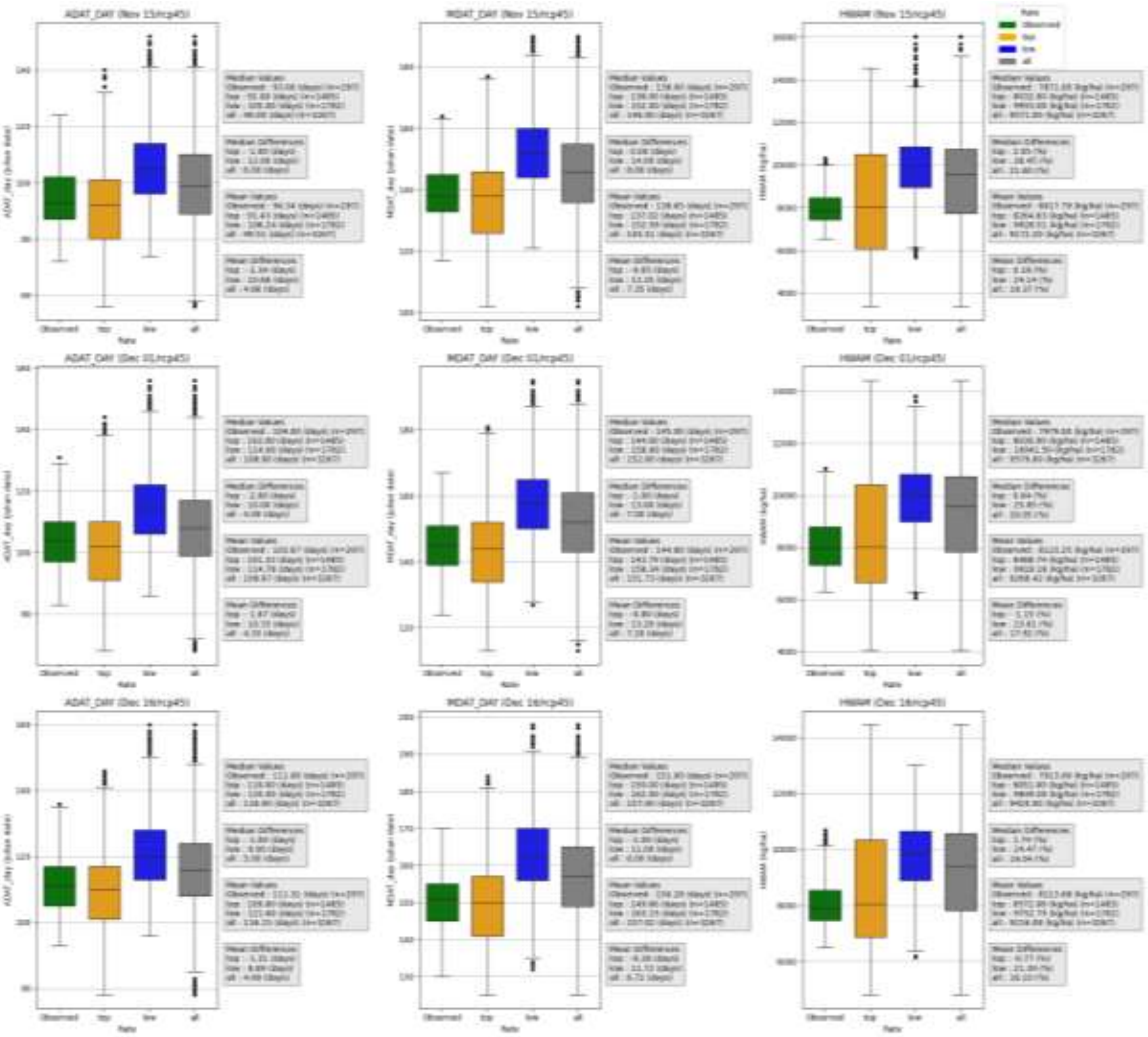

Figure 2. Comparison of anthesis days, maturity days (Julian dates), and yield (kg/ha), as derived from CERES-Wheat, for three different planting dates (15 November, 1 and 16 December), for the RCP4.5 scenario, for the observations (Observed), the total (all), the best (top: sRPI>50%) and worst (low: sRPI<50%) simulation scenarios. The sample size (total n of growing seasons) for each category is also presented.

Conclusion

This study highlights the critical role of climate model selection in enhancing the accuracy of crop model predictions. The study’s findings underscore the importance of selecting appropriate climate models for reliable agronomic predictions. The calibrated CERES-Wheat model, when driven by the best-performing climate models, provided more accurate predictions of wheat anthesis, maturity and potential yield, enabling better adaptation planning. Further research should continue to refine model calibration and explore additional adaptation measures to address the evolving challenges posed by climate change.

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THE INFLUENCE OF AIR TEMPERATURE TRENDS ON CHERRY PLUM (*PRUNUS CERASIFERA* EHRH) IN THE BLUE-GREEN INFRASTRUCTURE

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Abstract

Air temperature variability and trends within the blue-green infrastructure (BGI) of the southwestern suburban zone of Belgrade, specifically within the municipality of Čukarica, were examined across various temporal scales. The study focused on seasonal fluctuations in the flowering phenophase of *Prunus cerasifera* Ehrh (Cherry Plum), between the second-order road P107 and the main road M19 to the settlement of Pećani. The study analysed the relationship between the flowering phenophase of Cherry Plum and air temperature, a key climatic factor, from 2007 to 2024. Findings indicate that the onset of the flowering phenophase was influenced by climate fluctuations, with significant correlations observed between flowering patterns and air temperature. It was confirmed that (a) as cumulative chill hours (CHt) increase, temperatures during the flowering period decrease, (b) as the number of days from full flowering (FF) to end of flowering (EF) increases, the flowering phenophase lengthens, (c) as air temperatures from FF to EF increase, temperatures during flowering increase, (d) as temperatures from BF to FF increase, the number of days from FF to EF decreases, and (e) lower temperatures from bud break to BF imply lower temperatures from BF to FF. Our research establishes a framework for understanding how Cherry Plum responds to complex environmental conditions, considering factors such as stress memory and acclimation. The analysis of variation in Cherry Plum flowering phenology proves valuable for BGI management and landscape design within the study area, characterised by a mosaic of forest communities, agricultural areas, meadows, and interconnected environmental corridors.

Keywords: *Prunus cerasifera Ehrh., blue-green infrastructure, landscape design, blooming period, climate change.*

Introduction

Climate change is accelerating, leading to a surge of adverse effects on ecosystems, particularly the intensification of air temperature rise (WMO, 2021), with the fragmentation of BGI further contributing to alterations in climate patterns (Ioja, 2018). Research findings indicate a trend of rising air temperatures since the latter half of the nineteenth century, with the periods of most intense warming observed between 1920 and 1944 and after 1975, particularly in the northern hemisphere, where the rate of warming has been reported to be twice that of the southern hemisphere (Berardi & Jafarpur, 2020). The influence of air temperature on vegetation manifests prominently through alterations in phenological patterns (Ocokoljić et al., 2023; Petrov et al., 2024), prompting research to examine their effects on vegetation (Malmqvist et al., 2018). Studying the relationship between phenological indicators and meteorological parameters, specifically air temperature, is imperative for gaining deeper insights into the relationship between climate change and phenology trends (Masson-Delmotte et al., 2021).

Therefore, our investigation focuses on the phenological flowering patterns of the early-flowering species Cherry Plum within Belgrade's Blue-Green Infrastructure (BGI), spanning the timeframe from 2007 to 2024. The study examines the phenological responses of the species to the rising trend of air temperature over 18 consecutive years. Our specific objectives included (a) evaluating the status of *P. cerasifera* within BGI ecosystems; (b) investigating variations in flowering phenological patterns influenced by air temperature; (c) establishing potential correlations between temporal air temperature trends and phenology and (d) proposing ecological conservation measures aimed at preserving BGI.

Material and Methods

The research area lies within the BGI of Belgrade in Serbia, between the second-order road P107 and the main road M19, extending to the settlement of Pećani (Figure 1) on the right bank of the river Sava in the southwestern territory of the Čukarica municipality, from Ostružnica (φ44º42'43.79” N, λ20º18'59.49" E) to Umka (φ44º41'39.11" N, λ20º18'55.74" E). The total land area under investigation is 2,555,476 m^2, with elevations ranging from 73 to 162 meters. The soil adjacent to the Sava River exhibits gleysol, followed by recent alluvial deposits and transitional alluvial pararendzinas, while slightly higher terrains feature drier gleysol soils (WRB, 2015).

Figure 1. Spatial transformations of BGI: a) from 2007 b) to 2024 with a red question mark indicating the zone of intensive human activity and Cherry Plum population in full flowering phenophase on 29 February 2024 within the research area.

The main characteristic of the terrain is its lack of urbanisation, coupled with the presence of an aquatic ecosystem in Pećani Pond (*Ser. Pećanska bara*), surrounded by meadows lined with narrow belts of forests and ecotones. Adjacent to the M19 road in the eastern sector there are also some cultivated fields. Within and outside the ecotones, a mosaic pattern reveals the presence of a large number of Cherry Plum trees, exhibiting invasive expansion over an eighteen-year research period (Figure 1). Within our research area, ten populations were selected to assess the influence of air temperature on species dynamics. Following the methodology outlined by Meier (1997), our investigation monitored flowering and fruiting patterns at the population level, encompassing critical developmental stages: bud break (BB), beginning of flowering (BF) defined as when over 10% of flowers are open, full flowering (FF) when more than 50% of flowers are open, end of flowering (EF) when over 80% of flowers have wilted, and full ripening (FR) when the fruit shows fully ripe colour. Observations were conducted visually every other day, with dates of key events noted. Crop abundance was assessed on a scale ranging from 0 to 5, where 0 indicated no fruiting and 5 denoted maximum fruiting. Cumulative chill hours (CHt) were computed from 1 November of the preceding year until the date of BB during the flowering year, using the formula proposed by Cosmulescu & Ionescu (2018). The data presented show the mean values for ten Cherry Plum populations. To determine the impact of temperature, hourly and daily meteorological data from the RHMS website (https://www.hidmet.gov.rs/index.php, accessed on 1 May 2024) were utilised, sourced from the Surčin meteorological station (φ44º 47' 54.44" N; λ20º 27' 53.35" Er). The term "normal" in this study refers to the climatological standard temperature normal for the reference period of 1991-2020. Statistical data analysis conducted using XLSTAT 2020 and Past4.11 software packages, included descriptive statistics, Spearman's rank correlation coefficient (ρ), and the Mann-Kendall trend test. Google Earth Pro was also utilised in this study.

Results and Discussion

In the study area, the structure, composition, and diversity of selected populations of Cherry Plum have undergone significant changes over 18 years of investigation (Figure 1), while grassland and forest habitats have remained stable. Therefore, our analysis focuses on assessing the influence of air temperature on the flowering and fruiting phenology of this species. Descriptive statistics revealed variations in CHt from 781h (2024) to 1441h (2015), with a mean value of 1125h (Table 1). CHt are crucial for breaking the dormancy and serve as the most decisive factor determining the flowering timing. The requirements for CHt result from plants’ prolonged climate adaptations (Ruiz et al., 2007). Our findings highlight fluctuations in CHt, which are directly linked to air temperature oscillations, with the record-low chill hours registered in 2024, aligning with the report of the RHMS indicating 2023 as the warmest year in Belgrade since 1888, with a deviation of 1.7°C from the norm. Considering the dependency of flowering timing on the taxon and its CHt requirements, as well as heat (WMO 2021, Ocokoljić et al., 2023), Table 1 includes mean daily air temperatures for each period of the flowering phenophase. Coefficients of variation indicate deviations of the analysed parameters from the mean, prompting us to analyse the significance of these deviations using the Mann-Kendall trend test. However, the obtained p-values, ranging from 0.0810 to 0.8202 for all analysed parameters (Table 1) suggest that observed trends towards increased air temperatures are not statistically significant.

Table 1. Descriptive statistics for CHt and mean daily air temperatures during key events of Cherry Plum flowering phenophases for the period 2007-2024 in the studied BGI

Variable / Statistical parameter	CHt	Tmean BB-BF	Tmean BF-FF	Tmean FF-EF	Tmean BF-EF
Mean	1125	5.87	8.67	8.94	9.16
Std. error	44.90	0.42	0.94	0.56	0.54
Stand. dev	190.48	1.7769	4	2.39	2.29
Variance	36282.94	3.16	16	5.70	5.23
Median	1149.5	6.2	8.2	8.75	8.8
Coeff. var	16.93	30.26	46.15	26.71	24.99

In Figure 2, a phenogram depicting flowering patterns over 18 consecutive years is presented. The earliest recorded BB was in 2024, while the latest occurred in 2009. The mean value for the start of the growing season was 56 days after 1 November of the preceding year. BF and FF started earliest in 2024 and latest in 2009, whereas EF showed its earliest onset in 2024 and its latest in 2013. These observations align with the findings reported by Petrov et al. (2024). The average duration of the flowering phenophase was 24 days, with a notable reduction in 2024 by 5 and 5.3 days compared to the periods spanning from 2007 to 2024 and 2007 to 2023, and by 15 days compared to 2008, when the flowering phenophase was at its longest, lasting 34 days. This reduction in the duration of the flowering phenophase can be attributed to increased air temperatures, as March 2024 was the warmest March on record with a daily mean temperature of 12.6°C (RHMS). Considering that phenophases vary depending on local climate and abiotic and biotic factors (Denny et al., 2014), Spearman's correlation coefficients were calculated (Figure 3) with a significance level set at $p < 0.05$. These analyses confirmed: A) strong positive correlations a) between the number of days from FF to EF and BF to EF (0.97) and b) between the daily mean air temperatures during the periods from FF to EF and BF to EF (0.84); and B) moderately negative correlations between a) CHt and the mean air temperatures from FF to EF (-0.59), b) the number of days from FF to EF and the mean air temperatures from BF to EF (-0.55), and c) the daily mean temperatures from BB to BF and BF to FF (-0.51). The results of the correlation analysis confirmed that an increase in the number of days from full flowering to the end of flowering affects the overall length of the flowering phenophase. Moreover, higher temperatures observed from BF to EF correspond to higher temperatures from FF to EF. Moderately negative correlations suggest that with an increase in CHt, temperatures from FF to EF decrease; higher temperatures from BF to FF lead to a reduction in the number of days from FF to EF, and lower temperatures from BB to BF correspond to decreased temperatures from BF to FF. Other correlations were negligible.

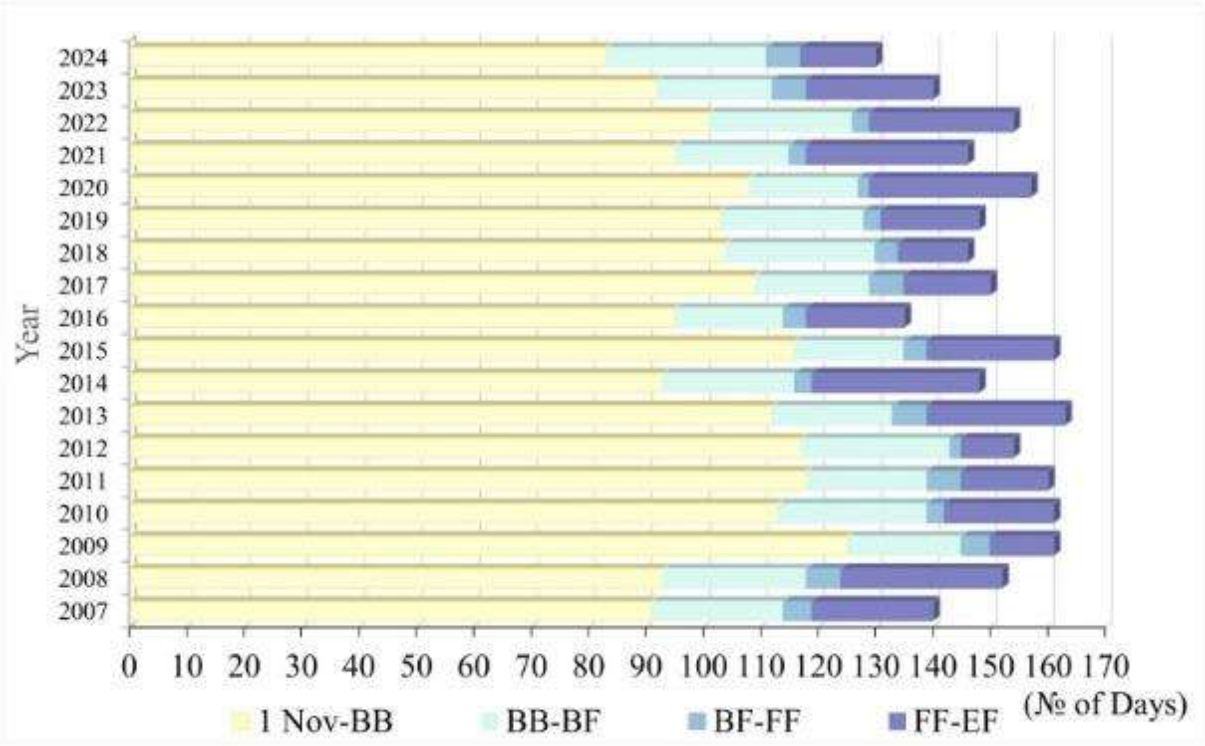

Figure 2. Phenogram of the number of days for flowering phenophases: 1 November-BB, BB-BF, BF-FF, and FF-EF (November 2006-March 2024) for cherry plums in the research area.

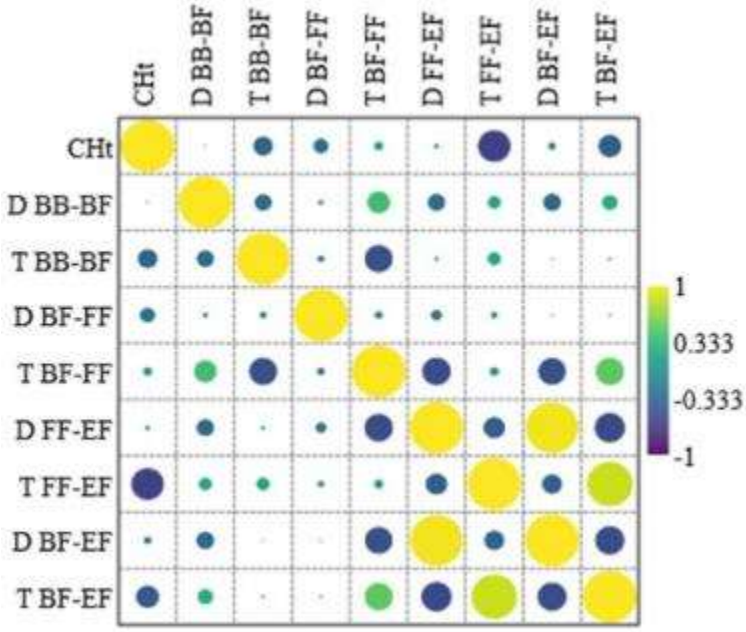

Figure 3. Graphical representation of Spearman's correlation coefficients for CHt, mean air temperatures (T), and number of days (D) for the periods BB-BF, BF-FF, FF-EF, and BF-EF, during the period 2007-2024.

The study area is a suitable habitat for amphibians, stressing the importance of preserving the surrounding forest belts, or ecotones with cherry plum trees and meadows around Pećani Pond, which these species use for hibernation. Furthermore, the non-urbanised status of this zone underscores its conservation importance. Satellite imagery analysis has revealed

significant anthropogenic activity, resulting in a marked shrinkage of the aquatic ecosystem from 2020 to 2024, with the remaining portions now at risk. We recommend the immediate protection of the studied BGI area to facilitate its defragmentation (Lindholm, 2019).
Given that the Sava River overflows and floods the surrounding land during spring and that only woody vegetation can endure prolonged surface water, it is noteworthy that populations of Cherry Plum, a xerothermic species, persist and even spread, particularly in areas formerly characterised by wetlands. Our investigation has confirmed the adaptability of *P. cerasifera* to fluctuations in air temperature. Therefore, phenological flowering patterns and sustained maximal fruiting (rated 5) over an eighteen-year period are pivotal for comprehending ecosystem functioning. They also lay a solid groundwork for devising landscape design directives aimed at fostering site identity through anticipation of forthcoming shifts in biological diversity. This is especially important considering the presence of permanently protected bird species, alongside hares, pheasants, and wild ducks, safeguarded by hunting restrictions (Batrićević A., Batanjski, 2014).

Conclusions

Our research results provide a framework for nature-based solutions, as the examination of phenological series at the population level of Cherry Plum can serve as a reference structure in BGI conservation efforts. Analysis of air temperature trends highlights key spatial and temporal aspects. Our study confirms the specific relationship between air temperature and phenology in Cherry Plum, indicating the species' adaptability in the context of past and future climatic changes. The significance of the investigated surface and linear elements of BGI with *P. cerasifer*a growing in extreme soil and hydrological and topographical conditions of the research area in the form of relict aquatic ecosystems lies in the multifunctional use of natural resources, support for additional ecosystem services, utilitarian values, and biodiversity conservation.

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GREEN INFRASTRUCTURE: IMPLEMENTATION OF WINTERSWEET IN LANDSCAPE DESIGN AND ENHANCEMENT OF ECOSYSTEM SERVICES

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Abstract

Green infrastructure (GI) represents a strategic network of green spaces meticulously designed to safeguard and enrich biodiversity while offering essential ecosystem services to human communities. While GI is embraced as a pivotal concept by urban policy-makers, there remains a limited understanding of the varying values and benefits of distinct types of green spaces and their synergistic relationships. Thus, this paper aims to illuminate the significance of Wintersweet as an integral component of Belgrade urban green infrastructure (UGI). The study delves into the interaction of climate and biodiversity, bridging the various aspects of fundamental vegetation science through a specific case study spanning 18 consecutive years (2007-2024) amidst climate change conditions. To assess changes in flowering patterns, the flowering phenology within the municipality of Čukarica was monitored and noted. A total of 1,620 phenological observations during autumn, winter, and spring were used to determine the stages and other key flowering events that regulate pollination, fruiting and use in GI. The findings affirm that winter flowers during the colder months not only ensure visual continuity but also serve as a manifestation of climate change through phenological alterations. The research outcomes yield novel insights and avenues for assessing the adaptability of Wintersweet amidst climate change. It is underscored that winter flowers not only contribute to vibrant visual displays during the colder months but also enrich biodiversity, while offering significant ecosystem services as honey-bearing plants.

Key words: *Chimonanthus praecox* (L.) Link, *ecosystem services, biodiversity, climate change, landscape design.*

Introduction

Urban green infrastructure (UGI) is advocated as a method to address significant urban ecological and societal challenges, such as climate change adaptation (Schwarz et al., 2011). It encompasses a range of green spaces, including designed green areas, segments of natural landscapes, suburban agricultural land, and abandoned fields undergoing vegetative succession. The designing of UGI components must be based on the principles of urban dynamics, with special consideration given to the impacts of gray infrastructure. As proposed by Schetke et al. (2010), IPCC (2014) and Ocokoljić et al. (2023), planning and designing of UGI contribute to biodiversity conservation and enhance cities' resilience to climate change. Moreover, the planning and design of UGI foster the growth of the green economy and hold great potential for transitioning towards sustainable and resilient urban development, achieved through the application of appropriate plant material (Schetke et al., 2010).
Hence, our study explores Wintersweet an adaptable, fast-growing deciduous alien shrub that was introduced to Europe from the mountainous regions and gorges of northern China (Ocokoljić & Petrov, 2022). It is an important aromatic and honey-bearing plant and one of the species dating back to the Tertiary period (Zhao et al., 2010). Wintersweet has a

cultivation history spanning over a millennium (Zhang & Liu, 1998). The blooming period and sharp fragrance make it one of the most prevalent species in China, where it enjoys state protection and is highly regarded as an ornamental plant with significant economic benefits (Zhao et al., 2010).
In Serbia, Wintersweet is an exceptionally rare species, prompting the paper to examine its flower development, phenological patterns, and resilience to climate change in Belgrade's GI. The species blooms during winter, thus exhibiting distinct flower development mechanisms compared to plants with a spring flowering phase. The flowering phenological patterns defined after 18 consecutive years of research will support the modification of green spaces towards the establishment of sustainable UGI, thereby ensuring invaluable additional ecosystem services.

Materials and methods

The research was conducted in the green area in front of the Faculty of Forestry along Kneza Višeslava Street, in the municipality of Čukarica (Banovo brdo), Belgrade, Serbia (Figure 1). The selection criterion for this location was based on the presence of the species *Chimonanthus praecox* (L.) Link, commonly known as Wintersweet chosen for its adaptability, resilience, and ornamental value during the winter season (Zhang & Liu, 1998; Ocokoljić & Petrov, 2022), which qualify it as an integral component of UGI providing other ecosystem services.

Figure 1. a) Wintersweet in 1995 near the Sculpture "The Deer's Roar", b) Research Area in Belgrade, and c) Wintersweet in February 2024

The terrain is gently sloped (3.9°) with a north-northwestern aspect, on Luvic Chernozem soil (Škorić et al., 1985), with coordinates 44°46'57.07"N and 20°25'20.15"E, at an elevation of 128m. According to the Köppen classification for the period 1991-2020 (RHMS), the research area is categorised as C(f)wa, indicating a humid continental climate with increasing continentality towards the northeast. Climate data for the reference periods 1991-2020 and 2021-2024 were obtained from the Main Meteorological Station (MMS) in Belgrade, located at 44°47'54.44"N and 20°27'53.35"E, at an elevation of 132m (https://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_godisnjaci.php and https://www.ogi met.com/synopsc.phtml.en, accessed on 1 March 2024).
Over eighteen consecutive years (2007-2024), phenological changes during the flowering phase were documented every other day for three individuals by noting the dates of critical occurrences (Koch et al., 2007), which were converted into day-of-year (DOY) values. All three individuals grew in the Arboretum of the Faculty of Forestry. One, approximately 40 years old, was near the faculty building, while the other two, approximately 30 years old, grew near the "The Deer's Roar" sculpture (by Jovan Soldatović), installed in 1995 when these two shrubs were planted as three-year-old seedlings. The range of DOY values was defined by the first date (BF), marking the onset of the flowering phenophase (in December)

and the date when this phenophase ended (in April). Therefore, DOY values were expressed on a scale from -27 DOY to 95 DOY. The negative sign (-) indicates that flowering began in the previous calendar year, where -1 DOY corresponds to 31 December, and 1 DOY corresponds to 1 January, and so forth. Key events of the flowering phenological pattern were designated according to the *BBCH* scale [Meier, 1997]: beginning of flowering (BF) – the day when more than 10% of the flowers were open, full flowering (FF) – the day when more than 50% of the flowers were open, and end of flowering (EF) – the day when more than 80% of the flowers had withered. To determine the growing degree days (*GDD*), the mean day-of-year (DOY) was first determined (for all investigated individuals) for each key event. Then, by summing the daily mean air temperatures from the beginning of each designated phenophase, according to Lalić et al. (2021), the required *GDD* for each of the 18 years was determined.
The morphometric analysis of flowers encompassed the measurement of two flower diameters, the total number of petals, and stamens in a sample of 300 flowers. Flowers selected for analysis were collected from the southern part of the canopy during the full flowering phenophase, with 100 flowers taken from each individual from the southern portion of the canopy. Petal colours were defined using the RGB (Red/Green/Blue) colour system. Morphometric analysis of flowers was executed using software (with the UTHSCSA Image tool). Quantitative data were statistically processed using descriptive statistics, the Mann-Kendall trend test, and the Spearman Rank test between variables, utilising the XLSTAT 2020 package.

Results and discussion

An extensive eighteen-year investigation has generated valuable insights into the morphological characteristics of Wintersweet flowers, contributing to our understanding of the species acclimatisation within the humid continental climate conditions of UGI. Given that Wintersweet blooms during the cold months, when a limited number of taxons are in the flowering phenophase within the GI of Belgrade, flower colouration was defined, and both metric and morphological flower traits were explored (Table 1). The flowers are yellow (RGB shade: 253/254/88), pendant, cup-shaped with almost translucent waxy petals arranged in two concentric circles. The petals within the inner circle exhibit a reddish-brown or purplish pigment at the base (RGB: 112/25/5). They emit a fragrance comparable to that of hyacinths and violets that can be detected from a distance of up to 50 meters (Zhang & Liu, 1998). This fragrance has scientific and economic significance as it serves diverse ecological roles, such as repelling herbivores, deterring pathogens and harmful insects, attracting pollinators, and drawing space users to the area (Lin & Chen, 2020).

Table 1. Descriptive Statistics for Investigated Wintersweet Flower Elements

Element / Statistical Variable	Flower diameter 1 (mm)	Flower diameter 2 (mm)	Total Number of Petals	Length of Yellow Petal (mm)	Length of Petal with Purple-Red Pigment (mm)	Number of Stamens
Mean	24.76	22.06	18.47	11.62	7.15	5.26
Stand. dev	4.38	4.24	2.40	1.62	1.35	0.51
Std. error	0.75	0.94	0.41	0.28	0.23	0.09
Median	25	21	18.5	12	7.5	5
Coeff. var	17.67	19.23	13.00	13.90	18.91	9.71
Variance	19.15	18.00	5.77	2.61	1.823	0.26

The dimensions of flowers and petals are crucial traits of ornamental flowering plants. Looking at the results, flower diameters ranging between 18 and 35mm stand out. The length of yellow petals varied from 8 to 15mm, while petals with purple-red pigment ranged from 5 to 10mm. The recorded diameters fell within and exceeded the dimensions of 25mm, as indicated in the literature (Lin & Chen, 2020). However, both the mean and median values aligned closely or were identical to those reported in the literature. The low values of standard deviation and coefficients of variation confirmed the low variability. The total number of petals ranged from 10 to 23, while the number of stamens ranged from 5 to 7. The recorded number of petals deviated from the findings of Lin & Chen (2020), who reported 15 to 21. The low values of standard deviation and coefficient of variation confirmed that the variability was statistically insignificant. Our results confirmed the significance of Wintersweet as a component of the UGI in Belgrade under conditions of climate change. The plant provides additional ecosystem services as its flowers serve as a source of nectar, its edible petals are a valuable food source, and the shrubs provide shelter for birds (Lin & Chen, 2020).

The lowest Growing Degree Days (*GDD*) required for the beginning of flowering (BF) occurred in 2013 (0.0004), while the highest was observed in 2018 (75), with a mean value of 22.72 for BF during the study period. For full flowering (FF), the lowest *GDD* was recorded in 2019 (0.2), and the highest in 2018 (93.4), with a mean value of 36.86. The end of the flowering phenophase (EF) occurred earliest in 2014, with the lowest *GDD* (44.5), and latest in 2024, with the highest *GDD* (350.2). The mean value for EF during the study period was 181.69. These results align with literature suggesting a significant influence of climatic parameters on early flowering species, as well as the direct correlation between accumulated heat sums and air temperatures during the flowering phenophase (Petrov et al., 2024).

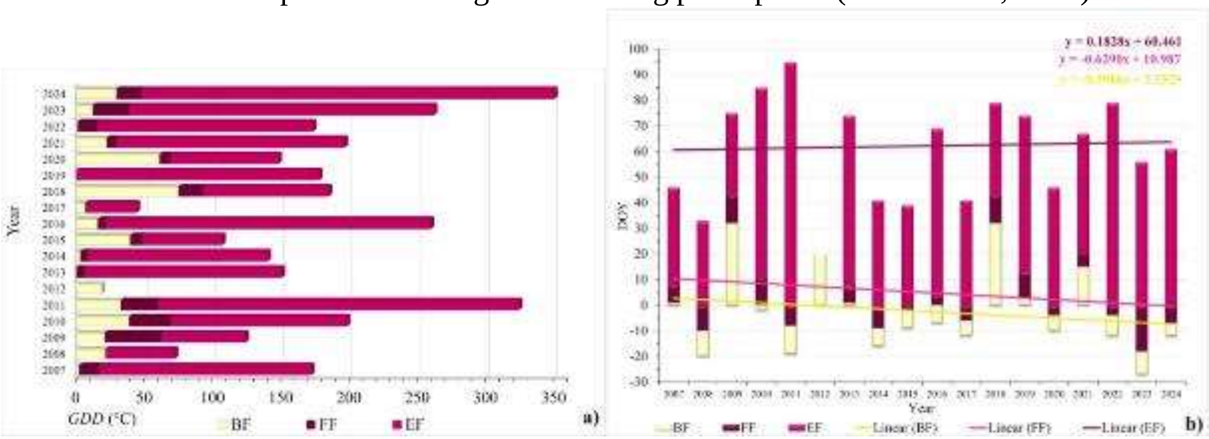

Figure 2. Temperature Sums a) *GDD* (°C) and DOY b) for the BF, FF and EF of Wintersweet

Figure 2b illustrates the phenogram for Wintersweet during the period 2007-2024. Notably, 2023 stands out for having the earliest BF DOY (-27) and FF DOY (-18), in contrast to 2018, which recorded the latest BF DOY (32) and FF DOY (43). The EF DOY was earliest in 2008 (33) and latest in 2011 (95). The average duration of the flowering phenophase was 63 days. The shortest flowering phenophase occurred in 2012, characterised by only BF (20 days), while the longest was observed in 2011 (114 days). Winter 2012 was classified as cold and very cold according to percentile methodology, with a surplus of precipitation, i.e., precipitation totals were significantly above normal values. February 2012 stood out as the coldest February on record, with snow cover persisting for over twenty days, surpassing the absolute maximum snow depth (RHMS). The longest flowering phenophase was observed in 2011, during which the mean monthly temperatures and monthly precipitation sums during the flowering period remained within the normal range according to the percentile methodology (RHMS). Decreasing trends in DOY were identified for the BF and FF, indicating an earlier beginning of flowering and full-flowering phases (Figure 2b). The absolute difference between the earliest and latest BF was 59 days and 61 days for FF.

However, trend analysis suggests a shift towards earlier flowering by 13 days over the past 18 years. Regarding the EF, an increasing trend in DOY was observed, confirming a prolonged flowering period. The absolute difference between the earliest and latest EF was 62 days, with the flowering period extending by 15 days over the eighteen-year study period. However, the Mann-Kendall trend test determined that the trends were not statistically significant (p: 0.13 to 0.94). Compared to the findings of Zhang & Liu (1998), who reported that the species blooms from late November to March in central southern and southwestern China, our research defines the flowering period from 5 December to 4 April in the UGI of Belgrade. To confirm the adaptability of the species to climate change, Figure 3 presents the mean minimum air temperatures over the eighteen-year study period alongside the number of ice days, with a separate depiction of these parameters for 2012.

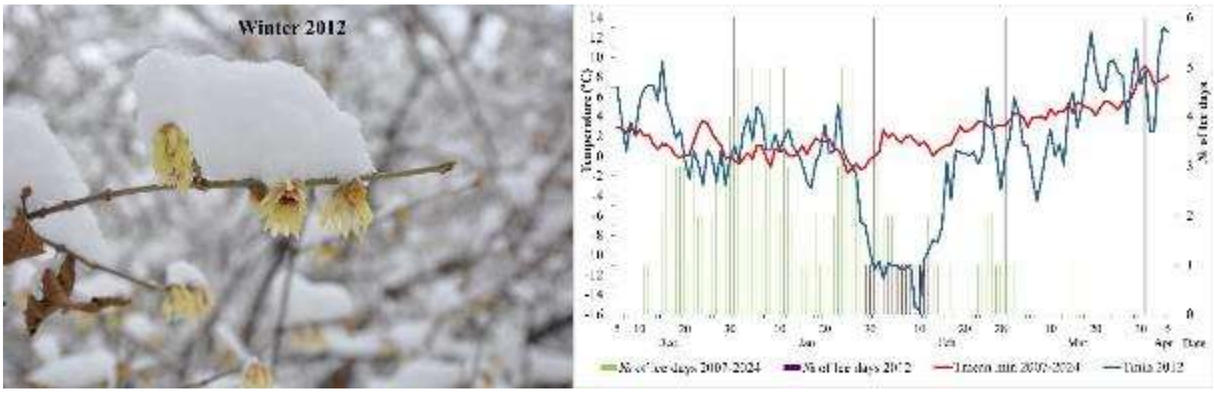

Figure 3. Mean Minimum Daily Air Temperatures and Number of Ice Days from 5 December to 4 April 2007-2024, alongside Minimum Air Temperatures and Ice Days during 2012 in Belgrade

It is clear that the flowering phenophase was disrupted by climatic parameters, leading to damage and frost injuries on the flowers. However, after the conclusion of extreme weather events, new flowers emerged, resulting in a longer duration until the end of flowering, as observed in the study. In 2012, only the beginning of flowering (BF) was recorded due to an ice spell. According to Zhao et al. (2010), Wintersweet can withstand temperatures as low as -25°C. However, consecutive ice days (17 in succession) interrupted the flowering phenophase. Despite this, all three individuals remained vital and continued to thrive in subsequent years, with all key flowering events up to the present (2024).

The Spearman's rank correlation coefficient (ρ) values for *GDD* and DOY, corresponding to the respective flowering phenophase periods, were statistically significant for BF DOY and FF DOY (0.98), as well as BF *GDD* and FF *GDD* (0.90). Similar to the findings of Ocokoljić et al. (2023), other correlations were not statistically significant (at the $p<0.05$ level), indicating no consistent increasing or decreasing correlations between other key events and their *GDD* and DOY. These results align with the findings of Lalić et al. (2021), confirming that integrating phenological data with climatic data yields specific insights, thereby identifying variations in phenophases that may span several weeks.

Throughout the study years, except in 2012, the presence of pollinators (bees and flies) during the flowering phenophase was observed, consistent with the findings reported by Du et al. (2012). Based on our findings, it can be concluded that the flowering phenology patterns of Wintersweet, at 30 and 40 years of age, affirm its adaptability to urban influences and climate change. Therefore, the recommendation is for designers to consider incorporating this species into UGI areas, as it offers additional ecosystem services during the cold seasons. However, in suburban areas where herbivores are present, it is essential to implement appropriate protective measures because the plant, apart from its flowers, contains toxic alkaloids that can pose a threat to their survival (Numan et al., 2016).

Conclusions

Over the course of eighteen consecutive years, Wintersweet has demonstrated high potential as a component of GI for the sustainable development of future urban environments. It has been confirmed that Wintersweet responds to changes in climatic parameters. However, the responses have been variable, indicating nonlinear reactions, species-specific thresholds, and cumulative temperature effects. Based on the findings, it is recommended to encourage and financially support the use of Wintersweet in GI, as it provides immediate ecological and economic benefits to ecosystems. Additionally, knowledge regarding extreme climatic events is essential for detailed projections of potential effects of climate change on GI elements and additional ecosystem services.

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TILLAGE SYSTEMS - ADVANTAGES AND DISADVANTAGES IN TERMS OF NUTRIENT CONTENT, YIELD AND ECONOMIC PROFITABILITY

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Abstract

In the process of plant production, soil is an irreplaceable resource that supplies plants with food and water, serves for their rooting, and in which numerous microflora develop. Tillage, which can be defined as any mechanical action in the pedosphere with the aim of creating a favorable soil structure for agricultural production, creating a favorable water-air regime of the soil, introducing nutrients into the soil, improving its physical and chemical properties, etc., occupies an important place in the complex of measures to increase the yield of agricultural crops. Although it can be done in different ways, i.e. by using different systems, properly performed tillage is the most effective way to increase soil fertility, and fertility is the main characteristic of soil as means of agricultural production. Tillage systems have different effects on soil properties, both physical and chemical, have different ecological effects and differ in economic profitability. Research shows that replacing the conventional system with a conservation one can have a positive effect on the soil structure, increase the content of organic carbon in the soil, reduce the risk of erosion to a minimum, improve the quality of the soil, but also protect it, given the impossibility of replacing it as a resource in agricultural production. The analysis of literature data points to the fact that the yield is only one of the factors that influence the decision on the choice of tillage system and it should not be considered separately from economic and environmental factors.

Key words: *Soil, Tillage, Yield, Soil properties, Climatic characteristic, Organic matter.*

Introduction

In order for the sown seed to germinate, and the sprouted plant to develop normally and give the desired yield, apart from other necessary conditions, the soil must be in a favorable condition. To bring the land to a favorable condition, it is necessary to cultivate it. Land cultivation is a way of preparing it to provide plants with favorable conditions during the growing season. By cultivating the land, we create a favorable substrate for cultivated plants, so on properly cultivated land, the plant grows better and achieves a higher yield (Šarić, 1985). However, the question arises whether soil cultivation is necessary in the process of growing plants and how do wild plants grow on natural grasslands and in forests without any soil cultivation? How does the processing process itself affect the soil in the long term, but also the environment in general? Although the conventional tillage system, which involves the use of a plough, is still the most prevalent in our production areas, modern tillage systems are based on reducing the number of operations and passes. In this way, the land is to a certain extent protected and preserved for future generations, since it represents an irreplaceable resource in plant production. By processing, the soil is crumbled, crushed, small, mixed, its physical properties change, such as porosity, ratio of macro and micro pores, compaction and soil bulk density. At the same time, its chemical and biological properties change, that is, these properties directly depend on the intensity of processing (Gaj et al., 2015, Jaskulski et al., 2015). Land cultivation can have a positive or negative effect on the mentioned property, depending on the inherent properties of the land and management practices. It is considered that soil cultivation is one of the biggest consumers of energy in agriculture, which is the reason for the emergence of research into different cultivation systems and their impact on fuel consumption. Using the right soil cultivation system can contribute to reducing energy consumption for cultivation, increasing profits, yields and improving soil conditions and protecting resources (Kovačević et al., 2019).

Based on the results of their research Chavarria et al. (2018) concluded that the tillage that implies leaving it out (no tillage) and that promotes soil health and system sustainability can reduce soil disturbance, improve its structure and provide better physical protection for the content of organic matter in to the soil, thus maintaining its fertility (Guo et al., 2021), the results of which Jiang et al. (2011) and Bogunović et al. (2018) showed that the omission of tillage exhibits disadvantages such as soil nutrient stratification, mechanical compaction, changes in its pH value and increased weed activity, all of which contribute to increased risk in terms of yield decline.

According to (Birkás, 2008), the application of new, more modern machines and the increased use of mineral fertilizers in the process of intensive agricultural production create better production conditions for plants, but the land is not given adequate importance. As a result, land degradation occurred, the consequences of which in modern agricultural production are manifested in different ways (Jug et al. 2010). By switching from a conventional to a conservation tillage system, it is possible to have a positive effect on the structure of the soil, increase the content of organic carbon in the soil, reduce the risk of erosion to a minimum, improve the quality of the soil and protect it as an irreplaceable resource (Busari et al. 2015).

The aim of this paper is to point out the advantages and disadvantages of certain tillage systems through their influence on the content of nutrients in the soil, the yield of agricultural crops and economic profitability

The influence of conventional and conservation tillage systems on the content of total and easily accessible nutrients in the soil

Conservation tillage practices range from zero tillage (No-till), reduced (minimum) tillage, mulch tillage, ridge tillage to contour tillage.

Šeremešić et al. (2016) examined the influence of conventional and reduced tillage on the change in the level of organic matter when growing wheat and sunflowers on chernozem type soil, and came to the conclusion that on the plots where reduced tillage was applied, the total content of organic matter was higher compared to conventional tillage. Reduced tillage was carried out with a heavy disc harrow to 15 cm for wheat and 25 cm for sunflower using the Horsch Terano harrowing tool. Conventional tillage for both cultures was carried out with a plough to a depth of 25 cm for wheat and 27 cm for sunflower. The mentioned authors came to the conclusion that the total content of organic matter (OM) in the soil was higher on the plots where reduced cultivation is performed compared to conventional plowing, and that the place of plowing and the amount of plant residues affect the accumulation of OM. Also, they found that the content of labile organic matter soluble in hot water (HWC) has a greater influence on the depth of cultivation compared to the cultivation system and the crop.

Soil cultivation breaks down its structural aggregates, as a result of which organic carbon from the soil becomes more susceptible to various types of degradation and decomposition. In addition, the complete removal of crop residues, high dependence on mineral fertilizers, burning of crop residue, practicing monoculture or growing a smaller number of crops, and less addition of organic nutrient sources are factors that intensify the decrease in soil organic carbon (Kassam et al. 2019). At the same time, the availability and large variety of selective herbicides, the availability of specialized machinery for sowing and introducing fertilizers to a certain depth, as well as the increased interest in the field of research and development front in the modification of nutrient release patterns from crop residues through different ways (Swarnalakshmi et al., 2013; Choudhary et al., 2016) also favour agriculture based on conservation tillage principles.

It is considered that the loss of organic matter (OM) in the soil is a consequence of the negative effects of intensive agricultural production. The organic part of the soil represents a complex system with an extremely dynamic character. It is the result of the continuous flow of organic compounds into the soil and their continuous transformation (Vidojević and Manojlović, 2010). The content of organic matter in the arable layer of the soil is determined by the texture of the soil, climate, vegetation and the way of use (Jenkinson, 1990). When OM is analyzed from the aspect of realization and long-term yield stability, it represents one of the most important indicators of the production potential of the agroecosystem (Reeves, 1997). It is one of the main carbon reservoirs (Clercq et. al 2015). According to Brady and Weil (2002), OM in the soil is considered the main source of nutrients mainly N, partially P, S and microelements, and it also favourably affects numerous physical properties of the soil (Tobiašová, 2011). As stated by Ismail et al. (1994) and Rahman et al. (2008) in the surface layer of soil that has not been cultivated, the content of exchangeable Ca, Mg and K is significantly higher compared to soil that has been subjected to a conventional cultivation system. Ali et al. (2006) found the lowest content of OM, N, P, K, Ca and Mg in the plots subjected to the conventional tillage system, explaining this as a possible consequence of the upper layer of the soil moving deeper and pushing it to the surface of the substrate, which is less fertile.

The research results obtained by Stankowski et al. (2022) show a significant increase in nitrogen content on plots where ploughless and direct sowing systems were applied compared to plots where classic ploughing was applied. Similar results are presented by Rajewski et al. (2012) indicating that the highest content of nitrogen in the whole arable layer (0-0.25m) was found precisely on those parcels that were not tilled. The above is confirmed by the results of Naeem et al. (2021) who also found the highest nitrogen content on soils with zero tillage. Zyłowski (2017) and Stankowski et al. (2022) determined that the total nitrogen content, which is conditioned by the application of reduced tillage, is directly related to the carbon content. Zyłowski (2017) pointed out that the increased content of organic carbon was determined in the surface layer of the soil, and this is not a rule for the entire soil profile. The results of Stankowski et al. (2022) show that in addition to the total content of nitrogen and carbon, the content of accessible magnesium was higher on plots with ploughless and direct sowing tillage systems, while the effect of ploughless and direct sowing when talking about the content of accessible potassium was determined only in the surface layers of the soil. Also, the same authors determined that tillage systems had no effect on the content of accessible iron, copper, manganese and zinc, and that the influence of pre-grass on the physical and chemical properties of the soil and its chemical composition was more significant compared to the tillage system. Maintaining and preserving the OM level, observed for a specific agroecological area, is one of the prerequisites for proper land use and environmental preservation (Körschens, 2004). Jaćimović et al. (2013) point out that in order to increase, but also to preserve the fertility of the soil as well as to reduce production costs, in Western countries there have been tendencies to reduce soil cultivation, to reduce its trampling and compaction, to increase and maintain the level of organic matter and humus, and to preserve structural , physical-chemical and microbiological properties.

The influence of conventional and conservation tillage systems on the yield and their economic profitability

Momirović et al. (1998) pointed out that the concept of sustainable agriculture implies the rational management of agricultural resources both from the aspect of meeting the changing needs of the population in food and raw fiber, and with the aim of preserving natural resources and protecting and improving the environment. In most farming systems, land cultivation is the most important item when we talk about energy needs, and the basis of sustainable agriculture is the productivity of the system, expressed through the ratio of inputs and outputs (Momirović et al., 2011). Conventional tillage systems showed greater efficiency in controlling weeds and creating favourable conditions for cultivated plants (Kovačević et al., 2008). In their research, the same authors came to results that indicate that by applying conservation tillage systems compared to conventional ones, the yields of winter wheat were lower by 25-35%, spring barley (5.72-51.85%), corn (24.62-24.90%) and soy (34.95-39.41%). Conservation technologies are more rational, but given that they give lower biomass yields than the conventional system, there is a need to examine their economic efficiency from the point of view of energy and soil protection from degradation. The application of conservation processing systems changes physical, chemical, microbiological properties, thus creating different conditions for the development of the root system of plants and its role in plant nutrition. It is mainly about the reduction of essential vegetation factors. Changed circumstances affect cultivated plants, weed vegetation, the appearance of diseases, pests, etc. In these conditions, the crop rotation and proper selection of plants are of great importance, and preference should be given to multi-purpose crops (Lorenzetti and Fiorini, 2024). However, for the successful realization of conservation systems, when all the necessary preconditions are created, there should be an adequate assortment, that is, there should be varieties that can tolerate more modest growing conditions. Wheat varieties Lasta, Pobeda, France gave a positive response with a higher yield to the reduction in tillage, fertilization system and low degree of weed protection. Such conditions were not suitable for varieties with very intensive, high demands and which can achieve better results only in conditions with conventional processing (Kovačević et al., 2008). Similar results were obtained by Biberdžić et al. (2020) stating that the highest yields of wheat were achieved under the conditions of application of the conventional processing system in the year in which the highest amount of precipitation was recorded, as well as the highest moisture content in the soil and the lowest compaction, and that the yield achieved was significantly higher than the yields achieved in other systems. land cultivation. They also stated that on soils with poorer mechanical composition, satisfactory yields of wheat can be achieved only under conditions of complete soil cultivation, which includes plowing and adequate soil preparation. Maize yield under a minimum tillage system is likely to be more sustainable compared with conventional tillage (Busari and Salako, 2013). The same authors stated that the best yield achieved by crops using minimal tillage can be explained by weak root development using zero tillage as well as rapid structural deterioration caused by slaking and dispersion under conventional tillage (Guzha, 2004) which were possibly not the case under minimal tillage. Drury et al. (2011) and Gruber et al. (2012) found that maize yields in temperate climate regions on no-tillage plots were similar or lower compared to yields achieved under conventional tillage conditions in cold and humid climates or on poorly drained soils. In a three-year study conducted by Liu et al. (2022) it was determined that the yield of corn obtained by applying CT was 1740 kg ha^{-1} higher compared to the yield obtained on plots where cultivation was omitted and this difference was statistically significant. Van de Putte et al. (2010) proved that the omission of processing can reduce the corn yield by 8.5%. (2010). Gaj et al. (2015) examined the effect of different tillage methods on the nutritional status,

yield and quality of sugar beets and determined that the reduced tillage systems in sugar beet cultivation did not result in either a yield decrease or a worse technological quality of roots.
As Pitelkow et al. (2015) stated in their work, their results indicate that there are some contexts in which the realized yield on uncultivated land is higher compared to the yield with conventional tillage systems. This is typical of arid regions, especially where water limits crop growth. According to them, yields are only one of the factors that influence the decision to apply no-till and they should not be considered separately from economic and environmental factors.
The yield results achieved with conventional and conservation treatment were comparable, so economic profitability is determined based on operating costs (Boselli et al. 2020). Based on the analysis, these authors concluded that operational costs result lower for the CA management of plots.

Conclusions

Choosing an adequate tillage system is a complex task that depends on the climate, soil, type of crop, harvesting system, etc. Given that the choice of soil cultivation system is conditioned by a large number of factors, it simultaneously results in different success on the same land from year to year. From the analyzed sources we can conclude that conservation tillage, especially minimum tillage, has a better effect on the chemical properties of the soil. Also, by omitting processing, erosion caused by water or wind is reduced, the rate of water infiltration increases, as well as its content in the soil. By reducing the number of operations during land cultivation, we reduce trampling and compaction, the level of organic matter and humus increases and is maintained, which positively affects its structural, physical-chemical and microbiological properties. Unlike conventional processing systems, the use of conservation systems reduces labor and fuel consumption, which makes them even more attractive compared to conventional systems.

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SOIL WATER RETENTION IN PANNONIAN CHERNOZEM

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Abstract

The content of different categories of water in the soil depends on the soil texture, organic matter, structure, compaction and chemical soil properties. The fertility of the soil directly depends on the soil's ability to store easily available water, especially in conditions of extreme weather caused by climate change. The research aimed to determine whether the values of different categories of water in chernozem soil type were still satisfactory after decades of intensive use of this soil. The samples were taken in a disturbed state from six localities (from three profiles at each locality and three depths from the genetic horizons Amo, AC and C). The water content values for filed capacity (pressure of -33 kPa) ranged from 29.32 to 34.20 in mass percentage. Values for lentocapillar water (- 625 kPa) ranged from 14.44 to 18.28%, while the values from 12.90 to 18.01% indicated wilting point i.e. the water content at a pressure of -1500 kPa. Easily available water varied from 13.22 to 18.36%, while less available water ranged from 0.37 to 1.63%. According to the findings, it has been concluded that the chernozem on the loess terrace has a high water retention capacity and can still support the cultivation of all crops, despite a considerable decline in organic matter content due to intensive farming practices over the past century.

Keywords: *retention capacity, available water, kPa.*

Introduction

Soil and water are critical components of agricultural production. Soil and water interact in a way that both, soil and water resource management, are strongly influenced by soil water retention. In the future, agricultural productivity will strongly depend on the ability of soil to retain water against gravity, making it available for plant uptake. Various soil properties, including texture, aggregate stability, organic matter content and compaction determine soil water retention. The potential response of these properties is a key indicator of the impact of agricultural management on the movement of water and chemicals through the soil (Rawls et al., 1991).

One of the key factors influencing soil water retention is soil texture (Geroy et al., 2011). Soils with high clay content have higher water retention capacity due to the high surface area of clay particles and high porosity, which allows holding water more effectively. On the other hand, sandy soils with larger particles have lower water retention capacity but better drainage, which can be advantageous in certain contexts. According to Rawls et al. (2003), adding information on taxonomic order and taxonomic order and organic carbon content to the textural class brought 10% and 20% improvement in water retention (-33 kPa) estimation, respectively, as compared with estimation from the textural class alone. Texture and structure interact together to affect water retention in the soil (Guber, 2004). Well-aggregated soils with good structure have a higher water retention capacity than compacted soils with poor structure. The distribution and aggregate size affect soil air and water capacity (Wang, 2015). This is because well-aggregated soils have more pore spaces of varying sizes, which can hold water and allow for better infiltration and root penetration. Ćirić et al. (2015) proved that the structure is strongly related to the content of clay and silt and also to water retention at -33 and -1500 kPa. Tamboli (1964) concluded that larger aggregates retained more moisture than smaller aggregates at pressures above 0.1 bar, indicating that larger aggregates had a larger volume of small pores. Soil water retention at pressures <1 bar is strongly influenced by soil structure (Tuller, 2004). For soils with high clay content and high aggregation, pore size distribution and water retention can be better predicted from aggregate size than from particle size distributions (Wu, 1990).

Organic carbon content is another important factor affecting soil water retention (Kern, 1995), but reports about the relationship between soil water retention and soil organic carbon content are contradictory (Rawls et al., 2003). According to (Saha & Kukal, 2015) organic carbon content exerts a strong influence on soil water retention, which is confirmed by many authors (Oguike and Mbagwu, 2009, Zhang et al., 2001). On the other side, Minasny and McBratney (2018) report that the increase in organic matter is still uncertain and may be overestimated, since the increase of 1% of soil organic carbon, on average, increases water content, field capacity, wilting point and available water capacity from 0.17 to 2.95 mm H_2O 100 mm soil^{-1}, respectively. Thus, the overall increase in available water capacity is very small. According to Bauer and Black (1991) and Stevenson (1974), cited by (Kern, 1995), there may be no change or an increase in plant-available water-holding capacity with changes in soil organic matter because of complex changes in water retention at both -33 and -1500 kPa. The influence of soil organic matter on soil water retention is more pronounced for water that is held in the soil by weaker forces (Saha & Kukal, 2015). Rawls et al. (2003) reported that an increase in organic matter content led to an increase in water retention in sandy soils and a decrease in fine-textured soils.

Soil compaction and soil crusting are the major management-created layers that significantly influence water infiltration (LAWR Technical Report, 1984). Changes in porosity, pore size distribution and pore connectivity refer to soil compaction (Alaoui et al., 2011). Compaction has a detrimental effect on the physical and hydraulic soil properties. Research conducted by Ngo- Cong et al. (2021) showed that a 10-20% increase in soil bulk density reduced cumulative infiltration by 55-82% and the available water storage capacity by 3-49%, depending on soil type. Improving soil management practices is essential for preventing further waterlogging and runoff.

The research aimed to determine whether the values of different categories of water in chernozem soil type were still satisfactory after decades of intensive use, and if not, to contribute to the creation of practical solutions for maintaining the water-physical properties of chernozem within optimal limits. Practices such as adding organic matter, conserving tillage, and using cover crops can improve soil structure and thus improve water retention capacity. Understanding the factors that influence soil water retention enhances soil management. Since soil water retention is crucial for crop productivity because it significantly determines water uptake and transport by plants (Razzaghi, 2020), improved soil management can enhance water retention, increase crop yields, and protect water resources. It is important to consider sustainable soil management practices due to the future consequences of intensive farming practices that have taken place in Pannonian chernozem over the past century.

Materials and methods

Field research of the soil profiles took place in six localities in Vojvodina province and Stig plain in East Serbia (Rimski Šančevi, Sombor, Kikinda, Zemun Polje, Požarevac and Pancevo), where experiments with corn hybrids were set up. In each location, samples were taken in triplicate from 1 profile and 2 semi-profiles. Disturbed and naturally undisturbed soil samples were collected from each genetic horizon (Ap-A-AC). Statistical processing of the data was performed with the "Statistica" program. Interdependencies between variables were determined by correlation analysis for a significance level of 5%.

Methods used for determining physical soil properties:
- soil texture, using the pipette method, preparation of samples for analysis with Na-pyrophosphate according to Thun (1955), textural class based on the classification according to Tommerup (1934)
- structural analysis (dry sieving using the N.I. Savinov (1936) method (Bošnak et al., 1997) and structure coefficient (Ks) by Вершињин (1958) method (Gajić, 2006).
- retention of H_2O (33 kPa), using a "porous plate" apparatus
- retention of H_2O (625 and 1500 kPa), using a "pressure membrane" apparatus
- plasticity index – calculated from the upper and lower limit of plasticity (upper limit of plasticity according to Casagrande, the lower limit of plasticity - according to Atterberg)

Methods used for determining the chemical soil properties:
- pH value in soil suspension with H_2O and soil suspension with 1M KCl, potentiometrically, using a pH-meter
- $CaCO_3$ content, volumetric, using a Scheibler calcimeter
- humus content, according to the Tyurin (1931) method

Results and discussion

According to the classification of the World Reference Base for Soil Resources (WRB), the soil is identified as Chernozem (Siltic), profile 1 (Rimski Šančevi), profile 2 (Sombor), profile 3 (Kikinda), profile 4 (Zemun Polje), profile 6 (Pančevo) and Chernozem (Clayic) profile 5 (Požarevac). In all localities, the chernozem is moderately humus in the arable Ap horizon, with a decreasing tendency toward deeper horizons. Chernozem is a neutral to medium alkaline soil in the arable Ap and sub-arable A horizon, while in the AC horizon, it has medium alkaline to strongly alkaline chemical reactions. Eluvial-iluvial migration of $CaCO_3$ is evident in all six localities (Table 1). According to the soil texture, the soil is classified as loam, clay loam or loamy clay (Table 1). The soil texture is homogeneous in the localities of Zemun Polje (profile 4) and Požarevac (profile 5), while it is somewhat more heterogeneous in the localities of Rimski Šančevi (profile 1), Sombor (profile 2), Kikinda and Pančevo (profiles 3 and 6), with no major textural differentiation between the horizons. The AC horizon contains less clay than the arable Ap and sub-arable A horizons in all localities. According to the structure coefficient (Ks) all soil profiles indicate that chernozem still has a good structure (Ks > 1.5) (Table 1).
The water content values at a pressure of -33 kPa ranged from 29.32 to 34.20% (Table 1.) which indicates medium to high water content for the chernozem of the loess terraces. Vučić (1964) showed field capacity values for chernozem loess terraces ranging from 23.93 to 27.94% for the entire profile, and from 26.1 to 26.49% for Ap and A horizons. The differences between the values obtained by this author and those obtained in this research arise from the differences between the methods used. Bošnjak (1992), stated that instead of field capacity values, values of water retention capacity can be used if we cannot determine field capacity or do not have its values. However, the water retention capacity values are significantly higher than the field capacity values. The differences are directly proportional to the air capacity of the soil (Vučić, 1964). Bošnjak (1997) pointed out that field capacity values in light and medium loamy soils range from 20-30%, and in heavy ones from 30-40%. The highest water content values at a pressure of -33 kPa were recorded in the A and AC horizons. Regarding the water content value at the pressure of -625 kPa and -1500 kPa observed by depth, they were mostly the highest in the A horizon. The mean values of water

content at a pressure of -625 kPa ranged from 14.44 to 18.28% and the mean values of water content at a pressure of -1500 kPa ranged from 12.90 to 18.01% (Table 1). Examining the chernozem of the loess terraces, Vučić (1964) stated values for wilting points (-1500kPa) ranging from 8.75 to 11.33% for the entire profile, i.e. from 10.92 to 11.22% for the Ap and A horizons, and also highlighted higher values obtained at a pressure of -1500 kPa from the actual water content at wilting point.

Non-draining available water ranges between field capacity (-33 kPa) and wilting point (-1500 kPa). Also, it is divided into easily available (between the field capacity and the lentocapillar water), and less available water (between lentocapillar water and wilting point). Easily available water content varied from 13.22% to 18.36%, which indicates common high values for soils with a loamy texture, such as chernozem, that provides the largest amount of easily available water. Less available water ranged from 0.37% to 1.63%. In the Požarevac locality (profile 5), the highest water content was recorded at a pressure of -625 kPa and -1500 kPa compared to the other localities, which can be interpreted as an increased content of clay and higher compaction of the soil, and therefore a higher content of fine pores that retain water that is more difficult to access and inaccessible for plants.

Table 1. Soil properties values for Ap-A-AC horizons for each of the six profiles

Profile	**Soil texture**	**Plasticity index**	**Ks**	**-33 kPa**	**-625 kPa**	**-1500 kPa**	**Organic matter**	**$CaCO_3$**	**pH H_2O**	**pH KCl**
		% mass.		**% mass.**			**%**	**%**		
P1	Loamy clay	11.42	6.85	30.80	16.15	15.79	2.59	0.14	6.96	5.94
	Loamy clay	13.96	3.94	32.15	16.55	16.51	1.70	1.67	7.97	7.07
	Clay loam	12.52	5.40	32.34	15.22	13.94	1.18	22.28	8.28	7.66
P2	Clay loam	7.68	3.88	30.25	14.44	14.20	2.86	9.58	8.23	7.55
	Clay loam	12.63	5.93	33.30	16.53	15.55	1.88	15.55	8.45	7.68
	Loam	10.77	5.23	32.89	15.15	12.90	0.79	33.93	8.53	8.02
P3	Loamy clay	12.43	4.28	29.32	15.83	15.30	2.49	0.91	7.41	6.08
	Clay loam	14.68	3.90	29.96	16.74	16.37	2.25	1.13	7.60	6.52
	Clay loam	14.54	4.35	31.51	16.33	15.22	1.19	12.27	8.47	7.50
P4	Clay loam	12.57	7.44	31.49	16.53	15.79	2.64	2.25	8.23	7.38
	Clay loam	11.11	8.33	32.11	16.45	15.82	2.41	4.28	8.32	7.41
	Clay loam	12.11	8.35	34.20	15.84	15.21	1.25	22.04	8.39	7.69
P5	Loamy clay	15.34	8.20	32.39	18.12	17.98	2.38	0.21	7.62	6.58
	Loamy clay	14.49	7.00	32.51	18.28	18.01	2.03	6.53	8.03	6.97
	Loamy clay	16.25	16.20	32.73	17.54	17.34	1.29	13.43	8.41	7.39
P6	Clay loam	12.26	4.39	32.31	15.69	15.56	3.28	16.31	8.40	7.43
	Clay loam	11.77	4.44	33.53	16.37	16.19	2.97	16.17	8.44	7.50
	Loam	11.27	9.03	34.12	16.41	15.98	1.90	22.55	8.55	7.70

Positive correlations were determined for a significance level of 5% between water retention at -625 kPa and the plasticity index (r= 0.79). A comprehensive analysis identifying the importance of specific soil properties on soil water retention will depend on which soil properties were measured and how much variation was exhibited by the measured properties (Rawls et al., 1991).

Conclusion

Soil water retention is of great importance for agricultural productivity and ecosystem health. The water content measurements at various pressures indicate that chernozem has field capacity (-33 kPa) from 29.32% to 34.20% in mass percentage. For lentocapillar water (-625 kPa), the range was 14.44% to 18.28%, and at the wilting point (-1500 kPa), the range was 12.90% to 18.01%. The easily available water content varied from 13.22% to 18.36%, while less available water ranged from 0.37% to 1.63%. We can conclude that Pannonian chernozem even after many decades of intensive conventional production is still favorable and satisfactory for the production of corn and other intensive crops, which require soils with a good water retention capacity.

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ASSESSING THE INFLUENCE OF NITROGEN PRESSURE ON NITROGEN COMPOUND LEVELS AND COMPOSITION IN SELECTED SURFACE WATER BODIES

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Abstract

With the global population on the rise, there is an increasing demand for food and water, prompting the need for intensified development of modern agricultural practices and proper water management. This population growth leads to higher water consumption and a greater volume of water requiring purification. Consequently, the increased agricultural production and wastewater generation contribute to higher nitrogen compound loads in water bodies. While fertilizer application is crucial for achieving high crop yields and quality products, its inadequate implementation presents various challenges. Moreover, the lack or insufficient functionality of wastewater treatment plants results in a significant amount of untreated wastewater reaching water bodies. This study focuses on investigating the impact of nitrogen compound pressures from various sources on the prevalence of nitrogen compounds in selected surface water bodies. The research aims to assess the extent of nitrogen compound contamination and identify the range of nitrogen compounds present in surface water bodies. By examining their correlation, the origin and transformation processes of nitrogen in the stream can be understood. The findings incorporate data from a five-year monitoring period of two rivers in Serbia – the Sava, and Velika Morava. These results contribute to a better understanding of nitrogen pressures on surface water quality, thus facilitating the development of strategies for sustainable agricultural practices and effective water resource management.

Keywords: *fertilizers, surface water, nitrates, nitrogen transformation.*

Introduction

Damjanović et al. (2001) concluded that non-point sources of pollution contribute to over 50% of total water pollution, as they contribute 90% of fecal and total coliform bacteria, 80% of total nitrogen, and 50% of total phosphorus. From the perspective of Water Framework Directive (WFD), the consequences of excessive use of fertilizers and pesticides, inadequate manure storage, and poor agricultural practices on agricultural land are of particular significance. Agriculture and land use are considered as one of the main drivers of diffuse source pollution emissions into surface and groundwater in the Republic of Serbia (WMP, 2023). Agricultural land covers most of the Serbia's land area (55%) and consequently poses a considerable potential for diffuse pollution, making it one of the major factors contributing to pressures and impacts on surface water and groundwater bodies (WPM, 2023). In addition to agricultural land use, livestock production is also a very important driver of surface and groundwater pollution. An analysis of data from the 2012 Agricultural Census conducted by the Serbian Environmental Protection Agency (SEPA) shows that in 2012, mineral fertilizers were used on 67% of agricultural land, while manure from livestock farms was used on 12% of agricultural land, and 21% of agricultural land was not fertilized. The use of mineral fertilizers in the Republic of Serbia has significantly decreased in the past, from 1,450,000 tons in 1985 to around 300,000 tons in 2000. It is estimated that currently 600,000 tons of mineral fertilizers are used annually. The average area of total agricultural land per capita, standing at 0.54 hectares, and the utilized agricultural land at 0.48 hectares per capita, highlight Serbia's significantly advantageous position compared to many European countries in this regard. For instance, France has 0.33 ha/cap, Italy 0.2 ha/cap, Germany 0.19 ha/cap, and the Netherlands 0.06 ha/cap. This situation signifies favourable food self-sustainability for the population of Serbia (NRP, 2016). The current agricultural practice regarding manure management is inadequate due to insufficient space for storage on livestock farms and a lack of equipment for its transportation and efficient application in the fields. Livestock contributes to total nitrogen pollution by 71% and about 70% of phosphorus, while households not connected to public sewage contribute to nitrogen pollution by 1.6%, phosphorus by 0.8%, and around 10% organic pollution (WMS, 2023).

Currently, 56% of the total population in Serbia is connected to sewage systems (approximately 3.9 million) (WMP, 2023). Public sewage systems receive about 300,000,000 m^3 of wastewater annually. Of the total discharge of wastewater into the public sewage network, 69% comes from households, around 19% from industry, and about 12% from other activities (WMP, 2023). In the Republic of Serbia, 49 wastewater treatment plants have been built. Out of the total number of plants, 32 are operational, with a smaller number achieving the designed effectiveness of the wastewater treatment process. An analysis of nutrient pollution pressures on surface water bodies in the Republic of Serbia is presented in Fig.1.

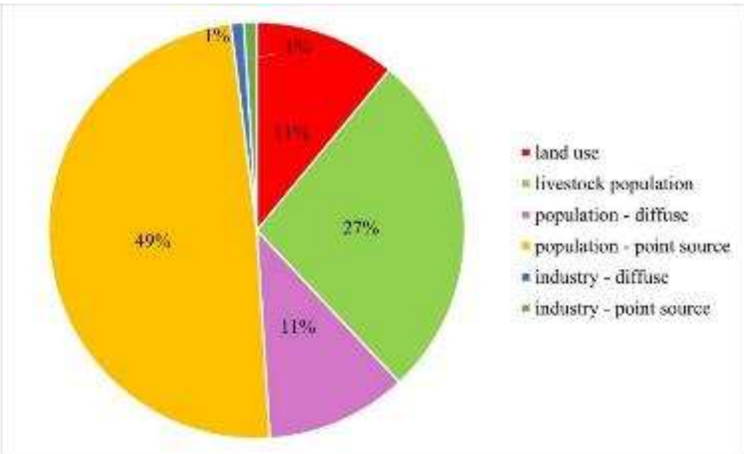

Figure 1. The pressure of nutrients on surface water bodies according to the source of pollution (according to WMS, 2023)

Relatively high specific pressures from nutrient pollution have been identified in less than 15% of the watersheds in the Republic of Serbia. However, it should be noted that even low specific pressures of nutrient pollution can cause significant impacts if the flow of water bodies is low (SUVRS, 2015; WMS, 2023). In conducted research, it was examined whether the current agricultural practice in Serbia and untreated wastewater discharge cause detectable changes in the quality of selected rivers concerning nitrogen compound concentration levels.

Materials and methods

Data from the state monitoring of surface waters (SEPA) have been processed for five-year period (2010-2014). Selected parameters of the Sava River quality are analyzed from 4 profiles: Jamena, Sremska Mitrovica, Šabac, and Ostružnica. The quality of the Velika Morava River is monitored at 5 profiles: Varvarin, Bagrdan, Velika Plana, Trnovče, and Ljubičevski Most. To group variables directly or indirectly involved in the nitrogen cycle, exhibiting similar behaviour patterns, and potentially having significant linear correlation, factor analysis was conducted. It included 13 surface water quality parameters (of the Sava and Velika Morava rivers). Considered physico-chemical parameters include: O_2, pH, electrochemical conductivity (ϰ), NH_4^+, NO_2^-, NO_3^-, organic nitrogen (ON), total nitrogen (TN), Cl, SO_4^{2-}, Ferrous, Manganese, and total organic carbon (TOC) (data on B concentration are not available for river water). The Kaiser-Meyer-Olkin test values above 0.6 and the significance of Bartlett's test of sphericity ($p < 0.01$) indicated that the applied factor analysis could be meaningful for processing the given datasets. Sample adequacy measure, represented by the average correlation value between observed parameters, in the principal component analysis applied in this study, exceeded 0.5. By evaluating factor loadings, primary components with the greatest influence were obtained. Oblique factor rotation was applied to clarify each factor and examine partial relationships alongside simple ones, reduce dimensionality, and identify the most suitable model with the fewest factors. The criterion of the eigenvalue was used to define the number of principal components (factors).

Results and Discussion

The quality of the river Sava based on selected parameters

Values of descriptive statistical data processing are shown in Table 1. The mean concentration value of nitrate nitrogen along the Sava River in Serbia is 0.62 mgN/l, and for ammonium ions, it is 0.08 mgN/l.

Table 1. Statistics of selected parameters for the river Sava for the period 2010-2014.

		O_2	pH	ϰ	NH_4^+	NO_2^-	NO_3^-	ON	TN	Cl^-	SO_4^{2-}	Fe_{rast}	Mn_{rast}	TOC
Unit		mg/l	/	μ/cm	mgN/l	mgN/l	mgN/l	mgN/l	mgN/l	mg/l	mg/l	mg/l	mg/l	mg/l
No of samples		104	104	104	104	104	102	102	102	102	102	75	76	98
Average		9.03	8.65	411.54	0.08	0.02	0.62	0.79	1.30	16.57	20.30	0.07	0.01	4.12
Median		8.93	8.00	403.00	0.08	0.01	0.60	0.46	1.29	15.25	19.00	0.04	0.01	3.70
Std. Dev.		1.88	7.00	55.89	0.05	0.01	0.27	2.07	0.42	8.24	6.37	0.08	0.02	1.67
Min		0.98	0.90	269.00	0.00	0.01	0.05	0.05	0.50	1.00	3.00	0.01	0.01	1.70
Max		13.80	79.00	550.00	0.30	0.06	1.17	21.00	2.60	44.00	37.00	0.45	0.09	10.30
Percentiles	25	7.81	7.96	378.25	0.04	0.01	0.40	0.23	1.00	10.30	16.00	0.01	0.01	2.70
	50	8.94	8.00	403.00	0.08	0.01	0.60	0.47	1.29	15.25	19.00	0.04	0.01	3.70
	75	10.10	8.10	440.75	0.10	0.02	0.89	0.85	1.50	21.00	25.00	0.10	0.01	5.35

Principal component analysis for the Sava River, according to the criterion of the eigenvalue, revealed 5 extracted factors (PC1-PC5), explaining 66.31% of the total variance of parameters (Table 3). The highest percentage of variance, 20.76%, is explained by the linear correlation between chloride, sulfate, and electrical conductivity, represented by the first extracted factor PC1. Since oxic conditions prevail in the river, these anions move similarly to non-reactive tracers, where their simultaneous increase in concentration with increasing conductivity is the most likely indicator of anthropogenic influence. PC2, explaining 13.51% of the variance, shows a strong correlation between nitrite, total, and ammonium nitrogen concentrations. The identified correlation indicates that the origin of total or ammonium and nitrite nitrogen in the

Sava is common, likely organic, potentially indicating the influence of sewage water, where the dominant form of nitrogen is ammonium ion, and the correlation with nitrites within the same factor may indicate the occurrence of nitrification processes. PC3 explains 12.32% of the total variance and shows a correlation between dissolved iron and manganese in the river. These metal ions are generally not present in significant concentrations under oxic conditions prevailing in the river and therefore exhibit the same behaviour pattern and are extracted into the same factor. PC4 explains 11.58% of the total variance and shows an inverse linear correlation between oxygen and organic carbon. This linear relationship may indicate that oxygen-rich water entering the river (large water volumes, high water levels) corresponds to the dilution of total organic carbon concentrations and oxygen consumption, as the most favorable electron acceptor, for the oxidation of organic carbon. PC5 explains the smallest percentage of variance, 8.14%, and indicates that nitrates (present in low concentrations) in the Sava exhibit a distinct behavioural pattern. Nitrates are not linearly correlated within the second factor, PC2, where predominantly nitrogen compounds are present, likely for three reasons: 1) untreated sewage water carries ammonium as the dominant ion, whose oxidation begins in oxic conditions prevailing in the river, generating nitrites, and then nitrates; 2) besides nitrates from nitrification, nitrates as such come within sewage water (besides ammonium ions); 3) there are other sources of nitrates (leaching from agricultural surfaces, atmospheric deposition).

The quality of the river Velika Morava based on selected parameters

The mean concentration of nitrate nitrogen is 0.90 mgN/l, with 75% of the samples having concentration values up to 1.20 mgN/l. The mean concentration of ammonium ions is 0.19 mgN/l, with 75% of the samples having concentrations up to 0.26 mgN/l (Table 2). The applied principal component analysis for the Velika Morava River revealed 4 distinct factors (PC1-PC4), which account for 48.44% of the total variance of parameters (Table 3). PC1, explaining 18.22% of the variance, shows a correlation between the concentration of ammonium ions, chloride, sulphate, and the electrical conductivity of water. The first identified factor confirms that the highest load of the Velika Morava watershed is noticeable based on the identified correlation, explaining the largest percentage of parameter variance. In the Velika Morava, ammonium cation correlates with other indicators of untreated wastewater discharge. PC2 explains 11.62% of the total variance and demonstrates an inverse linear correlation between the concentration of nitrates and organic nitrogen. Although the concentrations of observed parameters are not high, the correlations revealed between them indicate detectable sources, i.e., the influence of untreated sewage. Factor PC2 is consistent with the explanation of the first factor and indicates the oxidation process of organic nitrogen (nitrification) and the generation of nitrates (reducing the concentration of organic nitrogen). PC3 explains 10.17% of the variance and shows that pH values in the Velika Morava behave according to a separate and independent pattern compared to the mentioned parameters. The fourth factor, PC4, explains 8.44% of the total variance and shows a connection between the concentration of nitrite ions and organic carbon, where their simultaneous decrease in concentration is recorded, indicating their common origin (organic matter and nitrogen components).

Table 2. Statistics of selected parameters for the Velika Morava for the period 2010-2014.

		O_2	pH	ϰ	NH_4^+	NO_2^-	NO_3^-	ON	TN	Cl^-	SO_4^{2-}	Fe^{2+}	Mn^{2+}	TOC
Unit		mg/l	/	μ/cm	mgN/l	mgN/l	mgN/l	mgN/l	mgN/l	mg/l	mg/l	mg/l	mg/l	mg/l
No of samples		221	213	221	220	221	221	211	211	219	221	162	160	204
Average		9.91	8.13	420.63	0.19	0.07	0.90	1.13	4.81	13.25	35.21	0.35	0.40	5.58
Median		9.90	8.10	428.00	0.13	0.04	0.80	1.08	2.20	13.00	34.00	0.06	0.01	5.05
Std. Dev.		1.92	0.27	78.99	0.19	0.10	0.56	0.70	25.59	4.29	11.52	1.92	4.44	2.71
Min		3.70	7.00	27.00	0.01	<0.005	0.08	0.01	0.80	3.30	3.00	0.01	0.01	1.70
Max		17.40	9.00	770.00	1.00	0.70	3.60	5.25	274.00	26.00	96.00	17.00	56.10	22.20
Percentiles	25	8.51	7.91	378.50	0.07	0.02	0.50	0.60	1.86	10.40	29.00	0.02	0.01	3.80
	50	9.90	8.10	428.00	0.14	0.04	0.80	1.08	2.20	13.00	34.00	0.06	0.01	5.05
	75	11.10	8.34	463.00	0.26	0.07	1.20	1.60	2.62	16.00	40.00	0.13	0.03	6.40

Table 3 Matrix of selected parameters for the Sava, Danube, and Velika Morava rivers.

	Sava					**Velika Morava**			
	Extracted Factors								
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**
O2				0.898					
pH								0.854	
ϰ	0.838					0.729			
NH_4^+		0.577				0.581			
NO_2^-		0.860							-0.689
NO_3^-					0.923		0.852		
ON							-0.697		
TN		0.731							
Cl	0.876					0.535			
SO_4^{2-}	0.775					0.717			
Fe_{rast}			0.620						
Mn_{rast}			0.845						
TOC				-0.529					-0.817

Conclusion

The average concentration of ammonium ion is higher in the Velika Morava River (0.19 mgN/l), the in Sava River (0.08 mgN/l). The average concentration of nitrate nitrogen in the Morava (0.90 mgN/l) is higher than in the Sava (0.62 mgN/l). The highest nitrogen compound load is observed in the Morava River basin. A common pattern observed in both rivers, according to the applied factor analysis, is the same pattern of changes in concentrations and linear dependence of chloride, sulfate, and consequently electrical conductivity. In the Velika Morava, which has the highest population load in the basin, along with chloride and sulfate, a simultaneous increase in the concentration of ammonium ions is evident. The observed inverse linear correlation between the concentration of organic nitrogen and nitrates in the Velika Morava indicates that nitrates in the river are predominantly derived from the oxidation of organic nitrogen. Although the Sava River is characterized by the lower concentrations of ammonium ions and nitrates, a strong correlation has been established between the concentrations of total nitrogen, ammonium ions, and nitrites. Due to the treatment of only approximately 10% of wastewater and the lack of precise data on the types and quantities of fertilizers used, examined surface water quality and nitrogen compound concentrations in Serbia appear to be satisfactory. Nevertheless, analysis using Pearson coefficients reveals a clear correlation between untreated water inflow and subsequent nitrification of organic nitrogen and ammonium ions.

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NITROGEN PRESSURE IMPACT ON GROUNDWATER NITROGEN COMPOUND LEVELS

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Abstract

Considering that roughly 70 percent of Serbian total land area is comprised of agricultural land, with about 90% of wastewater being discharged untreated, we have investigated these two potential sources of nitrogen to determine if they have a discernible impact on shallow groundwater quality. The research aimed to analyze nitrogen compound concentrations, dependencies, and correlations in specific groundwater bodies to understand their transformations and origins. The presence and trends of concentration levels of the following species: ammonium (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-), organic nitrogen (ON), total nitrogen (TN), sodium (Na), chlorides (Cl), sulfates (SO_4^{2-}), iron (Fe), manganese (Mn), arsenic (As), total organic carbon (TOC) and boron (B) were analysed along with state parameter pH and electrical conductivity. Selected parameters provide comprehensive insights into the chemical composition, nutrient dynamics, pollutant levels, and overall water quality, which are essential for environmental monitoring and management. The analysis revealed that 75% of the groundwater samples had nitrate content below 2.54 mgN/l and ammonium content below 0.33 mgN/l. The study examined groundwater quality and levels of nitrogen compounds at 55 sites within shallow alluvial aquifers, as part of the regular annual national monitoring program spanning from 2011 to 2016. Correlations indicated that ammonium cations in the groundwater are primarily of autochthonous geological origin, while nitrogen presence under oxic conditions can partially be attributed to anthropogenic influences, linking the nitrification of organic nitrogen and boron. The objective of the conducted research was to ascertain whether there were indications of anthropogenic influences on the shallow alluvial aquifers, leading to an increase in the concentration of nitrogen compounds in groundwater.

Keywords: *anthropogenic impact, groundwater, nitrogen.*

Introduction

Approximately 75% of the EU population relies on groundwater for water supply. The significance of groundwater in the Republic of Serbia as a water supply source is remarkable, evident from the share of groundwater used for water supply, which stands at 75%. Over 50% of the groundwater used for water supply in Serbia originates from alluvial groundwater sources. Hence, it is of utmost importance to ensure that the concentrations of polluting substances potentially entering groundwater remain below the legally prescribed limits. In managing groundwater resources, it is crucial to consider the self-purification potential of the aquifer, which can be particularly significant in the case of nitrogen compounds (Perović and Dimkić, 2021). Processes such as sorption, biodegradation, oxidation-reduction, along with the effects of dispersion, diffusion, and advection, contribute to significant improvements in water quality (Perović and Dimkić, 2021). Sources of nitrogen compounds include organic waste, wastewater, inorganic and organic fertilizers, natural processes occurring in the soil (mineralization), animal manure, septic tanks, unsanitary dumps, and atmospheric precipitation (Nikolenko et al., 2018). Elevated concentrations of nitrate ions in water (NO_3^-)

have been observed in numerous aquifers worldwide, typically attributed to agricultural activities since the mid-twentieth century (Pasten-Zapata et al., 2014). The significance of agricultural nitrogen emissions to the environment is reflected in a specific Nitrate Directive adopted by all European Union members, which limits the nitrate concentration to 50 mg/l in all freshwater bodies (Directive 91/676/EEC, 1991). Several European Union member states (Denmark, Belgium, France, Germany, the Netherlands, Luxembourg, England, and Finland) have documented and addressed the issue of agricultural nitrogen emissions in National Reports as part of the implementation of the Nitrate Directive (Dimkić et al., 2008). Nitrogen compound leaching from agricultural areas not only negatively impacts groundwater quality but also affects the global nitrogen cycle due to the slow movement of groundwater, accumulation, and biological transformations of nitrogen compounds (Nikolenko et al., 2018). Nitrogen predominantly exists in organic form in the soil (about 98%), which is not directly available to plants and is not prone to leaching. Reserves of inorganic nitrogen (nitrate and ammonium ions) in the soil are typically very small and quickly depleted through uptake by plants and microorganisms. Inadequate irrigation as well as heavy rainfall can cause leaching of mobile and stable forms of nitrogen compounds, such as nitrates, below the root zone. Nitrogen can be removed from the soil in several ways: through evaporation, leaching, uptake by crops, denitrification, soil erosion, and surface runoff. Inadequate application of nitrogen fertilizers, inappropriate quantities, meteorological conditions, types of fertilizers, soil types, as well as the presence of septic tanks, contribute to elevated NH_4^+ concentrations (Bohlke et al., 2006). The potential impact of nitrogen from two significant sources (agricultural production and sewage discharge) is being investigated to determine whether it is detectable in shallow groundwater or if there are any observable nitrogen transformations. The highest number of groundwater bodies in the Republic of Serbia is in the Morava region (55), followed by the Sava (33), Danube (30), Banat and Bačka (21), Belgrade (9), and Srem (5) regions, respectively. Most of the water in Serbia is abstracted from alluvial sources (more than 50%). The next most utilized are karstic (slightly over 20%), while fractured aquifers are the least utilized groundwater sources (Stevanović, 1995; Dimkić et al., 2007; Milovanović et al., 2010). Potentially the most pronounced anthropogenic influence can be observed on resources characterized by intensive and dynamic water exchange, as well as variable hydrochemical conditions, which mainly relate to karstic, and shallow alluvial sources. For population water supply, the most used aquifers are those with a free water table in the alluvial plains of major rivers (Danube, Sava, Velika Morava, and Drina), as well as confined aquifers within the Neogene basins of Vojvodina and central Serbia (FMG, 2018). Alluvial aquifers represent the richest groundwater resources, but at the same time, they are areas of intensive hydrodynamic exchange, fluctuating redox conditions, and are sensitive to potential anthropogenic influence.

Materials and methods

The quality of groundwater in alluvial aquifers in Serbia is systematically monitored through an observation network, which was previously under the jurisdiction of the Republic Hydrometeorological Service and has been under the jurisdiction of the Environmental Protection Agency since 2011. Statistical analysis of selected data on the quality of shallow alluvial groundwater was conducted in the study, covering 55 sampling locations over a period of 6 years (2011-2016) (Figure 1). To reduce the number of variables without losing significant ones, and to identify and understand patterns of change in concentration levels of selected parameters in shallow groundwater in Serbia, Principal Component Analysis (PCA) was applied. PCA enabled determination of data correlations within underlying structures between seemingly unrelated variables.

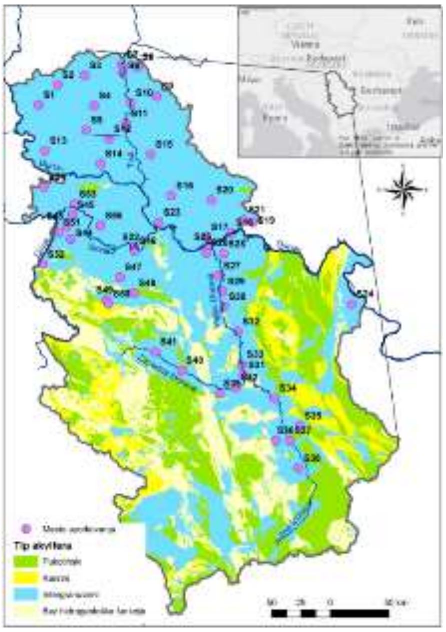

Figure 1 Sampling locations of shallow alluvial aquifers in Serbia. (Perović, 2019)

Results and Discussion

After six years of monitoring across 55 sites, several key conclusions regarding the origin and transformation of nitrogen in examine groundwater can be generally inferred (Table 1). Due to the significant diversity in the baseline quality and quantity of groundwater covered by regular monitoring in Serbia (Figure 1), discussion can be conducted regarding the interquartile range, concluding that 75% of groundwater samples have oxygen concentrations up to 4.03 mg/l, ammonium ion concentrations up to 0.68 mg/l, and nitrate concentrations up to 3.53 mgN/l.

Six out of 52 sites, where occasional measurements of dissolved oxygen concentration are available, have an average concentration below 1 mg/l (12%) (S1-Sombor; S4-Njegoševo; S5-Vrbas; S13-Bač; S14- Novi Sad; S18-Dubovac). Four sites, or 8% of samples (S6-Kanjiža; S10-Padej; S23-Borča; S49-Valjevo), have an average dissolved oxygen concentration between 1 and 2 mg/l. The majority of samples from 42 piezometers (80%) exhibit oxic conditions (>2 mg/l) (S2-Aleksa Šantić; S3-Subotica Mikićevo; S8- Banatsko Aranđelovo; S9-Kikinda; S11-Burza; S15-Zrenjanin; S16-Debeljača; S17-Kovin; S19-Kusić; S20-Banatski Karlovac; S21-Vračev Gaj; S22-Zabrežje Savska; S24-Negotin; S25-Šalinac, S26-Dubravica Lipe; S27-Lozovik Vlaški Do; S28-Požarevac; S29-Velika Plana Žabari, S30- Markovac Svilajnac; S31-Varvarin Ćićevac, S32-Bukovče Glogovac; S33-Obrež Ratare, S34-Žitkovac ciglana; S35-Brzi Brod Selo; S36-Žitorađa; S37-Doljevac; S38-Leskovac; S39-Tobolac Trstenik; S40-Sirča; S41-Stančić Selo; S42-Kruševac; S43-Bogatić, S44-Duvanište; S45-Noćaj, S46-Obrenovac; S48-Bogovađa; S50-Petnica Vrelo; S51-Badovinci, S52-Loznica polje; S53-Laćarak, S54-Šid; S55-Nikinci).

Of 55 piezometers, the mean concentrations of ammonium ions in 28 examined piezometers (51%) exceed the drinking water level of > 0.1 mgN/l. Six piezometers: S1-Sombor, S8-Banatsko Aranđelovo, S9-Kikinda, S10-Padej, S15-Zrenjanin, S20-Banatski Karlovac (11%) have mean concentration values above 1 mg/l. Significantly elevated concentrations of ammonium nitrogen (>3 mg/l) were recorded in the piezometers: S10-Padej and S20-Banatski Karlovac. Mean concentrations of nitrate nitrogen for 75% of groundwater samples from shallow aquifers are lower than 3.53 mgN/l. In 13 piezometers (27%): S14-Novi Sad, S21-

Vračev Gaj, S22- Zabrežje Savsa, S27-Lozovik Vlaški Do, S28- Požarevac, S32-Bukovče Glogovac, S33- Obrež Ratare, S43-Bogatić, S44-Duvanište, S48-Bogovađa, S51-Badovinci, S54-Šid, and S55-Nikinci, the mean concentrations of nitrate nitrogen exceed 3 mgN/l. The highest mean concentration values of nitrate nitrogen (>10 $mgNO_3$-N/l) were recorded at stations: S21-Vračev Gaj and S54-Šid.

Table 1. Statistical analysis of selected parameters for shallow groundwater in Serbia for the period 2011-2016.

		pH	Ec	NH_4^+	NO_2^-	NO_3^-	Fe_{uk}	Mn	Fe_{rast}	As	B	TOC
No. of samples		327	327	325	327	327	233	247	254	152	160	214
Unit			µS/cm	mgN/l	mgN/l	mgN/l	µg/l	µg/l	µg/l	µg/l	µg/l	mg/l
Avg		7,5	957,6	0,35	0,01	2,29	1090,77	290,10	148,33	18,31	97,43	6,51
Median		7,4	915,0	0,07	0,01	0,40	294,00	97,80	11,25	1,40	75,05	4,00
Std. Dev.		0,3	356,3	0,64	0,02	4,05	2045,36	544,05	576,82	63,38	92,10	6,99
Range		1,7	2021,0	4,16	0,11	23,65	12519,50	4626,50	4700,75	520,15	594,70	36,75
Min		6,6	309,0	<0,02	<0,002	<0,2	<10	<10	<10	<10	<10	<0,5
Max		8,3	2330,0	4,16	0,11	23,65	12520,00	4627,0	4701,00	520,40	595,20	37,00
Perc.*	25	7,3	707,0	0,03	0,00	0,20	84,35	22,80	0,50	0,25	33,53	2,50
	50	7,4	915,0	0,07	0,01	0,40	294,00	97,80	11,25	1,40	75,05	4,00
	75	7,6	1127,0	0,33	0,02	2,54	1115,00	307,80	90,25	3,60	126,58	7,80

*Percentile

Based on the eigenvalue scores, four principal components (PCs) were extracted using PCA analysis (Table 2). PC1, explaining 41.68% of the variance, indicates a strong positive linear correlation between concentrations of NH_4^+, Cl^-, SO_4^{2-}, and TOC, with an increase accompanied by elevated values of electrical conductivity. As this factor explains the highest percentage of groundwater sample variance, it can be concluded that a significant portion of detected ammonium cations in the examined shallow groundwater has a common origin with organic matter (indicating mineralization of organic matter, autochthonous origin). However, since the increase in Cl^- and SO_4^{2-} concentrations is also present in the linear correlation, part of the ammonium cations may also have an anthropogenic origin. Considering that the examined groundwater is from shallow "first" aquifers, above which agricultural production is prevalent and there are numerous small settlements without sanitary sewage, with predominantly recharge from surface water receiving untreated wastewater, a certain percentage of NH_4^+ originates from anthropogenic activities (application of manure and inorganic fertilizers, wastewater). The second extracted factor, PC2, explains 34.27% of the total variance and shows a very strong linear correlation between concentrations of NO_2^-, ON, TN, and B, which are inversely correlated with concentrations of dissolved iron and arsenic. This extracted factor explains the origin of nitrogen under oxic conditions and is a consequence of anthropogenic influence linking nitrification of organic nitrogen and boron, occurring in environments lacking dissolved iron and arsenic, i.e., oxic conditions. The third extracted factor, PC3, explains 12.64% of the variance and shows a decrease in conductivity, sodium, and sulfate concentrations with increasing pH values. The fourth extracted factor, PC4, explains 7.16% of the variance and shows a very pronounced linear correlation between concentrations of Fe, Mn, and As, with increased concentrations corresponding to elevated NH_4^+ cation concentrations. In inverse relationship with this increase are concentrations of nitrates and total nitrogen. This factor is an unambiguous indicator of autochthonous geological origin of ammonium cations in certain examined groundwater samples in Serbia.

Arsenic in groundwater can be released by reductive dissolution of ferruginous arsenite minerals and arsenic-sulfide minerals. If the organic matter content is naturally high, decomposition will cause a decrease in pH and the occurrence of anoxic conditions, resulting in reductive dissolution of iron oxides and iron oxyhydroxides and migration of sorbed As^{3+} ions (Weng et al., 2017). The observed correlations may also be due to carbonation of arsenic-sulfide minerals (arsenopyrite) in groundwater, characterized by high alkalinity (Berg et al., 2001).

Table 2. Cluster matrix for examined aquifers in Serbia.

	pH	Ec	NH_4	NO_2	NO_3	ON	TN	Na	Cl	SO_4	Fe*	Mn*	As*	TOC	B*
PC1		0.82	0.89		-0.67				0.96	0.85				0.94	
PC2				0.95		0.98	0.93				-0.66		-0.68		0.86
PC3	0.98	-0.65						-0.94		-0.53					
PC4			0.60		-0.91		-0.55				0.85	0.96	0.81		

*Dissolved
Ec- electrical conductivity

Conclusion

In addition to the application of nitrogen-based mineral fertilizers, unregulated sewage discharges and leakage, the presence of septic tanks, and manure application represent the most significant sources of elevated nitrogen compound concentrations in groundwater. Certain types of aquifers, due to their inability to retain water in the vadose zone and the effect of self-purification potential (karst and fractured), are characterized by a higher natural sensitivity. The hydrogeological characteristics of the terrain will define the flow of water, and therefore the potential for the influx of certain pollutants. After considering potential pressures and natural conditions for the retardation of pollutants, it is necessary to understand the chemical composition of water, which is influenced by: the porosity of the aquifer, the mineral composition of rocks in the aquifer, the permeability of the vadose zone, as well as the method of aquifer recharge and drainage. For nitrogen compounds, the amount of oxygen, the value of the electrochemical potential and the concentration of Fe^{2+}, Mn^{2+}, H_2S define the conditions for reducing nitrate concentration (nitrogen oxide emissions) or nitrogen conservation (reduction to NH_4^+ cations). In addition, concentrations of SO_4^{2-}, C^{l-}, Na, and B indicate the occurrence of transformation processes or the origin of nitrate anions. If linear (direct or inverse) correlations can be observed and quantified between changes in the trends of these parameters, interpretations of the fate and origin of nitrogen compounds in groundwater can be a reliable complement to hydrogeological and hydrodynamic considerations conducted for the same purpose. Based on gained results the 75% of the analysed samples have nitrate content below 2.54 mgN/l, while the same share has the ammonium content below 0.33 mgN/l. Revealed correlations indicated the portion of groundwater ammonium cations is of autochthonous geological origin, while the origin of nitrogen under oxic conditions is shown to be a consequence of anthropogenic influence (linking nitrification of organic nitrogen and boron). Vulnerability to nitrate infiltration into the aquifer decreases with increasing thickness and decreasing permeability of the vadose zone, reducing the amount and infiltration of precipitation, a favorable configuration of the hydrogeological complex with adequate protection from surface water infiltration, and having less sensitive intergranular porosity compared to fractured karstified aquifers.

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BIO-CONTROL OF THE FIVE MOST INVASIVE WEEDS AS A NARATIVE FOR THE SUSTAINABLE GOVERNANCE OF BALKAN NATURAL RESOURCES

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Abstract

Classical biological control remains the only tool available for permanent ecological and economic management of invasive alien species that flourish through absence of their co-evolved natural enemies. As such, this approach is recognized as a key tool for alien species management by the Convention on Biological Diversity (CBD), the European and Mediterranean Plant Protection Organization (EPPO) and the European Strategy on Invasive Alien Species (ESIAS). Successful classical biological control programs are present around the world, despite disproportionate attention being given to occasional and predictable non-target impacts. Despite more than 130 history cases in Europe against pests, no exotic classical biological control agent has been released in the EU against an alien invasive weed. This has occurred in the face and threat of increasing numbers of exotic invasive plants being imported everywhere almost taking over our National Parks, even „Virgin forests “, which is within first regime of protection areas in this region. The picture is the same on all continents. This paper considers the five most potential (with reference to Balkan) weed targets for classical biological control from ecological and socioeconomic perspectives using the criteria of availability of biological control agents, taxonomic isolation from European native flora and likely invasiveness outside Europe. Also, the paper considers the value to primary industry and horticulture (potential for conflicts of interest) regarding the five most invasive weed species (four woody species). Using standard entomology and phytopathology methods, results were presented in non-usual scale-number system, which was not done before, but in more plastic, interesting approach.

Key words: *Reynoutria, Ailanthus, Amorpha, Robinia, Bio-control.*

Introduction

Effective agents do not require regular application, as they spread naturally through the invader populations. Classical biological control has a historically tarnished reputation largely due to early unscientific and uncontrolled releases of vertebrate predators to control pests (e.g. releasing cats to control rats on islands). Even when this led to success, these predators also decimated native species. More recent cases of insect biological control agents spreading beyond their intended targets (e.g. the spread of *Harmonia axyridis* Pallas across Europe; Roy *et al.*, 2005). Plenty of cases also resulted from historically unregulated use of biological control against insect pests in Europe. Classical biological control of weeds now adopts a precautionary approach using the most specific antagonistic invertebrates and microorganisms against selected targets and follows best-practice scientific risk analysis and regulatory approval prior to release (Sheppard *et al.*, 2003). When conducted as such, negative effects have proved almost entirely predictable (Pemberton, 2000). In Europe, and on Balkan where weed biological control is still in its infancy, it will be important to identify initial weed targets that will have the widest ownership and be acceptable to even the sternest critics of biological control (Lonsdale *et al.*, 2001). With this in mind, the list was then assessed for biological control potential based on (i) historical success of biocontrol against these targets, ecological homologues and related species, (ii) taxonomic isolation of these weeds from geografic origines native flora (as a measure of risk of non-target damage), (iii) likelihood of suitable natural enemies being available as potential agents, (iv) target value to agriculture, horticulture and forestry (potential conflicts of interest) and (v) whether species were significantly invasive outside the area (opportunities for international collaboration; see Sforza & Sheppard, 2006). Impacts on European biodiversity could not be used, as so far data are available with reserve, butthey are presented where known. Each of five species“ independently considered these five questions“ and the number of positive responses was tallied for each species across all authors and used to prioritize the weeds for biological control. Where the number of positive responses was the same for different species, the relative geographical distribution and local abundance within and between closest countries was used to further prioritize the species. It was not possible to leave out the most important species with actual or potential conflicts of interest, because in a continent as culturally diverse as Europe nearly all alien species are valued by someone. It also has been reviewed why classical biological control of European exotic plants remains untested, considering problems of funding and public perception. Finally, consideration managed - of the regulatory framework that surrounds such biological control activities within constituent countries of the EU to suggest how this approach may be adopted in the near future for managing invasive exotic weeds in this region.

Materials and methods

The Risk Model uses numbers to represent the relative magnitudes of frequency, probability, and consequences, but the model cannot be considered “quantitative” in the sense of scientific accuracy. Using numbers simply allows various risk factors to be weighed systematically during the risk evaluation process. Description- the following description highlights the model for evaluating the risks associated with approving an operational plan for suppressing IS in sustainable and biodiversity preserving precondition. When an SDM receives an operational plan for approval the acceptability of the controlling- suppressing plan, must considered, all things reviewed, including following: The risks to a wide range of environmental, social and economic values of ISHE; The proposed mitigation of identified risks; The potential benefits of the proposed control or plan component; The Risk Model that follows offers guidance on the key steps in assessing risk. The approach leads decision-makers to draw risk conclusions by considering: Values of Concern; Potential Detrimental Invasive species hindering effect (Invasive species adverse effect; Frequency of Loss Event; Probability of Consequences Given a Invasive species adverse effect; Consequences of Loss Invasive species hindering effect -our „five all could be described like this, but finding perfect match for BC will connect the perfect pairs . Instructions -to ensure consistent application of this model, users should not assign numerical values other than those suggested in any of the categories. When in doubt choose the higher risk value. The initial entry in the Risk Assessment Matrix identifies the project or component under consideration. In the study of probability, invasive species hindering effect that will definitely happen are assigned a probability of 100 percent. Incidents that are impossible are given a 0 percent chance of occurrence, and everything else lies somewhere between these two extremes. Consider the following three levels of probability: Probable (7 points). The likelihood or probability of the consequence occurring is greater than 50 percent. Possible (4 points) The probability of the consequence falls between 20 and 50 percent. Unlikely (1 point) .There is less than 20 percent chance that the consequences will occur. The probability factors considered in selecting among the three broad categories of probability should be recorded in the comment space. Most Likely

Consequences - The next set of factors deal with the potential consequences of the plan or component, given a loss event, such as a landslide. In large part, this step requires decision-makers to consider the resources and other values that may be affected by the plan or component. Four categories of potential consequence are available: Catastrophic 10 points; Major 7 points; Serious 4 points; Minor 1 point. Briefly record the factors and features considered in making a determination of potential consequences. Total Risk Level-In the evaluation of risk, consider all three factors: Frequency of invasive species hindering effect presence, probability that consequences will occur, and the extent of consequences to things of value. Combining these related risk elements is a mental process that defies a strictly scientific or structured approach. However, the model suggests a simple method that has been adopted in other types of risk decision-making: Add the values for frequency and probability, then multiply the sum by the consequence value to determine a total risk score.

Results and discussion

Results suggest that each of a species, we are considering for matching is very competitive, resistant to contaminants or new control procedures, and one that can delay its dispersal until it has established a "beachhead" in the PA landscape. The beachhead patch ensures that there is a source for further migration into the landscape. The implication is that a fragmented or patchy environment will be more likely to contain an invasive because of the increasing number of areas that can be colonized and used as beachheads for further colonization. If the invasive can remain cryptic so that eradication efforts are limited until established in several refugee patches (PA habitats), the probability of a successful invasion should increase. The models confirm work by many other researchers (see Anderson *et al.*, 2004; Marvier *et al.*, 2004) that there is a clear interaction between the landscape, competing species, and the invasive species. Spatial structure must be incorporated if an understanding of the possible outcomes is to be factored into the risk analysis – special chalenge for Balkan countries.. The interaction between control measures and the native species demonstrated that only in extreme – high risk events influence had badly the outcome of the invasion (Watrud *et al*. 2004; Landis *et al*. 2000). Five alien plant species were identified through this process/ risk model as having positive responses to the posed questions. These are listed in Table 1.

Table 1. Exotic invasive plants in prioritized as potential biocontrol targets arranged by lines into groups of decreasing priority, but of similar priority within each group

Species Life form* Area of origin EU climate distribution Genus native to Europe

Species	Life form*	Area of origin	EU climate distribution	Genus native to Europe	Conflict of interest†	Past or current biological control programs/ publications
Fallopia japonica	Ge	Japan	Temperate	Yes	No	Yes
Fallopia bohemica(*Polygonum cuspidatum*)	Ge	Hybrid	Temp/Med	No‡	No	Yes
Amorpha fruticosa	Ph	N. America	Mediterranean	No‡	No	Yes
Ailanthus altissima	Ph	China	Temp/Med	No‡	No	Yes
Robinia pseudoacacia	Ph	N. America	Temperate	No	F	No

Legend for Table 1:
Freeman T.E.& Charudattan R. (1985)
*Ph = Phanerophyte, Ge = geophyte, Hy = hydrophyte, He = hemicryptophyte, Th = therophyte, Ch = chamaephyte.

Conflict of interest: †Past or current biological control programs/ publications
†O = current ornamental interest, F = value as forestry tree – simple aesthetic value of certain aliens weeds is not considered a conflict of interest as biocontrol will only reduce their density not eradicate them.
‡Family or subfamily also not native to Europe. 1. ***Fallopia japonica*** **(Houtt.).** 2. ***F. × bohemica*** **Chrtek & Chrtkova**

1. ***Fallopia*** (Polygonaceae) contains 24 species worldwide of which seven are considered weeds. ***Fallopia japonica*** **var.** ***japonica*** **(Houtt.) Ronse Decr.**, the most invasive clone (Bailey, 1994), is referred to as ***Reynoutria japonica*** **(Houtt.)** in some parts of Europe, Balkan also. Its firs appear on west of Serbia, then all across the country near roads and forest edges.
2. ***Polygonum cuspidatum*** **Sieb.& Zucc.** are also invasive, although their relative importance in Europe and Serbia is still being studied as partially *Fallopia japonica* and fact that mantioned hybrid appears to spread faster than either parent (Mandák et al., 2004), makes these two species among top five Serbia’s and Balkan’s aggressive aliens.

   <u>Existing and potential biological control for both plants:</u>
   Biological control is now recognized as the only longterm, sustainable solution to *Fallopia* spp. (Shaw & Seiger, 2002), but a full programme has been a long time coming. Since 2003, apredominantly UK consortium runthrough CABI has conducted field surveys in Japan, where the plant is heavily damaged compared with the exotic range. Selected insect sand range screening including a weevil (*Lixus* sp.), a psyllid (*Aphalara* sp.) (Fig. 1C), a rust (currently identified as *Puccinia polygoni amphibii* var. *tovariae* Arthur) and a leaf-spot disease (*Mycosphaerella* sp.) (Fig. 1D).
3. ***Amorpha fruticosa*** **L.** Origin, life history and ecology - According to some sources it was introduced in the Balkan Peninsula at the beginning of the twentieth century, precisely in 1900 (Petračić, 1938). False indigo bush L. (Fabaceae= Papilionaceae: Astragalae) reproduce generative, with pods, dispersed by water, and vegetative with a strong power of sprouting. Pods yield natural insect toxic chemical, and if demanding systematically control measures by combination of chemical and mechanical measures trailed this forest weed as woody plant are practically invincible (Gagić *et al*., 2008). Existing and potential biological control without finding solution that would exclude combined application of too expensive mechanical suppressing measures and environmentally eligible suspected pesticides it is possible to predict unstoppable expansion of this plant and facing with serious major problem in the near future. Table 2. as just seed enemies as bio control agents are more then enough for False Indigo Bush , because of power, or thanks to it’seed beetle opportunity even to attack dry seed so even 96% of whole samples could be destroyed.

Existing and potential biological control:

Table 2. Here just natural enemies of *A. fruticosa* seeds or it’s spermatophagous

Insect	***Biology and host preference of A. fruticosa pod pests***
Acanthoscelides pallidipennis* Motschulsky. *Coleoptera: Bruchidae: Bruchinae	***Indigo bush weevil, bruchid beetle found feeding in pods (from 1/3 to 2/3, which is significant agent)***
***E. vesicularis*; *E. urosonus* (Hymenoptera: Chalcidoidea: Eupelmidae), in significant trio with *D. acutus*, (Hymenoptera: Chalcidoidea: Pteromalidae)**	ectoparasitoids of weevil larvae
***Syntomaspis* sp. and *Torymus* sp. (Hymenoptera: Chalcidoidea: Torymidae)**	possibility of seed predation and hyper parasitism, both need to be proven
***Tetrastichus* sp.; *Aprostocetus* sp (Hymenoptera: Chalcidoidea: Eulophidae)**	known to encompass parasitoids of the first and second order, so it is needed to proceed the research in order to determine their status -hyper parasitism phenomena demands experimental “tricks”
(Hymenoptera: Proctotrupoidea: Scelionidae)	Reared one specimen as fresh bruchid beetle egg parasite. Investigation needs to be continued in a goal of getting more specimens, data, status confirmation and species determination
(Hymenoptera: Proctotrupoidea: Diapriidae)	Hyperparasitoid of *Eupelmus* and *Torymus* genera, until now one specimen had been reared and prepared
Acari, Pyemotidae	Predators of weevil larvae and pupa
***Pyemotes* spp. (=*Pediculoides*) verticosus, National Academy of Sciences 1978**	

4. *Ailanthus altissima* (Miller) Swingle. *Ailanthus* (Simaroubaceae) contains 10 species confined to Asia and Australia of which A. altissima (from temperate and subtropical China), the only member of the 156 family in Europe and Balkan, it is considered an invasive species in most temperate regions of the world. Mediterranean coasts of Eurasian countries where it can suppress many native species through allelopathy (Heisey, 1996). *Ailanthus altissima* roots cause significant structural damage and exposure to the sap through cuts and abrasions in the skin can cause cardiac problems (Bisognano *et al*., 2005).

Existing and potential biological control:

Surveys conducted in China by USDA Forest Service identified nine specific plant pathogens and four arthropods (Zheng et al., 2004). Of these, two weevils [*Eucryptorrhynchus brandti* (Harold) and *Eucryptorrhynchus chinensis* (Olivier)], one heteropteran (*Orthopagus lunulifer* Uhler), three fungal pathogens (*Alternaria ailanthic* Zhang & Guo, *Aecidium ailanthi* , Zhuang and a *Coleosporium* sp.) have been selected for further study which could be through collaboration with Europe (Sheppard *et al*., 2006). A commercial stump treatment product (Stumpout^{TM}) based on the fungus *Cylindrobasidium laeve* (Pers.) *Chamuris* is used in South Africa killing 80% of treated stumps (Lennox *et al*., 1999).

5. Robinia pseudoacacia L. *Robinia* (Fabaceae) contains four species from North and Central America, all of which are considered as weeds worldwide. Now is extremely widespread in many habitats on Balkan and Serbia, also within Europe. Existing and potential biological control-more recently, classical biological control of weeds has also undergone significant criticism from within the ecological and evolutionary scientific community (Louda *et al*., 1997), despite there being only a few predictable non-target impacts and the release decisions for the causal agents being made at a time when society was more risk accepting.

Picture 1 *Mycosphaerella sp. Almost covers leaf of Reynoutria japonica (Houtt.)*

Picture 2 *Ailanthus altissima are sensitive to Eucryptorrhynchus sp. weevils*

Existing and potential biological control:

Three North American insects have already established on *R. pseudoacacia* in Europe. The gracillariid leaf mining moth, *Phyllonorycter robiniella* (Clem.), is found from Switzerland (since 1983) to Poland and Germany to Italy, whilst the cecidomyiid gall midge, *Obolodiplosis robiniae* (Haldeman) from the eastern USA, is ten years afo found in Italy, Slovenia and in the Czech Republic, in whole Europe along with the widely distributed locust borer *Megacyllene robiniae* (Forster) (Cerambycidae). *Phyllonorycter robiniella* causes premature leaf drop that negatively influences tree appearance and as such has itself been the target of a biological control program in Italy (Wojciechowicz-Zytko & Jankowska, 2005). Relatively high infestations of *O. robiniae* also because leaf fall, but the tree soon produces regrowth (Duso *et al*., 2005). *Megacyllene robiniae* tunnels serve as entry points for the fungus *Phellinus rimosus* (Berk.) Pilat (syn. *Fomes rimosus* (Berk.) Cooke) (Hoffard, 1992), which causes extensive wood decay and root rot (Hoffard, 1992) almost like *Vascular Wilt Disease* – almonst destroy alms and many oaks. Augmenting these species in Europe could provide one biocontrol strategy, but *R. pseudoacacia* also has other natural enemies in its native range that could be targeted at less desirable parts of the plant, e.g. the seeds. So today, different experiment with its serious seed predator *Bruchophagous robinie* Zer. are perform, within Entomology laboratories on Institute of forestry, Belgrade in a foal of maximum destroying generative host potential (Freeman & Charudattan, 1985). Something usually is cover mechanisms of infestation are the hyperparasitoids (Chalcidoidea: Eulophidae; *Tetrastichus*) phenomena so even results are newer superior. Even, some balanced results could be expected soon – lower generative potential and germinative obstruction.

Conclusions

Classical biological control offers environmentally sound and public good solutions to group of where affiliate some of Europe’s worst alien five but strong and powerfull invasive plants. It would assist EU commitments to reducing chemicals in the environment and controlling alien invasive species, while applying the precautionary approach to intentional introductions of such beneficial exotic organisms. Europe has no shortage of potential targets for classical biological control using coevolved exotic natural enemies. Indeed, some of these weeds have been subject to successful biological control elsewhere. This review highlights just 5 of these and suggests, with the full support and in the context of the CBD and the ESIAS, the time is ripe for classical biological control of weeds to break into the mainstream, alongside public demand for action and national commitments to reduce chemical use and protect biodiversity. However, this will continue to be delayed if suitable government-assisted funding streams are

not established alongside processes for assessing conflicts of interest and raising public awareness on the issue of the costs of invasive species and the available solutions to them. Furthermore, the EU and its member states need to enact legislation and associated regulations as recommended under the ESIAS for restricting the importation of harmful and potentially harmful exotic organisms. Appropriate regulations can still allow releases of beneficial exotic species used in classical biological control, based on ISPM 3 and EPPO standards (2000) of risk assessment. By doing so, the EU would ensure all biocontrol agents proposed for release meet international risk analysis standards. The use of plant pathogens as classical biological control agents in the EU needs to be facilitated more than any other agent type through revision of the 91/414/EU directive, or at least its interpretation by member states, so that it is only applied as originally intended to formulated products. Rapid progress must be made, if all the invasive alien species management tools are to be available and Europe is to catch up with the rest of the world.
So for Balkan's countries there's a summary: *Fallopia* (both of) could be endengered the mostly by weevil (*Lixus* sp.) and serious cover by *Micosphaerella* sp. coverage on leaves; *Ailanthus altissima* are also sensitive to *Eucryptorrhynchus* weevils, *Amorpha* could be practicly destroyed through generative power by seed beetles and their parasitoids, and black locust could serious be attacked by combination of *Megacyllene robiniae* and fungus *Phellinus rimosus*, which causes extensive wood decay and root rot almost like Vascular Wilt Disease known as killer of Ulmus and oaks verticillosis.

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RESTORATION AND CONSERVATION OF THE PROTECTED TREE *QUERCUS ROBUR* L. AT THE SITE “JOZIĆA KOLIBA”, SERBIA

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Abstract

The "Jozića Koliba" memorial is located within the "Obrenovački Zabran" forest complex, which also includes the "Jozića Koliba English oak group", which is a level III protected monument. This natural monument consists of six English oaks (*Quercus robur* L.) with an average age of 250 years. Due to the effects of climate change and anthropogenic activities, all trees require intervention to maintain the overall attractiveness of the area. The tree number 1 requires immediate action to slow down its ageing and decay. The aim of this study was to analyse the state of health and the degree of deterioration of the protected tree number 1. Based on the results obtained, measures were defined to remedy the damage caused by biotic and abiotic factors. The investigation included recording the habitat-ecological conditions, dendrological parameters and the health of the tree. Boot damage was determined by visual inspection, sampling for laboratory tests and the use of an increment borer from inside the boot. The vitality and decorative value of the tree were assessed on the basis of these analyses using a modified VTA method. The analyses showed that the tree is dying due to its age and that this process is accelerated by ecological and, above all, anthropogenic factors. Based on the measurements, a cross-sectional model of the tree was created at the root collar and at a height of 1.5 metres. The percentage of the healthy part of the tree is between 10-85% of the total cross-section. The rehabilitation of the tree included cleaning the rot in the boot, disinfecting the cavity and filling it. Finally, a crown reduction was carried out by removing damaged branches. Support pillars were designed to improve and ensure the stability of the tree. The proposed restoration measures will extend the life of the tree and allow the area around the protected tree to be used safely.

Keywords: English *oak, arboriculture, Obrenovački Zabran, VTA.*

Introduction

The "Jozića Koliba" memorial is located within the "Obrenovački Zabran" forest complex on the left bank of the Kolubara River, near the village of Veliko Polje, and covers an area of 2 hectares. By the decision of the City Assembly of Belgrade, the area "Group of English oaks in Jozića Koliba" was designated as a natural monument and declared a protected natural area of protection level III - significant natural area (No. 501-8/96-XIII-01 of 1 February 1996, Official Gazette of the City of Belgrade, No. 1/1996) (Veselinović et al., 2014; Veselinović et al., 2015). The maintenance of this natural asset has been entrusted to the Public Enterprise for the Protection and Improvement of the Environment in the Municipality of Obrenovac.
The natural monument consists of six English oaks (*Quercus robur* L., syn: *Quercus pedunculata* Erh), which are on average about 200 years old, with their horizontal crown projections. The protected area covers 16.25 ares. The main objective of the protection is to preserve these English oaks as important components of the native forest communities of oak and ash (*Querceto-fraxinetum serbicum*) that were once widespread in Serbia (Gajić, 1984;

Tomić and Rakonjac, 2012). The site "Group of English oaks near Jozića Koliba" is under level III protection, which allows selective and limited use of natural resources as well as controlled interventions and activities, provided that they are consistent with the functions of the protected natural area or are associated with traditional forms of economic activity and housing, including tourism development (Veselinović et al., 2014; Veselinović et al., 2015). These activities must not jeopardise the authenticity of the flora and fauna, hydrographic, geomorphological, geological, cultural and landscape values, unless they maintain or create a natural balance and fulfil the functions of the natural area in accordance with the established protection regime and its significance. The protected oaks are located on the cadastral parcel Veliko Polje No. 1571/1 and are marked with numbers from 1 to 6. The observed damage and decay of the protected trees require interventions to preserve the trees themselves (Gruber, 2008) and thus maintain the authenticity and attractiveness of this place (Mitrović et al., 2023). The protected tree with the number 1 is the subject of this study.

Materials and methods

In order to achieve the restoration and conservation of the protected tree No. 1 in the natural monument "Group of English oaks in Jozića Koliba"," a methodology was applied that included the recording of habitat ecological conditions in the study area, a detailed analysis of the tree's health and degree of damage, mapping of damage, recording of dendrological parameters and assessment of the tree's condition (Zorzenon and Campos, 2014; Suchocka et al., 2022; Suchocka et al., 2023). Based on the results obtained, measures were defined to remedy the damage caused by insect pests and pathogens to the oak (Bond, 2006; Matheny and Clark, 2009). Basic data on the habitat-ecological conditions of the site were collected using climate data for the town of Obrenovac and laboratory analyses of soil samples. Samples with characteristic disease symptoms were taken to analyse the health status of the tree in order to identify the cause of the disease in time and make a diagnosis. A laboratory analysis of the available data on the prevalence and presence of insect pests and pathogens was carried out.

All symptoms recorded were categorised as follows:

- Changes in the morphological appearance of the entire plant or individual plant organs;
- Changes in the colour of the plant organs (chlorosis, spotting, redness, etc.);
- Changes in the content of the plant tissue (destruction), manifested by the appearance of rot or exudate; and
- Presence of foreign bodies on plants (vegetative organs of fungi, fruiting bodies of fungi, reproductive organs of fungi).

The identification of the saprophytic and parasitic fungi present was carried out using standard phytopathological methods: microscopic analysis of fruiting bodies and reproductive organs or isolation from plant parts in the case of fungi that do not form fruiting bodies in nature. Identification was based on the appearance, growth rate and pattern of the mycelium, the formation of fruiting bodies in culture, sporulation, appearance of hyphae, etc. The identification of the fungi was based on the descriptions in the following publications: Breitenbach and Kranzlin (1986); Hagara (2014); and Stalpers (1978). For each pathogen and pest, the sensitivity of the plant to the pathogen, characteristic symptoms and possible protective measures were listed. To evaluate the condition of the crown, a five-point scale was used, where: 0 - healthy tree with no dry branches in the crown; 1 - tree with single dry branches in the crown; 2 - drying has affected the top of the crown (dieback); 3 - tree with more than 50% dry branches in the crown; and 4 - dead tree. Damage to the boot was determined by visual inspection, taking samples from the surface of the tree and using a drill inside the boot. Based on the analysis of the samples, the degree of damage was determined and a model of the boot decay and a cartographic representation with the positions of the recorded damage were created. The vitality and ornamental value of the protected oak were assessed on the basis of the VTA (Visual Tree Assessment). The vitality and ornamental value were determined using the VTA (Visual Tree Assessment) method (Mattheck and Breloer, 1994; He et al., 2022) and a rating from 1 - poor to 5 - excellent was assigned on the basis of all analyses.

Based on the analysis of the extent of tree damage, the vitality of the trees and the ecological influences (wind, etc.), measures to improve stability were proposed. Based on all the information collected and the analysis carried out, proposals were made for specific measures and conditions to protect and improve the condition of protected tree No. 1.

Results and discussion

The basic climatic characteristics of "Jozića Koliba" are characterised by a temperate continental climate with warm summers and cold winters. Due to its complete openness to the north and north-west, it is often influenced by cold air masses. The valleys of the Drina and Kolubara rivers play an important role in the direction of the air currents. The wind usually blows from the south-east, while it blows less frequently and with less intensity from the north and south. The primary substrate at the Jozića Koliba site is black meadow soil – semigley. This is a hydromorphic soil formed under the influence of groundwater. Here, black meadow soil is formed on alluvial deposits, which makes the soil predominantly clayey and very well supplied with plant assimilates. The humus and total nitrogen content is high. A close C/N ratio indicates that the mineralisation of organic nitrogen and its conversion into mineral and plant-available forms is good. The soil is very well supplied with potassium, which is easily accessible to plants. The once widespread native forest community of English oak and ash (*Querceto-fraxinetum serbicum*) at this site is an indicator of a high groundwater table. The disappearance of this community and the state of its remnants are clear indicators of the retreat of water to greater depths. In addition to climate change as the main cause, the construction of a dam upstream on the Kolubara River (after the major floods in 2014) on the one hand and the raising of an embankment for the Miloš Veliki motorway on the other have further contributed to the lowering of the groundwater level. The analysis of the health status of English oak No. 1 revealed the presence of several pathogens and pests that threaten the survival of the entire tree. Among other things, pathogenic fungi were found: *Ganoderma adspersum* (Schulz.) Donk (Figure 1), *Stereum hirsutum* (Willd.: Fr.) S.F. Gray and *Microsphaera alphitoides* Griff. and Maub. The greatest damage to this tree was caused by *G. adspersum*, which causes white rot in the wood. Insects present include: *Microsphaera alphitoides* Griff. and Maub., *Parthenolecanium rufulum* Chll., *Pulvinaria betulae* (L.), *Asterodiaspis variolosa* (Ratz.), *Coroebus bifasciatus* Ol., *Cerambyx cerdo* L., *Clytus arietis* (L.), *Curculio glandium* L., *Archips xylosteana* L., *Cydia splendana* Hbn., *Cydia amplana* Hbn., *Ancylis mitterbacheriana* Den. Schiff., *Ticheria complanella* Hbn., *Caliroa varipes* (Klug.), *Andricus quercuscalicis* (Burg.), *Andricus collari* (Htg.), *Andricus hungaricus* (Htg.), *Andricus caputmedusae* (Htg.), *Neuroterus quercusbaccarum* (L.) and *Vespa crabro* L..

Figure 1. Ganoderma adspersum (Schulz.) Donk, Microsphaera alphitoides Griff. And Neuroterus quercusbaccarum (L.)

On the basis of the analysis of the extent of damage to the examined tree, it was established that the tree is very old and is gradually dying due to its age, with the process being considerably accelerated by the pathogens and pests present. A visual inspection of the tree revealed damage to the bark and cavities spreading inwards towards the centre of the tree, with a large opening on the south-east side. The most significant damage was found at the root collar and just above the soil surface. Examination of cores taken from the tree at a height of 1.50 metres shows that the hyphae of the rot fungus have not yet penetrated to a depth of 20 cm from the surface in this part of the tree, with the exception of part of the tree on the north-west side, where bark damage was found at a height of 1.60 metres.

Figure 2. Damage to the tree

The observed cavities are a direct consequence of the tree rot caused by *G. adspersum*. These areas in the tree (cavities, damaged bark, etc.) are ideal places for various animals and birds to settle and further damage the already diseased tree (Figure 2).

The measurements taken within the squares placed in the field enabled the creation of a damage model inside the tree that predicts the necessary remedial measures (Figure 3). The squares were positioned at the base of the tree, with a side length of 230 cm, orientated in the following directions: a – north-east, b – north-west, c – south-west, d – south-east. Using this model, it was found that the degree of damage at the base of the tree reaches up to 90%, while the central part of the tree is completely affected by the rotting process. The percentage of rotten wood decreases as the height of the tree increases, so that already at a height of 1.80 m this type of damage is no longer significant.

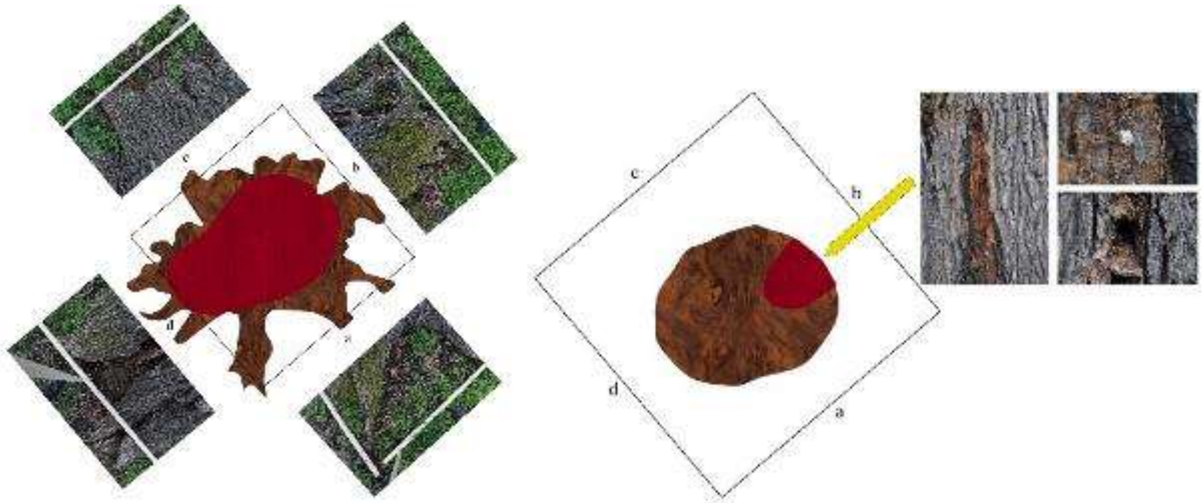

Figure 3. Schematic representation of the damage (rot) in cross-section at the root collar (left) and at a height of 1.50 metres (right). Arrows indicate the areas where the rot has affected the tree directly under the bark. Red color represents the area of rot in cross-section of trunk.

Based on the analysis of the core samples and the shape of the tree, the decay model inside the tree resembles a truncated cone with a base area of 2.07 m², a surface area of 0.12 m² and a height of 1.80 metres.

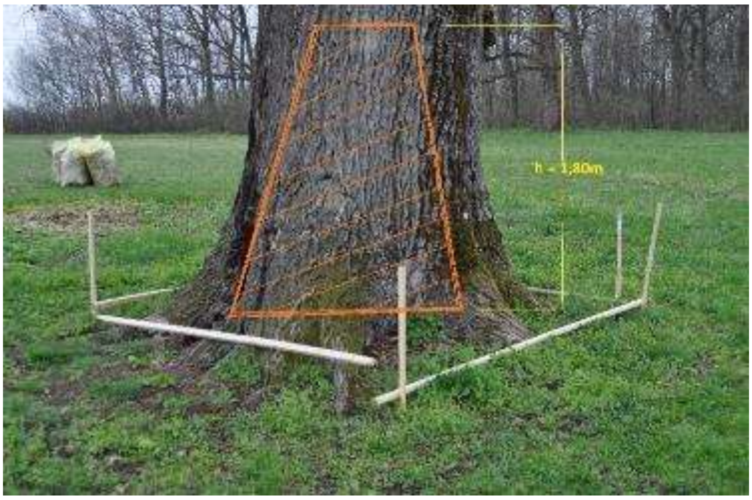

Figure 4. Schematic representation of damage in the shape of a truncated cone

According to the formula $M=\pi(R+r)*\sqrt{H^2+(R-r)^2}$ the surface area of the truncated cone is 5.73 m², increased by the upper surface area (0.12 m²), i.e. a total of around 6 m². According to the formula $V=\pi H/3*(R^2+rR+r^2)$, the volume of the truncated cone is approximately 1.5 m³.

he tree is damaged up to a height of 1.8 m, which corresponds to about 52%. According to the investigations carried out, the condition of the tree above this height is satisfactory. In this area, movements of ants were observed, which have settled in the decayed part of the tree and do not contribute to further destruction, but are a consequence of the condition of the tree. The decayed part of the tree has lost both its physiological and mechanical properties, thus jeopardising the stability of the entire tree. Apart from the sporadic occurrence of thin, dry branches and occasional broken branches in the tree crown, no major mechanical damage was observed. The health of the tree crown is satisfactory. The tree crown is asymmetrical, which also affects its stability. Most of the branches are orientated to the north-west, west and south-west.

1 2 3

Figure 5. Schematic representation of the reduction of the asymmetric canopy of oak tree No. 1 (1) and the planned corrections of the canopy on the northeast side (2) and northwest side (3).

Fumigation was carried out to eliminate the insects present in the boot. The tree was treated with a fumigant and wrapped in plastic for 24 hours. After removing the plastic, the area was observed for several days to see if the pests would reappear. The rot in the heartwood of the tree was then tackled in the following stages: Removal of the rotted and insect-damaged wood, formation of the cavity, disinfection of the cavity walls and coating of the cavity walls with waterproof materials (grafting wax). Aesthetic corrections were then made to the boot, including closing the cavity. The gap remaining after removal of the rotten wood was filled with expanding foam. In order to preserve the aesthetic features of the tree and blend it harmoniously into its surroundings, the outer surface of the opening was treated to make it look as natural as possible. It was painted with environmentally friendly paint that matches the colour of the tree bark. In addition to the crown reduction, support pillars were installed under certain branches to withstand lateral wind loads and prevent large branches in the tree crown from breaking off.

Figure 6. Repaired tree damage caused by *Ganoderma adspersum* (Schulz.) Donk

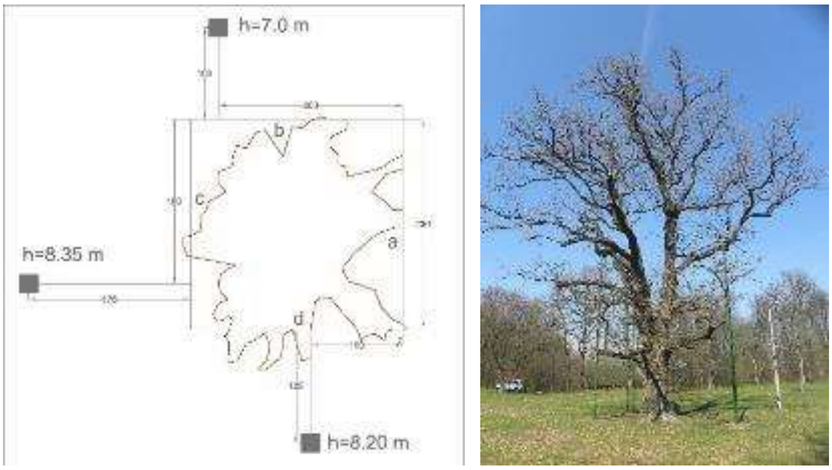

Figure 7. The position and spacing of the supports in relation to the base of English oak no. 1

Conclusion

The damage and decay of the protected tree no. 1 required urgent measures to preserve the tree. This ensured that the authenticity and attractiveness of this site was preserved. The proposed measures are in line with the protection conditions of this area and take into account the conservation and preservation of the biological integrity of the tree through the application of all available biological and technical care and protection measures. Considering the current satisfactory ecological factors, the measures taken to restore the condition of the protected oak will help to slow down the process of deterioration and extend its lifespan. This will allow the tree to exist safely at the "Jozića koliba" excursion site without jeopardising the safety of space users in the immediate vicinity of the tree.

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EVALUATING CORROSION AND INCRUSTATION RISKS IN SELECTED WELLS IN DANUBE ALLUVIUM

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Abstract

The determination of corrosion and incrustation risks in drainage wells is crucial for the maintenance of water extraction infrastructure, ensuring the required efficiency in groundwater levels maintaining, and safeguarding the functionality and longevity of water extraction systems. Analytical methods have long been established for assessing water aggressiveness, providing insights into the groundwater environment susceptibility to corrosion or incrustation of underground structures. The assessment of groundwater aggressiveness is facilitated by the Langelier (LSI) and Ryznar indices (RSI), while Johnson's Classification aids in understanding incrustation phenomena. Based on conducted groundwater sampling campaign in 2021, for four drainage wells in Danube alluvium, the risks of chemical corrosion and incrustation were calculated. Revealed results indicated that groundwater was characterised with oxygen level of 2.5 mg/l – 3.75 mg/l and redox values in range of 362 mV to 583 mV, with very variable iron content. Based on the negative LSI values, examined groundwater from B-1, B-2, and B-4 is undersaturated and tends to dissolve calcium carbonate. The calculated indices for B-3 suggest mild incrustation tendencies, indicating a propensity for carbonate precipitation. Groundwater in zone B-4 stood out as highly corrosive. Saturation indices indicated groundwater tendencies towards either calcium carbonate dissolution or precipitation. The choice of construction materials significantly influences corrosion susceptibility, necessitating pre-emptive measures. Understanding groundwater behavior regarding scaling and corrosion is pivotal for effective prevention and maintenance planning. By implementing tailored strategies, such as pH adjustment and corrosion-resistant coatings, the detrimental effects of scaling and corrosion can be mitigated, ensuring the longevity of drainage well systems and minimizing operational costs. Regular monitoring of groundwater chemistry enables timely interventions, fostering sustainable water management practices.

Keywords: *groundwater, corrosion, incrustation, Langelier, Ryznar.*

Introduction

The most common processes that contribute to the inevitable reduction in yield, known as "well aging," after long-term exploitation, are corrosion and incrustation-chemical scaling. The inclination of water towards either can significantly impact the transmission and distribution of water, influencing both economic considerations and public health concerns (Derakhshannia et al., 2020; Lodha et al., 2023; Mokhtari et al., 2010). Corrosion represents a set of chemicals, electrochemical, and biochemical processes that lead to damage and physical degradation of well construction. The basic precondition for the occurrence of corrosion of well construction metal parts is high mineralization, as well as the activity of microorganisms in water, especially sulfate-reducing bacteria. Favorable factors for the formation and development of corrosion include increased acidity of groundwater, increased content of dissolved oxygen (>2 mg/l), sulfides (>1 mg/l), and humic acids (>50 mg/l), presence of organic acids, low water hardness, and increased mineralization (>1000 mg/l). Incrustation or chemical scaling is the process of depositing insoluble materials in the pre-filter zone of the well or in the well's filtration construction itself; it distinguishes mechanical, chemical, biochemical, and biological scaling. Chemical scaling is the process of forming precipitates through chemical reactions, usually in the form of scale on the filtration construction and in the pre-filter zone of the well. Favorable factors for the formation and development of incrustation include increased alkalinity of groundwater, increased content of bicarbonates (>300 mg/l), iron (>2 mg/l), and manganese (>1 mg/l), and oxygen content below the aerobic boundary of the environment (<0.5 mg/l). Pumping of groundwater leads to disturbance of the natural chemical equilibrium of groundwater as a result of hydrodynamic disturbances in the aquifer, contact of groundwater with atmospheric oxygen, loss of dissolved gases, and deposition of corresponding substances. Filtration and pre-filter zone scaling are conditioned by disturbances in the carbonate equilibrium of water and redox processes in the narrow zone of the well. In order to predict examined groundwater aggressiveness, the Langelier and Ryznar number were calculated, and Johnson's Classification were estimated.

Materials and methods

The settlement of Vinci in Serbia is located on the right bank of the Danube River. Situated in the coastal area, Vinci occupies terrain elevations ranging from 70 to 75 meters above sea level (mnm), with the majority of the settlement positioned between 71 and 73 mnm. The lower-lying section of the settlement, below 72 mnm, lies adjacent to the Danube River, extending westward towards the "Vinci" water source (supplying the Golubac municipality), and partly towards the north and northwest, reaching the lower terrains of the Vinci-Požarevac drainage system.

In 1994, a drainage system consisting of a total of 4 operational wells (B-1 to B-4) with their own pumps and outlets into the Danube River was constructed to protect the lowest parts of the terrain in the developed area of the Vinci settlement (Figure 1). Following their construction, the wells were equipped with hydraulic and electrical equipment and put into operation. The primary role of this drainage system is to safeguard the lower coastal area of the Vinci settlement from the increased Danube levels. For these wells the Langelier and Ryznar indices were calculated as well as Johnson's Classification was determined.

Figure 1 The locations of the monitored wells

The Langelier index and Ryznar number
The Ryznar Stability Index (RSI) is a parameter used to assess the scaling potential and corrosiveness of water (Ryznar, 1944). It helps determine whether water tends to form scale (such as calcium carbonate deposits) or if it is corrosive. The Ryznar index is calculated using water analysis data, including parameters like pH, conductivity, total dissolved solids (TDS), calcium concentration, bicarbonate concentration, and water temperature. Calculated pHS value, corresponds to the equilibrium state in the solution of carbonate compounds. The pHS value represents the hydrogen ion index corresponding to the equilibrium saturation of groundwater with carbonic acid compounds. The pH value reflects the real concentration of hydrogen ions in groundwater when measured directly on-site. Conversely, pH values obtained in laboratory settings typically register higher than those measured directly on-site. In light of analyzed literature data (Shankar, 2014), a practical formula has been proposed to calculate pHS values as follows (Eq.1):

$$pH_S = 9,92 - \frac{t\left[{}^{0}C\right]}{40} - \log\left[Ca^{++}(mg/l)\right] - \log\left[HCO_3^{-}(mg/ekv/l)\right] + 0,2\log\left[S.O.(mg/l)\right]$$

(1)
t - temperature of groundwater (°C),
S.O. - dry residue (mg/l)

In terms of the stability of carbonate systems in water, the Ryznar criterion or number is introduced, expressed as:
RSI=2pHS−pH
RSI has only positive values, and the higher the values, the more corrosive the water is (Table 1).

Table 1. Ryznar index and water tendency towards incrustation or corrosion

RSI value	Indication of water aggressiveness
4-5	Highly incrustive
5-6	Mildly incrustive
6-7	Mildly incrustive or corrosive
7-7.5	Corrosive
7.5-9	Highly corrosive
>9	Extremely corrosive

The Ryznar index complements the Langelier Saturation Index (LSI), which also assesses water scaling potential. While Ryznar gives an indication of water aggressiveness, the LSI considers both scale and corrosion potential (Eq. 2).
LSI=pH−pHS
(2)
If the measured pH value is less than the calculated pHs value (LSI < 0), the concentration of dissolved CO_2 in groundwater is above equilibrium, and groundwater can dissolve carbonate compounds. If, however, the pH value is greater than the calculated pHs value (LSI > 0), the concentration of dissolved CO_2 in groundwater is below equilibrium, and calcium carbonate can precipitate from groundwater.
Values of LSI can be interpreted as follows:
- Positive value: water tends to precipitate calcium carbonate.
- Zero: water is in equilibrium.
- Negative value: water is undersaturated, tends to dissolve calcium carbonate.

Johnson's Classification is relevant to incrustation processes. This classification helps in understanding the propensity of groundwater to form incrustations, which can be valuable for various applications, including water supply management and infrastructure maintenance. According to Johnson's Classification the process of incrustation occurs in groundwater with the following characteristics: the water is alkaline, i.e., pH is > 7.5, carbonate content in water exceeds 300 mg/L, indicating the potential for calcium carbonate precipitation. The iron content in water is greater than 2 mg/L. The process of iron precipitation from water is intensified by biological processes - the action of iron bacteria. The manganese content in water is over 1 mg/L.

Results and Discussion

Considering the results of water quality testing, pH values ranged from a minimum of 7.16 in zone B-1 to a maximum of 7.62 in zone B-4, ranging from neutral to slightly alkaline values (Table 2). Carbonates were not detected, except in zone B-4, manganese content was >1 mg/L, and iron content varied widely, from 0.3 mg/l to even 5.42 mg/l (in B-2 and B-4). Along with high manganese values in groundwater in all four examined zones, microbial-mediated precipitation of iron and manganese and the formation of iron and manganese incrustations (iron and manganese oxide-hydroxides) are likely. Based on the negative values of LSI calculated according to the above relation, groundwater in the zones of hydro-technical facilities B-1, B-2, B-3, and B-4 is undersaturated and tends to dissolve calcium carbonate. The LSI value in zone B-3, according to the parameters calculated from measurements conducted in 2021, tends towards carbonate precipitation (Table 3). Based on the Ryznar number, groundwater in zones B-1 and B-2 fell within the range of corrosive waters, while zone B-3 exhibited characteristics of both weakly incrustive and corrosive waters. Groundwater in zone B-4 stood out as highly corrosive.

Table 2. Selected groundwater quality parameters

Sampling site		**B-1**	**B-2**	**B-3**	**B-4**
Parameter	**Unit**				
pH вредност		7.16	7.23	7.62	7.53
Ec	µS/cm	499	488	583	362
Eh	mV	371.5	394.2	363	389.9
DO	mgO_2/l	2.5	3.59	3.75	2.96
Iron (II)	mg/l	0.72	0.4	0.21	0.26
Iron	mg/l	2.77	5.42	0.72	0.3
NH_4	mgN/l	1.36	0.72	1.02	0.14
NO_2	mgN/l	0.006	0.054	0.038	0.028
NO_3	mgN/l	0.15	0.81	0.62	2.83
Cl	mg/l	17.6	17.44	17.49	11.07
SO_4	mg/l	3.33	15.5	18.35	25.47
H_2S	mg/l	<0.04	<0.04	<0.04	<0.04
TOC	mg/l	2.48	1.98	3.8	3.89
OP	mgP/l	0.218	0.34	0.08	0.262
TDS	mg/l	322	302	360	243
Total hardness	mg $CaCO_3/l$	222.4	233.4	275.4	151.3

Table 3. Saturation indices in groundwater

Well	**Redox (mV)**	**LSI**	**Ryznar**
B-1	371.5	-0.1	7.4
B-2	394.2	-0.08	7.4
B-3	363	0.42	6.8
B-4	389.9	-0.10	7.7

Several factors contribute to the corrosion of well materials. High mineralization levels in groundwater, along with the presence of dissolved gases and organic matter, can accelerate corrosion processes. Additionally, the pH level of the groundwater plays a crucial role, as acidic conditions can promote the dissolution of metallic components. Common materials used in well construction, such as steel, cast iron, and galvanized steel, are prone to corrosion if not adequately protected. Corrosion can lead to structural deterioration, leakage, and ultimately, the failure of the well system. Therefore, it is essential to select corrosion-resistant materials or apply protective coatings to extend the lifespan of wells and ensure the reliability of groundwater supply systems. Furthermore, proper design and construction practices, along with regular inspection and maintenance, are essential for mitigating corrosion risks in well infrastructure. By understanding the factors influencing corrosion and employing appropriate preventive measures, the integrity and functionality of well systems can be preserved, ensuring the sustainable management of groundwater resources.

Conclusion

Understanding the groundwater potential for chemical scaling and corrosion is important from several aspects: managing prevention of scaling, corrosion control, and maintenance planning. Groundwater with high mineral content, particularly calcium carbonate, can lead to scaling in drainage well systems. This scaling can clog pipes, reduce flow rates, and interfere with the operation of pumps and other equipment. By knowing the groundwater potential, appropriate measures can be taken to mitigate scaling, such as adjusting pH levels or installing filtration systems. By assessing the potential for corrosion based on groundwater characteristics such as pH, dissolved oxygen content, and mineral content, protective measures like corrosion-resistant coatings or cathodic protection systems can be implemented to prolong the lifespan of drainage well infrastructure. Understanding the potential for chemical precipitation and corrosion allows for proactive maintenance planning. Regular monitoring of groundwater chemistry parameters can help identify changes over time, allowing for timely interventions to prevent or mitigate scaling and corrosion issues. This proactive approach can minimize downtime, reduce repair costs, and ensure the efficient operation of drainage well systems.

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SHEEP WOOL BRIQUETTES

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Abstract

The problem in sheep farming is that a large amount of unused sheep wool is produced for which there are no suitable disposal sites. Sheep wool can be used as an organic fertilizer. It contains nitrogen, sulfur, potassium and other elements. It also has a great ability to absorb and retain water. By pressing sheep wool into briquettes, we reduce the volume of sheep wool and simplify its storage, transportation and handling. In the study, the sheep wool was pressed into briquettes using the Profilis MAXX briquetting device due to the simplicity of the process. We found that for briquetting it is necessary to grind the wool beforehand. If we did not grind the wool beforehand, we could not press the briquettes. The briquettes were made from ground wool of two different fractions and two different temperatures (80 ^{0} C and 140 ^{0} C). The briquetting process is easier if the fraction of incoming wool is smaller. Some farms also produce small amounts of wood residues as waste, which can also be used as organic fertilizer. The mixture of sheep wool and wood residues complements each other well, as wool has a much higher nitrogen content and wood takes longer to decompose. We have pressed briquettes from a mixture of sheep wool and wood residues where the mass ratio of the mixture being 1:1. The results of the quality analysis of the produced briquettes show that both the pressing temperature and the fraction of ground wool influence the quality of the briquettes.

Keywords: organic fertilization, sheep wool, briquettes, wood residues, bulk density.

Introduction

Since the middle of the last century, more and more sheep farmers have been confronted with the problem of the (non-)use of sheep wool, as the demand for sheep wool has declined significantly with the increasing interest in synthetic fibers. Once profitable animal by-products that were widely used in the textile industry have become waste that often ends up uncontrolled in the environment and pollutes. According to European Commission Regulations 1069/2009 and 142/2011, unprocessed shorn wool is defined as an animal by-product (category 3) and must be disposed of as special waste. These costs, together with the costs of shearing the sheep, which is usually done twice a year, affect the profitability of the activity (Bhavsar et al., 2021; Del Prà et al., 2024; Rabaniejad et al., 2019; Trop 2013). The problem is particularly acute for low-quality wool and where there is no concentrated sheep farming or where carp farming is scattered and practiced as a secondary activity or to avoid overgrowth of the landscape.
On the other hand, the EU Regulation (2019/1009) on organic fertilizers and the EU Decision (2022/591) on a general environmental action program up to 2030 offer the opportunity to improve the use of sheep wool as an organic fertilizer in outdoor and greenhouse cultivation. The use of wool waste as a fertilizer is therefore in line with the EU Green Deal targets to reduce the use of chemical pesticides by 50%, and the use of fertilizers by 20% and to reduce

nutrient losses by at least 50%. Sheep wool contains on average 44% C and 10% - 11% N and can be considered both a soil amendment and a source of nutrients due to its C and N content (Del Prà *et al.*, 2024).
Europe is the second largest producer of sheep after Australia, with 58 million sheep in 2023 (Eurostat, 2024). The challenge of beneficial use of sheep wool isbeing addressed in many environments, and Rajabinejad (2019) providesa detailed overview of the potential uses.
Slovenia is a country with a relatively small but constant number of sheep. In December 2023, there were about 116,000 of them (SiStat, 2024), which, assuming that one sheep produces an average of 1.4 kg of wool per year (Trop, 2013), corresponds to 162 tons of wool. Sheep are primarily bred for meat and milk production, with wool being a by-product of very low economic value. Shearing the sheep and removing the wool is now considered an unnecessary expense for the breeder, as the selling price of the unwashed wool does not cover the cost of shearing. Currently, the purchase price in Slovenia varies between 0.5 and 1 EUR/kg, depending on quality and sorting, and is mostly purchased on the principle of compensation. According to the latest available data (Kancler *et al.*, 2013), only 25% of wool is purchased, 15% is composted, and the rest is thrown away or mostly ends up as waste in nature (Trop, 2013). The main reasons for the current situation in Slovenia are the fragmentation of sheep farming and thus the disorganization of wool collection, sorting and purchase, the low purchase price, the legal framework that does not allow municipal companies to purchase wool, as well as the declining interest of the textile industry and the resulting non-operation of wool laundries. In addition, it should be emphasized that the wool of indigenous Slovenian sheep species, among which the Jezersko Solčava sheep dominates, is less suitable for the textile industry, as the fibers are larger in diameter and more robust, which makes it more difficult to compete on world markets.
Agricultural farms often also own forests and, and have primary wood processing operations. The residues resulting from this processing can be used as bedding for livestock or as fuel. In cases where the residues are larger and have a higher moisture content, they are less suitable for burning and are unfortunately still considered waste. Wood as a lignocellulosic material consists of carbon (41% - 51%), hydrogen (6.2% - 6.3%), oxygen (24% - 44%) and a small amount of nitrogen, sulfur, chlorine and fluorine (Obernberger and Thek, 2010). Due to their high carbon content, wood residues can also be used as a natural fertilizer, while cellulose and lignin accelerate the formation of humus and organic matter in the soil (Del Prà *et al.*, 2024). It can also be used as mulch and for soil loosening.
Briquetting is a relatively simple material compression process in which the requirements for the input raw material are somewhat lower than for pelleting. Compaction usually takes place at an elevated temperature, which depends on the type of compressed material. The briquetting systems themselves are also simpler, cheaper and suitable for different production quantities compared to pelleting systems. Briquetting increases the density of the material, which reduces the required storage and transportation space and facilitates handling (Anžič, 2021; Dinesha, 2019).
As part of the research, the possibilities of producing briquettes from sheep wool processed in three different ways, were investigated. The aim is to produce briquettes from pure sheep wool and briquettes from wool and hardwood residues (shavings, sawdust) in a mass ratio of 1:1 and to determine the selected properties of the briquettes produced.

Material and Methods

The wool obtained from the Jezersko-Solčava sheep and shorn in spring was stored in a covered air space for about three months. Three fraction sizes were used for the production of

briquettes: Unprocessed wool (WU), grinded wool without mesh in the mill (WR) and grinded wool with a mesh size of 9 mm grid (WF) (Figure 1 a, b and c).
The grinding was carried out with a self-built mill usied for grinding bio and artificial materials. The nominal power of the mill's drive motor was 7.5 kW at a rotation speed of 1440 rpm. The blades of the mill were sharpened before grinding. The dosing of the sheep wool was done manually.
The wood residues (W), which consisted mainly of beech and maple, were obtained from a wood processing plant. The wood residues were a mixture of wood shavings and sawdust (Figure 1 d).

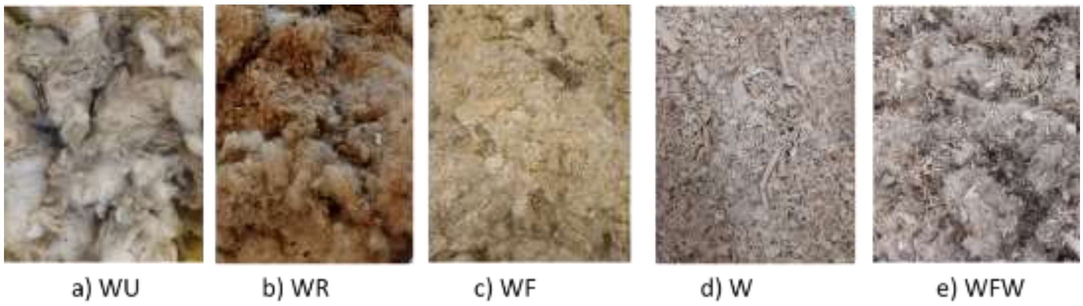

Figure 1. Input material for briquette production.

The determined moisture content (SIST EN ISO 18134-2:2017) and bulk density (SIST EN ISO 17828:2016) of all input materials (WU, WR, WF and W) are shown in Figure 2. The particle size distribution of the wood residues (Figure 1 d) was as follows (14.9% > 5.6 mm; 20.4% > 3.15 mm; 6.6% > 2.8 mm; 34% > 1%; 23.3% < 1 mm).

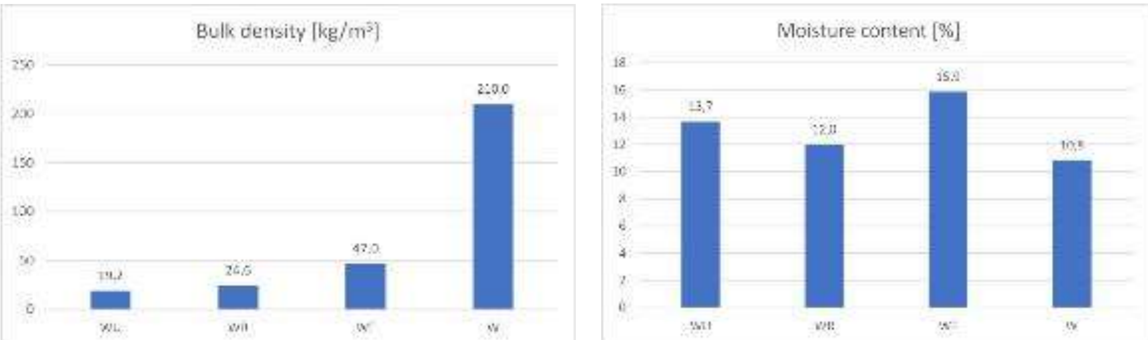

Figure 2. Bulk density (a) and moisture content (b) of input material (WU, WR, WF and W).

Unprocessed wool (WU), rough wool (WR) and fine wool (WF) as well as mixtures of these wool types and wood residues (WRW and WFW) were briquetted in a mass ratio of 1:1 (Table 1). The mixtures WRW and WFW (Figure 1 e) were produced immediately before briquetting.
Briquetting (Figure 3) was carried out on a Profilis MAXX (at the Biotechnical faculty, UL in June 2024) with a connected eletricity power of 4.5 kW and a capacity of 10 to 20 kg of briquettes per hour. The working pressure of the briquetting machine is 170 bar with a pressing force of 80,000 N. The briquettes have a nominal diameter of 54 mm. The briquetting machine also allows the pressin temperature to be regulated. In the research, briquettes were produced at temperatures of 80 ºC and 140 ºC. After 24 hours of conditioning at 24 ± 2 ^{0} C and a relative humidity of 65%, the density, moisture content and absorption rate of the briquettes were determined.

To determine the absorption rate and retantion, the briquette was weighed and then dipped for 5 s, 10 s, 15 s, 20 s, 30 s and 60 s in cold water. After each immersion, the briquette was drained for 30 s. The mass of absorbed water was recorded. The results given are the average of three repetitions. To determine the water retention capacity, the samples were weighed again after 5 days of storage in a normal air climate and the moisture content was calculated.

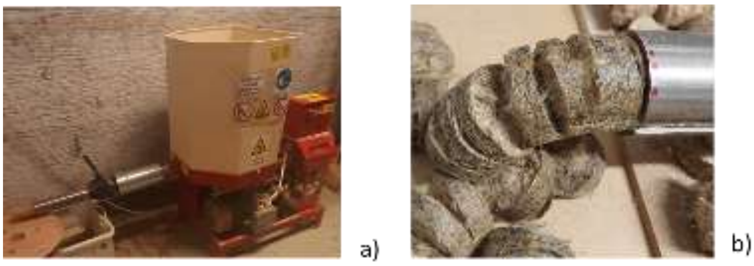

Figure 3. Profilis MAXX briquetting machine (a) and briquetting process (b).

Results and Discussion

In the study, we wanted to produce briquettes from unwashed (raw) sheep wool. Briquetting is a simpler process than pelleting, which has already proven to be successful (Del Prà et al., 2024). To our knowledge, no information is available on the production of sheep wool briquettes. The sheep wool were prepared in three ways (the analysis of the input materials is shown in Figure 2), but the production of briquettes from unprocessed wool (WU) was not successful (Table 1), so in the following we only give the results for the briquettes that we successfully produced (Figure 4).

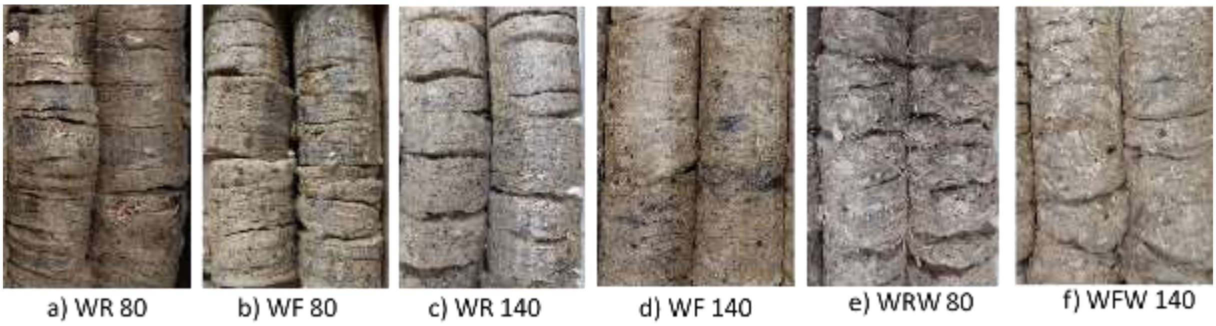

Figure 4. Briquettes from sheep wool.

Table 1. Composition and labeling of briquettes.

Label	Composition	Ratio	Compression temp. [^{0} C]	The success of briquetting
WU80	Unprocessed wool	100	80	No
WR80	Rough wool	100	80	Yes
WF80	Fine wool	100	80	Yes
WU140	Unprocessed wool	100	140	No
WR140	Rough wool	100	140	Yes
WF140	Fine wool	100	140	Yes
WUW140	Unprocessed wool : wood residues	50:50	140	No
WRW140	Rough wool: wood residues	50:50	140	Yes
WFW140	Fine wool : wood residues	50:50	140	Yes

Briquetting increases the density of the material considerably, as the briquettes produced have a density of between 349 kg/m^3 and 676.6 kg/m^3 (Figure 5 a), while the wool intended for briquetting had a bulk density of between 18.2 kg/m^3 and 47.0 kg/m^3. The moisture content of the briquettes varied between 11.5 % and 13.9 % (Figure 5 b).

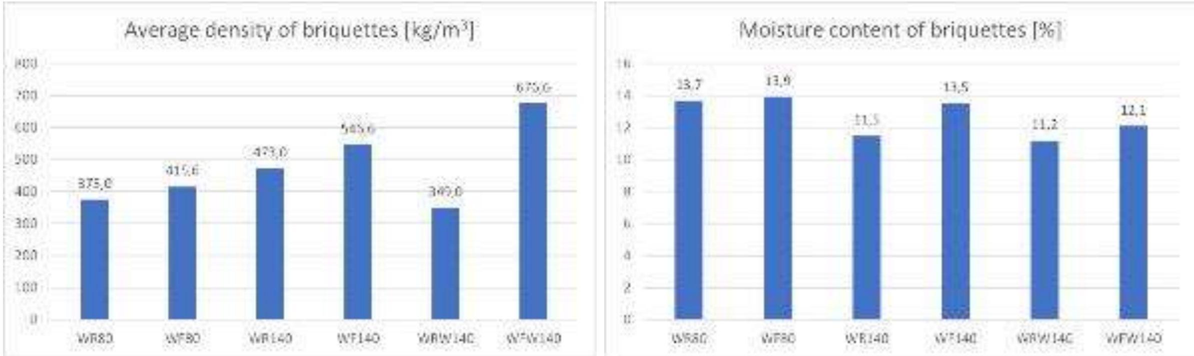

Figure 5. Average density (a) and moisture content (b) of the produced briquettes.

One of the important properties of sheep wool is its good water absorption, which was determined in the manner described in the Methods chapter. As can be seen from Figure 6 a, water absorption was extremely rapid within 10 s, and the moisture content changed little with prolonged immersion. The calculated absorption rate in first 5 s (Figure 5b) is the fastest for the WFW140 briquettes. It should be emphasized that with the test performed we only wanted to obtain basic information about the speed of absorption and did not take into account all the parameters influencing the measurements (e.g. density and dimensions of the briquettes,...). We also confirmed that the wool retains moisture perfectly by weighing the samples after five days of storage in a normal air climate. The moisture content of the briquettes ranged from 58.3% for WRW140 briquettes to 75% for WF140.

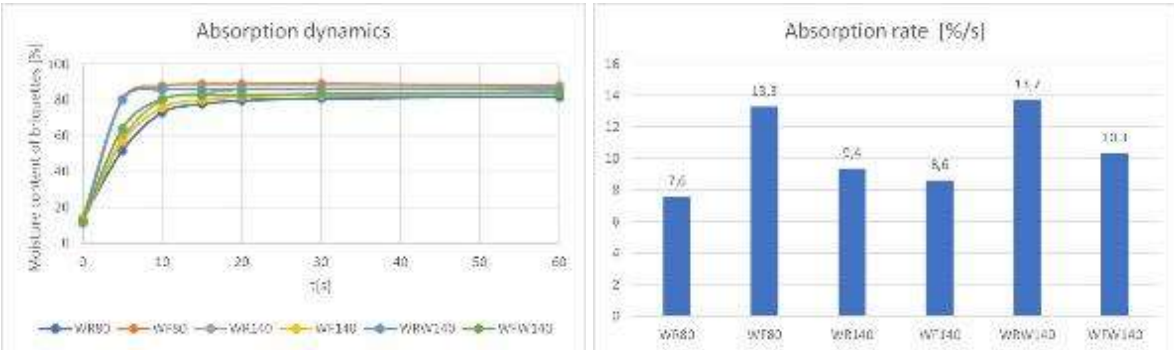

Figure 6. Absorption dynamics (a) and absorption rate of briquettes in the first 5 s (b).

Conclusions

Sheep wool, a byproduct of sheep farming is nowadays considered as waste (category 3), the collection and disposal of which is associated with costs for the breeder, which causes often uncontrolled disposal in nature. Such disposal burdens the environment and poses a risk for the spread of pathogenic organisms. Sheep wool has a potential to be use as a fertilizer, and can also reduces the need for artificial fertilizers. The production of pellets from sheep wool is already known, while there is a lack of information on the production of briquettes. Based on the research, we can conclude:

- for successful briquetting, sheep wool must be properly processed (ground),
- the briquetting of ground wool is fast and unproblematic,

• briquettes can be produced without large investment and processing costs with a positive impact on the profitability of sheep farming
• the briquettes have a higher density as uncompresed wool, which greatly facilitates the storage, transport and handling,
• by briquetting a mixture of wool and wood residues, two biomaterials which often appear as waste on farms, can be used effective and
• briquettes have a high water absorption potential, which is the greatest in the first 10 seconds.
Since briquettes are produced at an elevated temperature (140 ^{0} C), the investigatin whether the briquetting process already meets the requirements for sterilization of the product should be done. In addition, it would be useful to investigate the influence of the addition of lignocellulosic materials on the degradation rate of briquettes and their nutritional value.

Acknowledgments

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SPATIOTEMPORAL VARIATION OF SNOWFALL AND SNOW COVER IN URBAN CENTERS OF THE WESTERN BLACK SEA REGION, TURKEY

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Abstract

In this study, spatial and temporal changes in snowfall and snow cover in the urban centers of the Western Black Sea Region in Turkey were analyzed. Data from a total of six city centers obtained from the General Directorate of Meteorology were used. Four decades were formed between 1980 and 2019 and temporal change was evaluated. The results obtained show that the number of both snowy and snow-covered days tends to decrease temporally in the urban centers. The average number of snowy days in the 1980-1989 period (P1) was 333.8. In the periods of 1990-1999 (P2), 2000-2009 (P3) and 2010-2019 (P4), these values were determined as 310.7, 294.3, and 196.8, respectively. Similarly, there was a decreasing trend in the number of days covered with snow in the urban centers between 1980 and 2019. The average number of days covered with snow in the P1, P2, P3 and P4 periods are 240.3, 229.0, 229.0, and 191.0, respectively. During this period, the averages of six different city centers were compared and spatial change was determined. While the number of snowy and snow-covered days was highest in Kastamonu in the P1 period, both parameters were at the highest level in Bolu in the P4 period. When the P1 and P4 periods are compared, the highest decrease in the number of snowy and snow-covered days occurred in Kastamonu, at the rates of 59.2% and 45.0%, respectively.

Keywords: *Climate change, Environmental risk, Global warming, Snow characteristics.*

Introduction

The duration and amount of snow cover has important consequences for many regions. Because snow cover and snow characteristics affect many fields such as hydrology, ecology, climatology and tourism. For example, snowpack is a very important component for the hydrological cycle and soil moisture, as it provides water storage by supplying glaciers, streams and groundwater (Özyuvacı, 2001; Brown, 2019; Bai *et al.*, 2019). It is very important from a hydrological perspective that up to one-third of the world's land surface is covered by seasonal snow cover at any given time (Vaughan *et al.*, 2013). Since snowpack is sensitive to global warming, it also has major effects on global and regional climate change (Klein *et al.*, 2016; Brown, 2019; Xuejin *et al.*, 2019) and is one of the most important parameters for climate change analyzes (Terzago *et al.*, 2010). Studies on snow characteristics reveal that snow depth, number of snow-covered days and snowy days generally tend to decrease (Terzago *et al.*, 2010; Luomaranta *et al.*, 2019). In mountainous areas on a global scale, the number of snow-covered days and area are decreasing (Notarnicola, 2022). The most important reason for the decrease in snowpack depth and snow-covered area is shown to be temperature increase and global warming (Banerjee *et al.*, 2021; Notarnicola, 2022). Considering that global warming has increased by 0.7 °C in the last 50 years (Stocker *et al.*, 2013), it is understandable that the number of snow-covered days decreases, and the snowy season starts later and ends earlier (Klein *et al.*, 2016).
Like the whole world, Turkey is under the effect of global warming. As a matter of fact, studies show that the temperature is increasing both throughout Turkey (Ciftci and Sahin,

2023) and on a regional and local scale (Mersin *et al*., 2022; Bolat and Şensoy 2023a; Bolat *et al*., 2023). Moreover, analyzes and studies conducted with different methods in the Western Black Sea Region reveal that there is an increase in temperature (Bolat *et al*., 2018; Şensoy and Ateşoğlu, 2018; Bolat and Şensoy, 2023a; Bolat and Şensoy, 2023b). It is estimated that this temperature increase on a regional scale may have many effects. Spatial and temporal change of snow cover in the Western Black Sea Region is one of these reflections. Based on this evaluation, this study aimed to reveal the change of some snowpack components over the years (1980-2019), such as the number of days with snow cover and days with snowfall, which are closely related to the increase in temperature.

Study Area

The study covers the cities of Bartın, Bolu, Düzce, Kastamonu, Sinop, and Zonguldak located in the Western Black Sea Region, Turkey (Figure 1). The latitude, longitude and altitude of the meteorological stations located in the centers of these cities are given in Table 1.

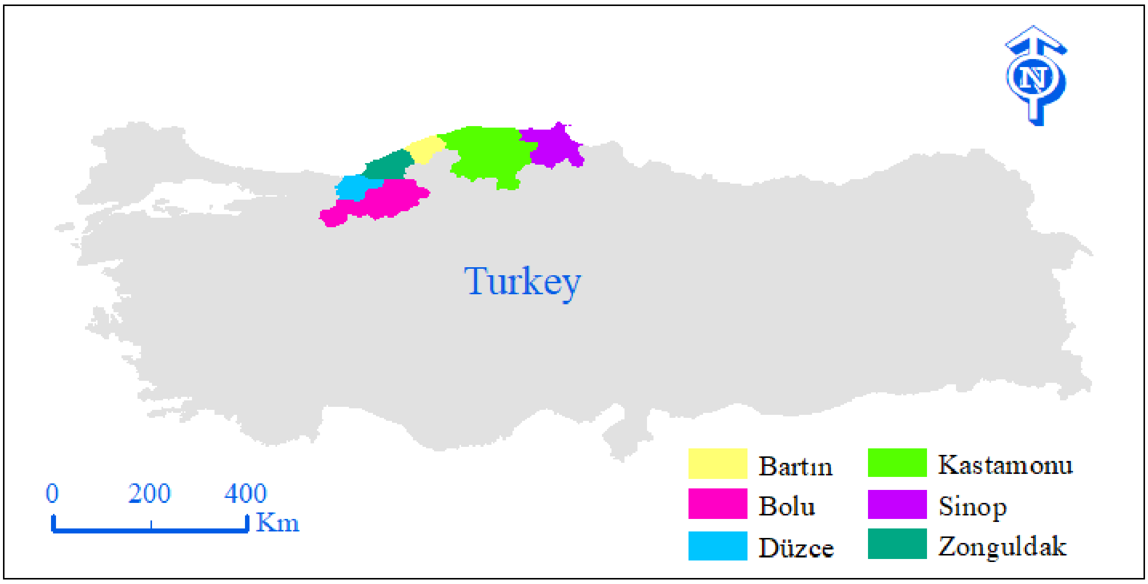

Figure 1. Locations of cities in Turkey.

Meteorological Data

In this study, daily snow-covered and snowy day data between 1980 and 2019 obtained from the General Directorate of Meteorology (TSMS, 2020) of the cities (Table 1) in the Western Black Sea Region were used and the variation of these data was evaluated. The data recorded by the meteorology directorate located in city centers was checked and it was determined that there was no missing data in the study period of 1980-2019. In order to make periodic comparisons, it is recommended to consider series containing at least ten years of data (Lemus-Canovas *et al*., 2019). In this study, four ten-year periods were created for analysis and the differences between them were evaluated. Days when the snow depth was 1 cm, or more were considered snow-covered days (Baltaci et al. 2020). Snow that fell but did not reach 1 cm on the ground was considered a snowy day. Daily data are grouped as decade and the spatiotemporal changes in the number of snow-covered and snowy days in the Western Black Sea Region are revealed. The results obtained were evaluated both numerically and proportionally.

Table 1. Information about the meteorological stations included in the study (URL-1, 2024)

Station Name	Station No	Altitude (m)	Latitude (North)	Longitude (East)
Bartın	17020	33	41°37'29.4"	32°21'24.9"
Bolu	17070	725	40°43'58.4"	31°36'07.9"
Düzce	17072	146	40°50'37.2"	31°08'55.5"
Kastamonu	17074	904	41°22'15.5"	33°46'32.2"
Sinop	17026	28	42°01'47.7"	35°09'16.1"
Zonguldak	17022	135	41°26'57.3"	31°46'40.5"

Results and Discussion

In the Western Black Sea Region, the average total number of snowy days in the city centers subject to the study in the period between 1980 and 2019 was determined as 1135.6. In the Western Black Sea Region, the ratio of the average number of snowy days to the total number of days during the study period was 7.8%. While the average number of days with snowfall in the first period (P1) between 1980-1989 was 333.8, this value was determined as 310.7 days in the second period (P2) between 1990-1999. In the third decadal period (P3) between 2000-2009 and the fourth decadal period (P4) between 2010-2019, the number of snowy days showed an average change of 294.3 and 196.8, respectively (Figure 2). On the other hand, the ratio of snowy days to the whole year in the P1, P2, P3 and P4 periods was determined as 9.1%, 8.5%, 8.1% and 5.4%, respectively.

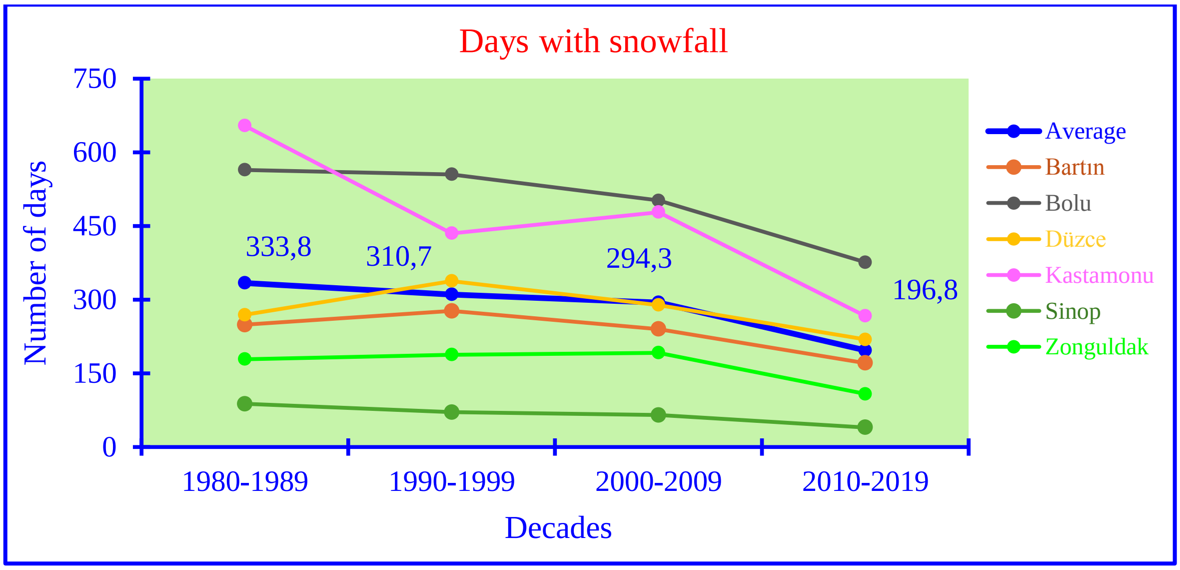

Figure 2. Spatial and temporal variation of snowy days in the Western Black Sea region

When city centers are analyzed separately for decadal periods, it is seen that the average snowfall days tend to decrease (Figure 2). This decreasing trend stands out very clearly, especially when the decades 1980-1989 and 2010-2019 are compared. The decrease is highest in Kastamonu with 59.2% and lowest in Düzce with 18.6%. The decrease rate in Kastamonu and Sinop centers is over 50%. The total number of average days covered with snow between 1980 and 2019 in the city centers of the Western Black Sea Region was determined to be 889.3. The proportional value of the number of days covered with snow is 6.1%. The total number of average days covered with snow in decades and its ratio to the total number of days are 240.3 (6.6%), 229.0 (6.3%), 229.0 (6.3), and 191.0 (5.2%) for the P1, P2, P3 and P4 periods, respectively. These values show that there is a decreasing trend in the number of days covered with snow, as well as in the number of snowy days (Figure 3).

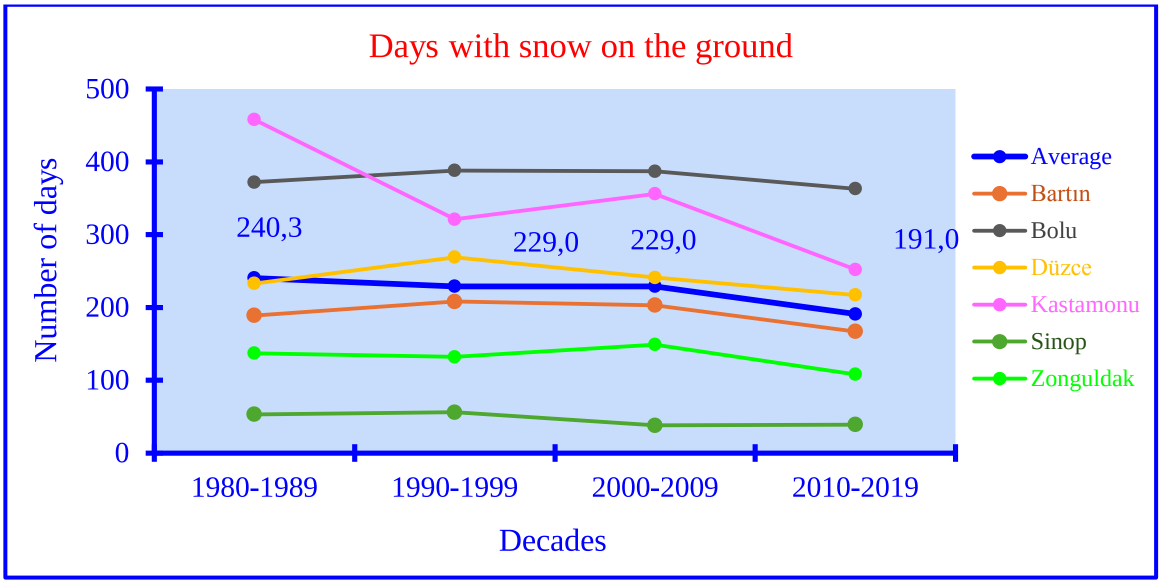

Figure 3. Spatial and temporal variation of snow-covered days in the Western Black Sea region.

Comparing the number of days covered with snow between the decades 1980-1989 and 2010-2019, a decreasing trend is observed in the entire region. This decrease was highest in Kastamonu with 45.0%, followed by Sinop with 26.4%. The center with the lowest decrease trend between the 1980-1989 and 2010-2019 decades was Bolu with a rate of 2.4%.
Previous studies on the subject generally report a clear decreasing trend in snow cover duration in the Northern Hemisphere (Choi *et al.*, 2010; Marty *et al.*, 2017; Beniston *et al.*, 2018). In this study, there is a spatial and temporal change in the number of days with snowfall and snow cover in the city centers of the Western Black Sea Region, and this tends to decrease. Many factors can affect the change in snow properties, such as global warming (Notarnicola, 2022), water content in the atmosphere (Bai *et al.*, 2019), average temperature (Brown, 2019). It has been emphasized in many studies that there is an increase in temperature averages throughout the region (Şensoy and Ateşoğlu, 2018; Bolat *et al.*, 2018, Bolat and Şensoy, 2023a; Bolat and Şensoy, 2023b). It is also stated that climate indices may change in Bartın, which is located within the region (Şensoy and Ateşoğlu, 2018). These results and evaluations largely explain the decrease in the number of snowy and snow-covered days. On the other hand, global and regional warming is one of the important factors that increase climate change. Considering that population growth has an impact on global warming (Dodson *et al.*, 2020), it is predicted that the population change of the region between 1980 and 2019 also has an impact on this result. As a matter of fact, the total population of the Western Black Sea Region in 2019 is 9.1% more than in 1980 (TUIK, 2024). The expansion of cities and the increase in the number of vehicles due to population growth may have caused some changes in the average temperature.

Conclusion

In the Western Black Sea Region, the number of snowy and snow-covered days has decreased temporally and spatially in city centers. As a result of the analysis in decades, when the 1980-

1989 period is compared with the 2010-2019 period, the average number of days with snowfall decreased by 41.0% and the number of days covered with snow decreased by 20.5%. A decreasing trend was observed in the number of snowy and snow-covered days in all six city centers where the study was conducted. Kastamonu was the center with the highest decreasing trend in the number of days with snowfall and snow cover. The lowest decreasing trend was in Düzce in the number of days with snowfall and in Bolu in the number of days covered with snow. These results show that increasing global warming due to both natural and anthropogenic factors also affect the Western Black Sea Region of Turkey. It is predicted that these decreases in snow cover, which is an important supplier of surface and groundwater, will negatively affect the hydrological cycle and soil moisture.

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THE EFFECT OF THE COMBINED USAGE OF HYDROFOBIC AND HYDROPHILIC AGENTS ON THE STALING OF BREAD

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Abstract

This study investigated the effects of combined usage of carboxymethyl cellulose (CMC) and beeswax (BW) on bread staling. Bread formulations were fortified with 0-5% CMC and 0-2% BW and the rheological and physical properties of dough and bread were monitored. Additionally, baked bread samples were stored for four days and staling characteristics were followed. According to the farinograph and extensograph data, the usage of CMC and BW led to increase in the water absorption and decrease in the resistance of the dough to extension ($p>0.05$). After four days of storage, the lowest moisture loss was found in the bread with the highest ratio of CMC and BW (1.09%). The usage of CMC and BW led to a decrease in the specific volume of the bread. The lowest staling index value was found in bread containing the highest ratio of CMC and BW to be 115%. In addition, the staling characteristics were calculated with Avrami kinetic model, and the lowest k and highest n constants were calculated for bread containing the highest ratio of CMC to BW. The k value indicates the rate of hardening of the bread and the lower the value of it, the slower the hardening process and therefore the staling. These results showed that the combined usage of CMC and BW was effective in retarding bread staling.

Keywords: *carboxymethyl cellulose, beeswax, dough rheology, bread staling, Avrami model.*

Introduction

Bread is a staple food in many countries, particularly those with cereal-based diets. Staling, a type of physical deterioration, is an important problem that reduces the consumption rate of bread. The staling process , which occurs after baking, adversely affects the sensory properties of bread, decreases its eating quality and shortens its shelf life. As a result of staling, the moisture balance is disturbed, starch retrogradation occurs, hardness increases, and sensorial features deteriorate (Simith et al. 2004).

Bread staling is directly related to starch retrogradation and becomes irreversible in later stages due to increased interaction with gluten in the dough. Wheat flour used in bread production contains approximately 84-88% starch. The starch polymer consists of straight chain amylose linked by α-1,4 glycosidic linkages and branched amylopectin structures linked by α-1,6 glycosidic linkages. When starch is heated in an aqueous medium, it swells by absorbing water, and the amylose is released from the granule and gelatinises. When the gelatinised starch is cooled below the melting temperature, physical structure of amylose and amylopectin fractions are disrupted. This is resulted with densely aggregation, increasing the viscosity of the food matrix and strengthening the gel. This phenomenon is defined as retrogradation (Atwell et al., 1988; Amigo et al., 2016; Gray and Bemiller, 2004).

The ingredients of bread are highly effective on retrogradation. Hydrophilic agents such as cellulose derivatives are associated with retarding staling by decreasing moisture loss in the crumb. In recent years, the demand for the use of cellulose derivatives such as carboxymethyl cellulose (CMC) in bakery products has increased due to their properties to improve texture, increase dough viscosity, reduce retrogradation and prevent water loss (Bousquieres et al.

2017). In a previous study, it was reported that increasing the concentration of CMC directly increases the specific volume values of bread (Sidhu and Bawa 2000), and this property is attributed to the hydrophilic properties of the carboxyl groups in its structure (Maleki and Milani 2013).

Hydrophobic food additives such as shortening and emulsifiers with high saturated fatty acid content can be used to delay bread staling (Mancebo et al. 2017; Yamauchi et al. 1992). There are several studies showing that the addition of fat has been delayed the staling of bread. Smith and Johansson (2004) reported that increasing the proportion of hydrogenated fats in bread formulations reduced staling. It is reported that the main reason for this result is that linear hydrocarbons in the hydrophobic formulation structure form a complex with the starch helix. The addition of emulsifiers such as lecithin and monoglycerides also slows down staling and retrogradation. These effects of emulsifiers were found to be achieved by increasing cell wall thickness and elasticity, preventing starch from losing water and gluten from hardening (Gray and Bemiller, 2004).

The aim of this study was to investigate the effects of combine usage of CMC as a hydrophilic agent and beeswax (BW) as a hydrophobic agent on bread staling.

Material and Method

In the study, carboxymethyl cellulose (CMC) and beeswax (BS) were added to the dough at ratios of 0-5% and 0-2%, respectively. To prepare control bread dough, wheat flour (1 kg), baker’s yeast (30 g), salt (15 g) and water (determined by farinograph unit) were kneaded in a mixing machine (Hobart N50, Offenburg, Germany) for 20 min. The dough was left to bulk fermentation at 30 °C for 30 min. After that, 100 g of dough was cut, placed into metal pans and proofed at 30 °C at 80–90% relative humidity for 60 min. The dough was baked in an electrical oven (Fimak Rokon Classic FRN10G, Konya, Turkey) at 240 °C for 15 min (AACC, 2000). The bread was packaged in polybags and stored for analysis at ambient temperatures for 4 days.

The rheological features of bread dough were determined by Farinograph and Extensograph instrument (Brabender, Duisberg, Germany). The specific volume of bread was calculated through the volume/mass ratio and expressed in mL/g (AACC, 2000). L*, a* and b* colour values of bread crust and crumb were measured by the colour meter (Chroma meter CR-400, Konica Minolta, Japan). Texture profile analysis (TPA) of bread crumbs was determined by a texture analyser (TA-XT plus, Stable Micro Systems, Surrey, UK) (AACC, 2000). Staling degree of bread was evaluated by following firmness, moisture loss, staling index value and thermal characteristics by differential scanning calorimeter (DSC) (Correa et al., 2015; Kerch et al., 2012). Staling kinetic was also calculated from Avrami equation by using firmness value (Armero ve Collar, 1998; Russell, 1983).

Result and Discussion

According to the rheological measurements (Table 1), CMC led to increase the water sorption of dough. It was found that the stability values of the bread doughs varied between 8.10 and 12.00 min and CMC utilization caused to decrease of stability. The extensibility resistance varied between 588 and 918 B.U, and the highest resistance was found in the control ($p<0.05$). When the energy values of the dough samples were analyzed, the highest value of 173 cm^2 was found in the formulation containing the maximum amount of CMC (5%) and BW (2%).

Table 6. Rheological properties of bread dough

Bread	Farinograph		Extensograph	
	Water absorption (%)	Dough stability (min)	Extensibility resistance (B.U.)	Dough energy (cm^2)
Control	54.90^{C}±0.30	9.05^{B}±4.65	889^{A}±86.86	134.33^{AB}±8.60
2.5 CMC+1 BW	66.40^{B}±4.20	12.00^{A}±2.60	675^{BC}±153.52	111.67^{B}±20.21
5 CMC+2 BW	80.75^{A}±1.45	8.65^{B}±0.25	918^{A}±10.97	173.00^{A}±6.93
5 CMC+1 BW	80.65^{A}±0.25	10.05^{BA}±0.55	588^{C}±57.84	91.33^{C}±9.94
2.5 CMC+2 BW	63.20^{B}±0.20	8.10^{B}±0.10	804^{AB}±44.60	138.67^{AB}±21.07

Specific volume, moisture loss and the change in texture profile are presented in Table 2. The utilisation of CMC and BW significantly affected the specific volume of bread ($p<0.05$). The highest specific volume was measured in control to be 4.47 mL/g and the additives decreased to specific volume of bread samples. During storage, moisture loss increased in all bread samples. However, the lowest raising ratio in moisture loss was detected in bread containing highest ratio of CMC and BW. There is a direct correlation between moisture loss and hardness increase during staling. In parallel with this result, the lowest hardness increase was determined in bread containing highest ratio of CMC and BW. The low moisture loss of this sample was also effective in obtaining this result.

Table 7. Specific volume, moisture loss and hardness change in storage

	Specific volume (mL/g)	Moisture loss (%)	Hardness (kg)	
			Day 0	Day 4
Control	4.47^{A}±0.02	2.67^{B}±0.52	2073^{A}±815	3441^{A}±496
2.5 CMC+1 BW	4.29^{AB}±0.04	3.25^{AB}±0.34	1220^{BC}±373	1851^{B}±184
5 CMC+2 BW	3.69^{B}±0.03	1.09^{C}±0.00	1127^{C}±237	1535^{C}±35
5 CMC+1 BW	2.96^{C}±0.04	4.83^{A}±0.79	1548^{B}±373	2192^{BA}±95
2.5 CMC+2 BW	3.34^{BC}±0.11	2.73^{B}±0.00	2232^{AB}±663	3382^{A}±126

The L*, a* and b* colour parameters were also measured in both bread crumb and crust. The addition of CMC caused a decrease in the L* colour value of the bread crust. The highest L* value of 60.92 was found in control bread. In parallel with the L* value, the addition of CMC caused a decrease in the b* value of the bread. The lowest b* value of 25.88 was found in bread samples containing 5% CMC and 2% BW.

Staling degree of bread containing CMC and BW was followed by staling indexmand Avrami kinetic parameters (Table 3). The highest staling index was detected in control sample with a value of 397.47. The combine usage of CMC and BW delayed significantly the staling of the breads ($p<0.05$). Descriptively, the lowest staling rate (115.11) was obtained in formulations containing the highest concentrations of CMC and BW.
Changes in bread firmness during storage are usually evaluated using the Avrami mathematical model. This equation is used to evaluate the degree of starch crystallisation occurring in a given solid matrix and the progress of crystallisation is expressed as an exponential inverse function of time (Armero ve Collar, 1998). The k value obtained from the model is associated with hardening rate of the bread and low k value indicates slower the hardening process and staling (Amigo et al., 2016; Russell, 1983). In the study, the lowest k value was found in bread samples containing the highest concentration of (5%) CMC and (2%) BW. As can be seen from these results, the combine usage of high concentrations of CMC and BW was an effective method of retarding of bread staling. This result may be sourced from that the hydrophilic CMC absorbs a high amount of water in the bread

formulation and the hydrophobic BW delays staling by reducing water loss from the amylose-amylopectin network after baking.

Table 8. Staling parameters

Bread	Staling index	Avrami coefficients	
		k	n
Control	397.47^{A}±112	0.036±0.005	2.90±1.28
2.5 CMC+1 BW	225.23^{B}±78.99	0.0011±0.001	5.78±1.30
5 CMC+2 BW	115.11^{B}±19.33	0.0005±0.0001	8.12 ± 3.52
5 CMC+1 BW	142.66^{B}±16.22	0.17±0.05	1.96±0.31
2.5 CMC+2 BW	184.90^{B}±21.90	0.11±0.01	2.12±0.84

Conclusion

As a result, it was found that combined usage of CMC and BW delayed the staling of bread. It was found that CMC with hydrophilic character absorbs a high amount of water in the bread formulation and BW with hydrophobic character reduces the water loss of the amylose-amylopectin network after baking and consequently slows down the staling mechanism.

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LESS CLEAN WATER FOR AGRICULTURE DUE TO MILITARY ACTIVITIES IN UKRAINE

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Abstract

Ukraine is the biggest country in Europe and occupies 19% of European agricultural land (Makarenko *et al.*, 2022). The military activities due to the Russian-Ukrainian war have far-reaching consequences for water and soil in Ukraine. According to our literature review, mre than 500 water infrastructure facilities (dams, wastewater treatment plants, and others) were destroyed, and around 742 million m^3 of water was lost (e.g. Strokal *et al.*, 2023; Strokal *et al.*, 24b). Moreover, the implications of military activities have led to water scarcity for crop production. For instance, more than 70% of irrigation systems in Ukraine were damaged in 2022-2023, causing crop yield losses of 17%. We analyzed the cause-and-effect relationships of military activities to identify drivers of water scarcity. We also assessed the risks for crop production and the supply of clean water for human activities. For instance, the risks for crop production are often associated with the loss of land degradation (35% of territories) and the contamination of soils by heavy metals (587,691 m^2 of polluted areas) due to mined agricultural lands. A risk to the water supply for agriculture is often associated with the less availability of clean water due to destroyed irrigation systems (more than 70%), contamination of water with 1.682 million tons of various pollutants, damaged reservoirs (18.200 million m^3 of water lost due to destruction of Kakhovka reservoir that was the main source of water supply for the South part of Ukraine).

Keywords: *water scarcity, military activities, agriculture, crop production, river and soil pollution.*

Introduction

Since the full-scale Russian invasion, Ukrainian territories have suffered from military activities such as shelling, mining, bombing, and occupying (Makarenko *et al.*, 2022). Many agricultural farms were damaged (Strokal *et al.*, 2023). The bombing destroyed soil covers and increased land compaction and erosion. Currently, approximately 30 % of soil degradation is reported with around 830 thousand m^2 of contaminated soil and around 19 million m^2 of littered land (data based on 01.04.2024) (EcoZagroza, 2024). Furthermore, water-related infrastructure plays an important role in agricultural production but has been largely damaged due to military activities. For example, 724 water supply systems were destroyed or damaged leading to soil pollution and water scarcity for crop production (Strokal *et al.*, 2024a). Another example is the damage of 160 wastewater treatment and sewage facilities that led to point source pollution of water and soil (Strokal*et al.*, 2023). As a result, the availability of clean water for the agriculture sector has decreased contributing to crop yield losses (Artuc*et al.*, 2023). In this paper, we distinguish the three main causes of the military activities with their effect on agriculture in Ukraine: (1) mined territories contributing to crop yield losses, (2) destroyed or damaged water infrastructure contributing to water scarcity, and (3) destroyed or damaged irrigation systems contributing to food shortage.

Materials and methods

In our research, we applied *the internationally recognized DPSIR framework* (Gari *et al.*, 2015) to show some potential impacts of military activities on the agricultural sector in Ukraine, especially on crop production. For this, we designed three frameworks. The original framework was often applied for different locations to analyze the cause-effect chain of certain environmental problems including drivers, pressure, state, impact, and responses (e.g., Shi *et al.*, 2021; Vannevel, 2018; Sun *et al.*, 2016). In this study, we applied the framework to describe the main *drivers* (*D*) to identify the *pressures* (*P*) on soil health, assess the *state* (*S*) of soil and water resources, and justify the *impacts* (*I)* of military activities that cause less clean water for agriculture. We highlighted the *responses* (*R*) for minimizing the influence of polluted soil and water on crop production and for enhancing soil productivity.

Results and discussion

Our three new DPSIR frameworks show the cause-effect relationships between less clean water availability for agriculture in Ukraine due to minted territories (DPSIR 1), destroyed or damaged water infrastructure (DPSIR 2), and irrigation systems (DPSIR 3, see Table 1). Below, we describe the results of the three DPSIR frameworks.

DPSIR 1 focuses on the cause-effect chain of the mined territories leading to crop yield losses (Table 1). On a monthly basis, the share of mined territories increases (*Driver*). This situation depends on the demining process and excessive military actions. According to the reports of the Ministry of Environmental Protection and Natural Resources of Ukraine (EcoZagroza, 2024), at the end of 2022, 22% of Ukrainian territories were mined (130 thousand km^2). According to the report of the State Emergency Service of Ukraine in 2023, before April, 30% of the territories were mined (174 thousand km^2), and after April this was 26 % (156 thousand km^2) (State Emergency Service of Ukraine, 2024). This situation pursued chemical and physical pollution (0.59 km^2 of polluted areas in 2023 and 0.83 $км^2$ in 2024) (*Pressure*) that increased levels of soil contamination (*State*). For instance, in the occupied territories in the Donetsk region where military activities are ongoing, territories are under active bombing. Those territories are mined. As a result, there are increased levels of concentration by chemicals (Cu, Zn, Ni, Pb, Sr, Cr) up to 3-35 times compared with standards (Splodytel*et al.*, 2023). According to this, land fertility and crop yield have been lost (*Impact*). The *Response* to address *state* issues (e.g., decrease soil contamination) is to execute soil recultivation such as phytoremediation, phytoextraction, and agrotechnical melioration (Table 1).

DPSIR 2 focuses on the cause-effect chain of the destroyed or damaged water infrastructures leading to increasing water scarcity issues (Table 1). Water infrastructures (dams, bridges, treatment, sewage facilities, and water supply systems) support largely the development of the agricultural sector in Ukraine (D’Odorico *et al*., 2020). Based on studies (Strokal *et al*., 2023), government reports (EcoZagroza, 2024), and information from the official online platform (Ecodozor, 2024), during the full-scale military invasion in Ukraine 123 bridges, 724 water supply systems, 160 water treatment plants, and sewage facilities, and 23 dams, including Kakhovka dam were already damaged (*Driver*). Due to damaged dams and bridges, 500 thousand hectares of agricultural land were flooded in the Southern part of Ukraine (Strokal*et al*., 2024b). Damaged water treatment and sewage facilities contributed to the discharge of untreated wastewater into rivers. For example, untreated urban waste was responsible for 20-62% of pollutants entering rivers in the Dnipro Basin based on a modeling study (Strokal*et al.*, 2023) (*Pressure*). Currently, water pollution levels increase. Approximately 1.7 million tons of pollutants such as nutrients, pathogens, and plastics entered water systems (*State*) (EcoZagroza, 2024). Due to damaged water infrastructures, especially in the agricultural territories of the Southern part of Ukraine water scarcity issues for crop production have increased (*Impact*). As a result, 6 thousand km^2 of land remained without access to clean water for irrigation especially in the Southern part of Ukraine (Solokha*et al.*, 2023). The *Response* to address *impact* issues is to provide climate-resilient smart agriculture with drought-resistant varieties of plants (Table 1).

DPSIR 3 focuses on the cause-effect chain of the destroyed or damaged irrigation systems leading to increasing food shortage (see Table 1). The Southern part of Ukraine requires irrigation systems for crop production due to its location in the Steppe Zone with a warm temperature, semiarid climate, and chernozems of soils (BioModel, 2024). However, due to the military activities, 94% of irrigation systems were destroyed or damaged in Kherson, which is dominated by agricultural activities and is located in a dry environment. This was 74% for Zaporizhzhia, and 30% for Dnipro Regions (*Driver*) (Centre for Economic Strategy, 2023). As a result, some of the agricultural lands have suffered from landslides and soil erosion (*Pressure*) which led to soil degradation (35 %) (Strokal*et al.*, 2023). Due to damaged irrigation systems, 18.2 km^3 of water was lost (*State*) and 14% of grain export decreased (*Impact*). The *Response* to address *impact* issues is to explore sustainable ways for saving water for irrigation and promote the use of treated wastewater to secure enough food production under a dry environment

Table 1. Designed three DPSIR frameworks with examples

	DPSIR 1	DPSIR 2	DPSIR 3
Driver	Mined Ukrainian territories	Destroyed or damaged water infrastructure facilities	Destroyed or damaged irrigation systems
	2022 – 22 % (130.000 km^2) *2023 – 30 % (174.000 km^2)* *2024 – 26 % (156.000 km^2)*	*123 bridges, 724 water supply systems, 160 water treatment and sewage facilities, 23 dams*	*94% in Kherson, 74% in Zaporizhzhia, and 30% in Dnipro Regions*
Response	To evaluate and assess technically mined lands: vegetables, grain crops and others	To estimate the degree of the destruction of water infrastructure on the agricultural sector	To provide new legislation that will make the use of treated wastewater that is safe for crop irrigation. To improve irrigation efficiency through altering farming practices (plant crops according to seasons and soil conditions)
Pressure	Emissions into the soil	Flooded territories, discharge of untreated wastewater in river	Landslides, soil erosion
	Flows of chemicals in the soil (0.59 km^2 of polluted areas in 2023; 0.83 kм² in 2024)	*500 thousand hectares of land are flooded in the Southern part of Ukraine due to the destruction of the Kakhovka HPP*	*Land degradation (35% of territories)*
Response	To identify a set of indicators that can be used for soil monitoring	To identify a set of indicators that can be used in water pollution monitoring	To reduce soil erosion: plant vegetation, especially including in sustainable crop rotation rye with clover seedings. To reduce landslides: provide technological methods for consolidation of soil slopes (terracing, building dams, shelterbelts)
State	Soil contamination levels with heavy metals and other pollutants	Water contamination levels with pollutants	The volume of water loss for irrigation. Clean irrigation water shortage
	The contamination of soils by	*The contamination of water with*	*18.2 km^3 of water lost*

	heavy metals up to 3-35 times compared with standards	*1.7 million tons of pollutants (nutrients, pathogens, plastics, etc)*	
Response	To execute soil recultivation (phytoremediation, phytoextraction, agrotechnical melioration)	To assess economic losses of water resources due to water pollution which was caused by damaged water facilities, using the national legislation that is adjusted for military activities	To switch from flood irrigation systems to sprinklers/drip irrigation systems. To provide new legislation that will make the use of treated wastewater to irrigation crops safe
Impact	Losses of crop yield and of fertile land	Water scarcity for crop production due to drought and pollution	Food shortages
	The crop yield losses have reached 17%	*6 thousand km^2 of land remained without access to enough water for irrigation with appropriate quality*	*Decreased 14% of the export of potential grain products*
Response	To involve potential stakeholders in the decision-making process regarding the goals of reclamation and evaluation of reclamation works	To provide climate-resilient smart agriculture with drought-resistant varieties of plants	To provide effective water filtration systems for ensuring the use of freshwater for irrigation. To explore sustainable ways for saving water for irrigation and promote the use of treated wastewater to secure enough food production under the dry environment

In general, agricultural production in 2022 decreased by 25 % compared with 2021 nationally. This was 95% for the Kherson and Donetsk regions and 32% for the Odesa region (Solokha*et. al.,* 2023). Including information during the full-scale military invasion, agricultural products were destroyed: 4 million tons in 2022 (Matsybora, 2023) and 300 thousand tons in the Southern part of Ukraine in 2023 (Strokal*et. al*., 2023).
Ukraine is a major exporter of wheat (accounts for 10% globally), corn (15%), barley (15%), and sunflower (especially oil – 50%) to world countries (KSE Agrocenter, 2024). However, the deterioration of soil fertility increases due to military actions. This causes crop yield losses. In addition, damaged water infrastructures provoke water scarcity for crop production in Ukraine. That is why, the agricultural sector in Ukraine is greatly challenged by less clean water and less productive land for crop production. These challenges contribute to decreased food exports to other countries in the world. Figure 1 shows the main concept of less clean water for agriculture due to military activities in Ukraine. This concept is based on the three DPSIR frameworks that are described above (Table 1).

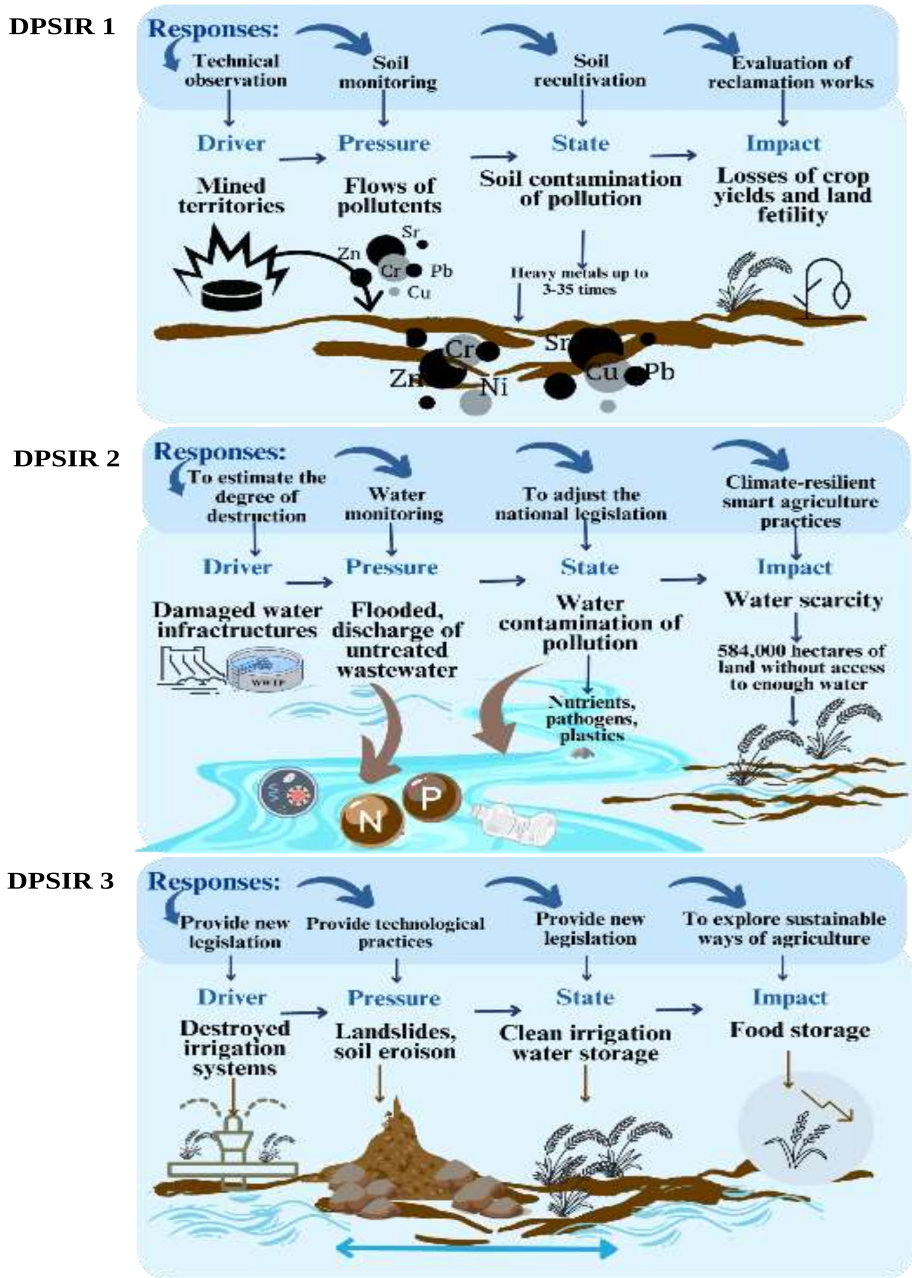

Figure 1. The main relationship between less clean water availability for agriculture and military activities in Ukraine. Source: the information is based on the DPSIR frameworks illustrated in Table 1.

Conclusion

Military activities considerably affect the agricultural sector within mined territories, and damaged water infrastructures and irrigation systems. In this paper, we designed the three DPSIR frameworks to determine the cause-effect aspects of (1) minded territories, (2) destroyed or damaged water infrastructure, and (3) irrigation systems. We showed the main challenges of those drivers for the decreased availability of clean water for agricultural production and crop yield losses. Our DPSIR analyses show the following implications on crop production: 35% of land degradation, 17% of crop yield losses, 14% of decreased grain product exports, the contamination of soils by heavy metals up to 3-35 times compared with standards (0.59 km^2 of polluted areas), the contamination of water with around 1,7 million tons of various pollutants. All these implications decrease soil health and contribute to food security and water scarcity. It is expected that water scarcity and soil deterioration will become more severe in the coming months and years, especially in the southern part of Ukraine. This will challenge the Ukrainian agricultural sector even more than today. National and international cooperation is needed to address the challenges.

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SOIL PROFILES IN GATACKO POLJE IN EASTERN REPUBLIC OF SRPSKA, BOSNIA AND HERZEGOVINA

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Abstract

Karstic areas are not only characterized by their unique geomorphological and floristic diversity but are also highly fragile ecosystems. Ensuring the sustainability of karst ecosystems requires a fundamental understanding of the actual site conditions. This research was carried out to ensure that the lands can be used again in agricultural production by implementing soil management and soil protection measures according to soil survey principles. This study carried out in Gacko Polje is part of Eastern part of Republic of Srpska (Bosnia and Hercegovia). According to the results, the soil profile formed by the main bedrock Carbonate has A-C-G soils. The general soil texture forms are Clay and Loamy. According to the WRB classification, the fluvial meadow (humofluvisol) soil order is Hydromorphic, and the soil class is Semiglay. In order to successfully reforest the study area, tree species should be selected taking into account ecological soil classes and variables such as parent material, soil type, depth, and stoniness. Karst groundwater in agricultural areas is vulnerable to salinization and contamination due to overuse of fertilizers, excessive irrigation, and poor soil management.

Keywords: *Soil profiles, Republic of Srpska, Gacko field.*

Introduction

Soil represents one of the most important natural resources. It is an invaluable, irreplaceable, immovable and non-reproducible resource for agricultural production and represents the good of humanity, not of one generation, nation, group or individual. Since soil profile development is an open, dynamic and natural system that reflects the ecological, geological and physiographic features that form it over time, their physical and chemical properties are also in close relationship with each other (Çepel, 1996; Kantarcı, 2000).

Karst landscapes developed on evaporite (anhydrite, gypsum, halite, sylvite) and carbonate (limestone, dolomite) rocks (Stevanovic, 2015). 12% of our planet's surface is made up of the Kars ecosystem (Liu, 2009). Unique geomorphological features, forests, soils, animals, water resources, archaeological sites, and potential ecological and economic assets are all present in karst environments. Although karst ecosystems' role in the global carbon cycle has not been fully understood, a number of its characteristics have been the subject of current research (Zokaites 1997). Karst areas have many geomorphological formations. Depressed areas specific to karst areas significantly increase soil organic carbon storage, especially in agricultural lands. Forest areas are more stable in carbon sequestration compared to other land uses, making depressed areas important geomorphological formations in carbon storage within karst ecosystems (Dindaroglu et al., 2019).

The area of Eastern Herzegovina is one of the most karstified regions in the world. Deep karst, sinking rivers, underground flows, temporary flooded karst poljes and lack of arable

land is main natural property of the region. Due to two kinds of misfortune, flood and drought, people have emigrated from this region searching for a better life Milanović (2023).
This paper includes a detailed field and laboratory investigation of the physical and chemical properties of natural soils, in this case humofluvisol, in the area of Gatački polje, that is, in eastern Herzegovina. This research was conducted with the aim of enabling part of the examined lands to be used again for agricultural production through the implementation of soil management and soil conservation measures based on scientific principles.

Material and Methods

Study area

The study area (Fig.1) covered Gacko of Eastern part of Republic of Srpska (Bosnia and Hercegovia). Total surface of Gatačko Polje is about 37.6 km^2, and its elevation is between 936 and 950 m a.s.l. It consists of two geomorphologicaly and hydrogeologicaly interconnected units: Gatačko Polje itself (31.83 km^2) and Malo Gatačko Polje (5.77 km^2), Fig. 1. (Milanović at all 2018).

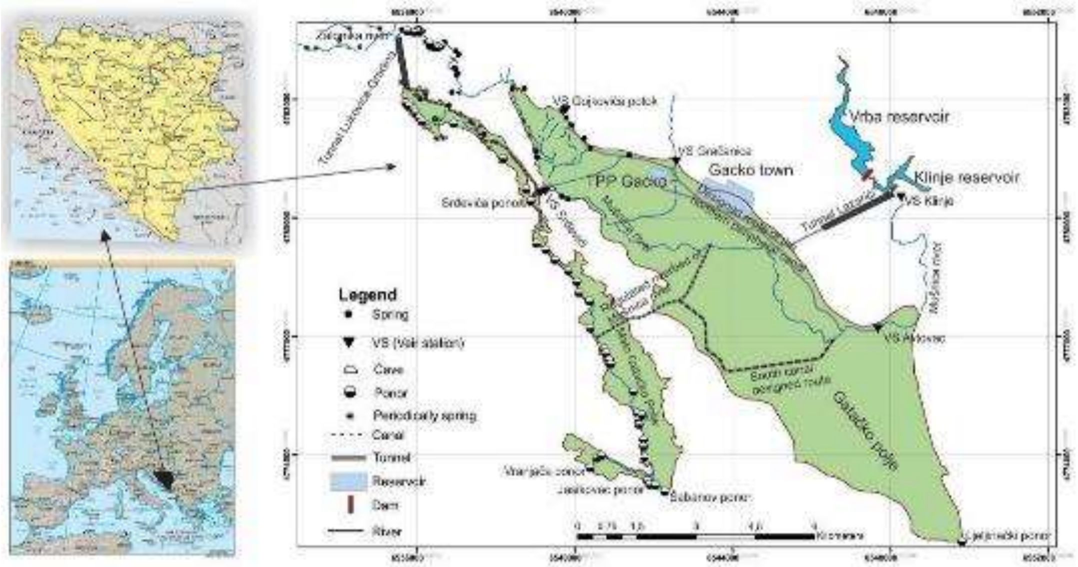

Figure 1. Geographical position and schematic map of wider area of Gatačko Polje Milanović at all (2018)

Geographical and climate characteristics

For this study data of geographical and climate characteristics were collected for examinated location Gacko, Republic of Srpska, Eastern part of Bosnia and Hercegoviana. Dataset of continuous monthly values for the period 2018 - 2022 covering a total of 60 months. These data include °E, °N, Altitude (m), precipitation (PRCR mm), mean air temperature (oC), Köppen–Geiger (Cfb), (Table 1). The data was provided by the Federal Hydro-Meteorological Institute of BiH.
In terms of altitude, Gacko is 950 m. This location fall under the classification of a temperate warm and humid climate (Cfb x''s), precipitation are 1065 mm and average air temperatures range are 8°C in Gacko.

Table 1. The basic geographical and climate characteristics of the examinated locations.

Location	°E	°N	Altitude(m)	PRCP (mm)	Tmean (^{o}C)	Köppen Geiger
Gacko	18°32'	43°10'	950	1065	8	Cfb x''s

Note: °E – longitude; °N – latitude; PRCP – precipitation; Tmean – mean air temperature; Cfb x''s –temperate warm and humid climates, Csa sx –Mediterranean climate with hot, dry summers

Field Research

Pedological profiles of fluvial meadow soil were opened in Gatačko polje at locations in Medanići (Profiles 1 and 2). Figure 2 at the Gatačko polje locality represents the fluvial meadow soil. Profiles 1 and 2 are fluvial meadow soils (humofluvisol) and according to the classification of the soil of Yugoslavia (Škorić et al., 1985), they belong to the order of hydromorphic soils, class semigley, subtype shallow gleyic soils with A-C-G profile structure. According to Resulović et al. (2008), based on the level of development, they can have a sequence of horizons, which is referred to as Ah-C-G or Ah-CG soils. According to WRB classification, fluvial meadow soil is Gleysols (FAO, 2006).

Figure 2. External morphology of fluvial meadow soils (humofluvisol), Gatačko polje (Tunguz, 2024)

The external and internal morphology is described for all pedological profiles (Munsell Soil Color Charts, 1954), soil samples in a disturbed state were taken for all genetic horizons and described (Škoric et al., 1985). Soil samples in undisturbed conditions were taken from individual genetic horizons, in three repetitions, by cylinders of Kopecký. The average soil samples were taken too (Belić et al., 2014).

Results and Discussion

Previous pedological research was worked aimed at inventorying and determining the classification of land resources. The level of such pedological research was defined for the purposes of creating a pedological map of Yugoslavia, Bosnia and Herzegovina, R=1:50000. The this areas where research was carried out included smaller or larger parts of four sheets (sections) of R=1:50000 maps. These are the sheets: Nevesinje 4, Gacko 1, Gacko 3 and 4 (Pedological map of Yugoslavia, Bosnia and Herzegovina, 1978-1984).

Profile 20
Location: Medanići
Altitude: 935 m
Relief: flat
Vegetation: meadow
On the pedological map of BiH R:1:50000, the soil was marked as: Mineral-marsh clay and organomineral-clay soils (70%+30%) MO+OG

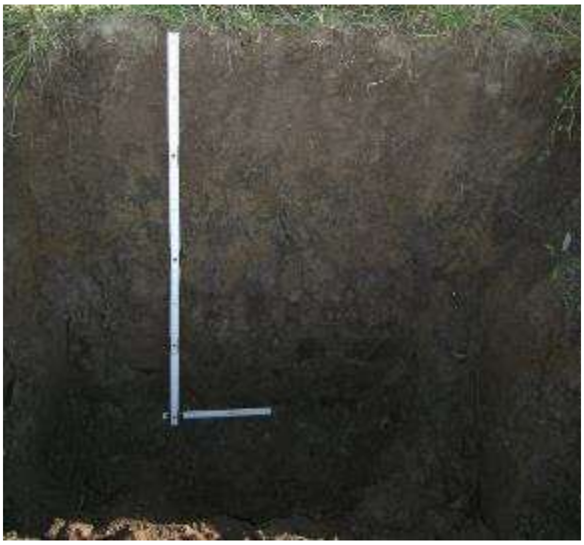

Figure 3. Internal morphology of fluvial meadow soils (humofluvisol), (Tunguz, 2015)

Order: Hydromorphic soils
Class: Semiglay
Type: Fluvial meadow (humofluvisol)
Subtype: Shallow shaped (L 100-150 cm)
Variety: Carbonate Profile: A-C-G Forms: Clay

Amo I (0-10cm) - molic, accumulative-humus horizon of light gray color (10 YR 5/2) in dry and gray color when wet (10 YR 4/2).Its texture is powdery clayey loam, powdery structure, carbonate, permeated with veins of vegetation.
Amo II (10-20cm) - light gray horizon (10 YR 5/2) in the dry and gray in the wet (10 YR 4/2).Its texture is powdery clay, powdery structure, carbonate.
CG I (20-40cm) - horizon of light gray color (10 YR 4/1) in dry and wet condition of gray color (10 YR 3/1).Its texture is powdery clay, lumpy structure, carbonate.
CG II (40-60cm) - horizon of dark gray color (10 YR 3/1) in dry and black gray color (10 YR 3/1) in wet condition.Its texture is powdery clay, prismatic structure, carbonate.
Gso (60-90cm) - gley horizon of dark gray color (10 YR 3/1) in dry and black gray color when wet (10 YR 2/1).Its texture is powdery clay, prismatic structure, carbonate.

Profile 21
Location: Medanići
Altitude: 930 m
Relief: flat
Vegetation: meadow
On the pedological map of BiH R:1:50000, the soil is marked as: Mineral-swamp clay soils and brown acidic soils on clays (50%+50%) MO+TB^2

Figure 4. Internal morphology of fluvial meadow soils (humofluvisol), (Tunguz, 2015)

Order: Hydromorphic lands
Class: Semiglay
Type: Fluvial meadow (humofluvisol)
Subtype: shallow-clay soils (G 100-150 cm)
Variety: Carbonate
rofile: A-C-G
Forms: loamy

Amo I (0-12cm) - mollic, accumulative-humus horizon of brown color (5 YR 3/4) in the dry and in the wet of brown color (5 YR 3/4).Its texture is powdery clayey loam, powdery structure, carbonate-free, permeated with veins of vegetation.
Amo II (12-31cm) - transitional accumulative-humus horizon of brown color (5 YR 3/4) in the dry and in the wet of brown color (5 YR 3/4).Its texture is powdery clay loam, powdery structure, carbonate.
C I (31-51cm) - brown horizon (5 YR 3/3) in dry and dark brown in wet (5 YR 3/).Its texture is clay loam, crumbly structure, carbonate.
C II (51-64cm) - brown horizon (5 YR 3/3) in the dry and dark brown in the wet (5 YR 3/2).Its texture is clay loam, lumpy structure, carbonate.
CG I (64-80cm) - horizon of brown color (5 YR 3/2) in the dry and dark brown color (5 YR 3/2) in the wet.Its texture is clayey loam, large lumpy structure, carbonate.
CG II (80-90cm) - brown horizon (5 YR 3/2) in dry and dark brown in wet (5 YR 3/2).Its texture is powdery clayey loam, large lumpy structure, carbonate.
Gr (90-120cm) - horizon of brown color (5 YR 2/2) in dry and dark brown color in wet (5 YR 2/1).Its texture is powdery clay loam, prismatic structure, carbonate.

The soil profiles of fluvial meadow soils were generally deep, with depths in the ranges of 120 cm (Fig. 3 and 4.).
Osher and Buol (1998) examined the relationships between soil properties, parent material, and physiography in Peru and found that the soils in the region are shaped by the texture of the parent material and land topography. Krasilnikov et al. (2005) studied the relationship between geomorphology and soil formation in an area covered by tropical forests in Mexico, reporting that the distribution of mountain soils is significantly influenced by geomorphological processes. They stated that the distribution in sediments leads to changes in

soil texture and mineralogical structure, and therefore, the observed variation in soil properties is due to land position. Yimer et al. (2006) found significant differences in the effects of plant community types and topographic aspect on the physical and chemical properties of soil, with variations occurring based on plant communities and topographic aspects.

Dindaroglu and Vermez (2019) investigated site characteristics in karst ecosystems, including parent material, physiographic characteristics (slope, aspect, elevation), soil properties, climate type, and vegetation structure. In the study area, five different parent materials (limestone, marble, breccia, diabase, and quartzite) were identified, with limestone being the dominant parent material. They identified and mapped 39 ecological soil series and 4 site characteristics (moist, fresh, slightly fresh, and dry) in the study area. Detailed classification of karst ecosystems and determination of their functions will increase the success of forestry activities planned in these areas.

In a study conducted by Vermez et al (2018) major bedrock is covered by limestone (mainly calcite minerals, some fossiliferous and others with brecciated texture), has soils with a moderately alkaline pH (8.1), high organic matter content (4.33%), a cation exchange capacity of 32.6 cmol kg^{-1}, and lime content of 10.77%. The soils were well-drained with medium water holding capacity. In the humid and dry forest sites, various tree species such as *Pinus brutia*, *Cedrus libani*, *Quercus cerris*, and *Juniperus excelsa* were spread on limestone bedrock. In the dry forest site, where marble, quartzite, breccia, and diabase bedrock exists, species like *Quercus coccifera*, *Laurus nobilis*, *Olea europaea*, and other shrubs were found.

Conclusion

Soil formation and development encompass factors such as plant root volume and soil properties that influence plant survival, including available water capacity, pH, organic matter, and texture in karst ecosystem. Reforestation efforts and silvicultural interventions can only be based on a solid foundation by understanding the site's environmental conditions. Therefore, providing planners and implementers with data on site conditions is essential. The soil profiles in Gacko Polje, developed primarily from carbonate bedrock, consist of A-C-G horizons and have a deep profile. The predominant soil textures are clay and loamy. These fluvial meadow soils, classified as humofluvisols, belong to the Hydromorphic order and fall under the Semiglay soil class.

Since any functional disruption in karst ecosystems will affect the entire ecosystem, it is crucial to avoid quarrying, overgrazing, and the unregulated use of groundwater in these areas, and to adopt soil conservation measures in agricultural activities. For successful reforestation efforts in the study area, tree species selection should consider ecological soil classes that take into account parent material, soil type, physiological soil depth, and stoniness. Excessive irrigation, overuse of fertilizers, and improper soil management practices in agricultural areas will negatively impact karst groundwater, increasing the risk of salinization and pollution.

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SOIL FERTILITY IN EASTERN REPUBLIC OF SRPSKA, BOSNIA AND HERZEGOVINA

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Abstract

Soil analysis gives insights into soil quality and identifies the nutrients required to improve it, helping to achieve higher crop yields. This paper reviews a part of chamical analyses of from Eastern part of Republic of Srpska Bosnia and Hercegovina. The soil sample were taken from ten different location (Vlasenica, Rogatica, Šekovići, Gacko, Foča, Bileća, Vojkovići, Trebinje, Nevesinje, Ilidža) from the depth of 0.30cm. The collection of the sample was done from 2018 - 2022. Analayses were preformed for the detemination of the soil pH in H_2O, pH in 1 M KCl, $CaCO_3$, Humus, total N, P_2O_5 and K_2O. The results shown fairly high pH in H_2O values (8.42 and 8.29) in Vlasenica and Bileća, and the hughest pH in 1 M KCl (7.50) in was Bileća. The higest content of $CaCO_3$ was found in Rogatica and Bileća (7.78 and 6.78), while the highest % of humus was in Trebinje and Šekovići (14.51 and 14.10). Regarding the content of N the lowest content was found in Nevesinje (0.23), as well as and the lowest content of K_2O (22.21) in the same location (Nevesinje), while the lowest content of P_2O_5 was found in Vojkovići (5.54). The obtained results could be useful to farmers as it helps optimize nutrient management, improve crop yields, and maintain soil health for sustainable farming.

Keywords: *soil properties, pH, $CaCO_3$, Humus, NPK.*

Introduction

Soil fertility is a critical factor in the agricultural productivity and sustainability of any region. Soil analysis is usually carried out to check in for soil quality, nutrient content, changes in various parameters of soil (Stupar *et al.*, 2023). Some of the most important chemical properties of the soil are pH, CaCO3, humus, and content of Nitrogen (N), Phosphorus (P) and potassium (K). Soil pH has a significant effect on the activity of the microbial communities and on the biogeochemical processes in which they participate, and it is vital in shaping microbial diversity and composition (Lauber *et al.*, 2009; Rousk *et al.*, 2010; Wang *et al.*, 2019). Microorganism activityas well as nutrients solubility and availability are some of themost important processes that depend on pH (Rodolfo *et al.*, 2018). For instance, in acid soils, most micronutrients are more available to plantsthan in neutral-alkaline soils, generally favoring plant growth (Lončarić, 2008). Calcium carbonate acts as a liming agent and is crucial for soil pH regulation. It helps neutralize soil acidity (low pH) and increase the pH level towards neutral, which can improve nutrient availability and promote better plant growth (Wan, 2018). In moderate quantities, CaCO3 is beneficial for soil structure, and is often used to neutralize acid pH in soils (Rodelo *et al.*, 2018). According to FAO (2021) when high $CaCO_3$ levels are present, this nutrient combines with other components, creating non-soluble compounds that are difficult to absorb by plants. Therefore, an excess of Ca may restrict plant availability of P, B, and Fe. The term humus is frequently substituted for SOM, since it is recognized as the “non-living, finely divided organic matter in soil, derived from

microbial decomposition of plant and animal substances” (Raphaël *et al.*, 2007; from Encyclopaedia Britannica, 1990). Humus is very rich in carbon and serves as a home for soil microorganisms and it plays a very important role in soil fertility and provides nutrients. Humus helps to retain moisture and improve soil structure (Piccolo *et al.*, 2018). Soil chemical properties such as NPK are important factors for plant growth and development (Liu *et al.*, 2021) and these elements often limit plant growth in created marshes (Stephen *et al.*, 2019). Monitoring of NPK levels is important to maintain the supply of fertilizer inthe soil for the sustainability of plant growth (Darmawan *et al.*, 2023). Nutrient deficiencies in soil–crop contexts and inappropriate managements are the important reasons for low crop productivity, reduced nutritional quality of agricultural produce and animal/human malnutrition, across the world. However, excess use of fertilizers causes environmental pollution as their residual and unused amounts will become pollutants for air, water, and soil (Rahman *et al.*, 2018; Chandini *et al.*, 2019). Therefore the agrochemical analysis of the soil are crucial for the obtaining data about the content of chemical soil properties.
In Eastern Republic of Srpska, Bosnia and Herzegovina, the diverse topography and varying climatic conditions create a unique set of challenges and opportunities for soil management. Analyzing the fertility of these soils is vital for optimizing crop production, ensuring sustainable land use, and supporting the livelihoods of local communities. Therefore, this paper compares the state of fertility based on agrochemical analyzes in the eastern part of Republic Srpska from ten different location from 2018 – 2022.

Material and Methods

Study area

The study area (Fig.1) covered ten locations (Vlasenica, Rogatica, Šekovići, Gacko, Foča, Bileća, Vojkovići, Trebinje, Nevesinje, Ilidža) of Eastern part of Republic of Srpska (Bosnia and Hercegovia). The map was created by using online mapChart tool.

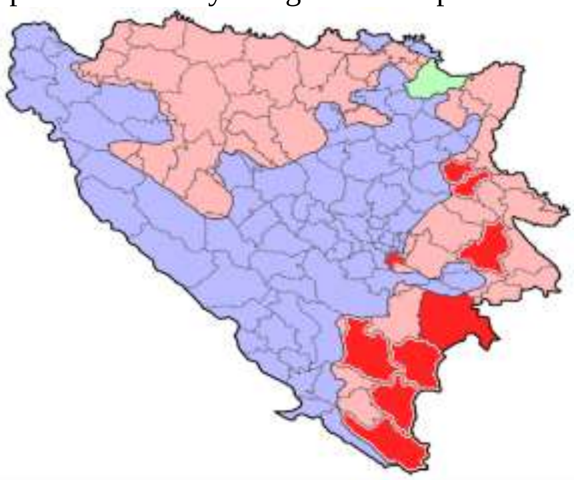

Figure 1. Study area—localization map of the 10 selected location is marked with red color.

Geographical and climate characteristics

For this study data of geographical and climate characteristics were collected for ten examinated location (Vlasenica, Rogatica, Šekovići, Gacko, Foča, Bileća, Vojkovići, Trebinje, Nevesinje, Ilidža) of Esteran part of Bosnia and Hercegoviana. Dataset of continuous monthly values for the period 2018 - 2022 covering a total of 60 months. These data include °E, °N, Altitude (m), precipitation (PRCR mm), mean air temperature (^{o}C), Köppen–Geiger (Cfb), (Table 1). The data was provided by the Federal Hydro-Meteorological Institute of BiH. In terms of altitude, the lowest WS is located in Šekovići

(360 m), while the highest is in Nevesinje (1070 m). All locations fall under the classification of a temperate warm and humid climate (Cfb x"s), except for Trebinje and Bileća which exhibits a Mediterranean climate (Csa sx“), and thus has distinctive climatic characteristics, including higher average air temperature (16 °C and 15 °C). The locatations Ilidža has a greater total precipitation (1350 mm) followed by and Trebinje and Foča (1300 mm). However, the locations differ in terms of annual precipitation totals and average air temperatures (Čadro *et al*., 2024). Precipitation ranges from 827 mm in Bileća to 1350 mm in Ilidža and average air temperatures range from 8°C in Gacko to 16°C in Trebinje.

Table 1. The basic geographical and climate characteristics of the examinated locations.

Location	°E	°N	Altitude (m)	PRCP (mm)	Tmean (^{o}C)	Köppen Geiger
Vlasenica	18°94'	44° 18'	660	1000	10	Cfb x"s
Rogatica	43°80'	19° 00'	528	1000	10	Cfb x"s
Šekovići	18° 51'	44°18'	360	1100	9	Cfb x"s
Gacko	18°32'	43°10'	950	1065	8	Cfb x"s
Foča	18°46′	43°30'	433	1300	11	Cfb x"s
Bileća	18° 43'	42°52'	468	827	15	Csa sx“
Vojkovići	18° 21'	43° 46'	600	1114	10	Cfb x"s
Trebinje	18°20'	42°42'	500	1300	16	Csa sx“
Nevesinje	18°18'	43°44'	1070	1000	9	Cfb x"s
Ilidža	18°27'	43°84'	500	1350	12	Cfb x"s

Note: °E – longitude; °N – latitude; PRCP – precipitation; Tmean – mean air temperature; Cfb x"s – temperate warm and humid climates, Csa sx – Mediterranean climate with hot, dry summers

Soil sample and laboratory analysis

For taking soil sample the next procedure was done: The surface of the soil was cleaned. Soil samples in the spring (March and April) every year were taken from a depth of 0-30 cm with several different meats in one plot. The sampling procedure was repeated on plots from other locations. All samples from one plot were then mixed in one container and a single sample was made from them. The soil sample were transferred to laboratory for the agrochemical analysis. The research was carried out in the soil fertility control laboratory of the Faculty of Agriculture of the University of East Sarajevo. The determination was done for: Active acidity (pH in H_2O), Substitution acidity (pH in 1 M KCl), content of $CaCO_3$, Humus, total N, P_2O_5 and K_2O. Soil classification were made according to JDPZ, 1966. The following methos was used for the determination of chemical properties in the investigation soil samples:

The pH in H_2O and pH in 1 M KCl was done electrometrically (inoLab pH 720, 2004) (JDPZ, 1966). Determination of $CaCO_3$ content in the soil using the Scheibler method (JDPZ, 1966). Determination of humus content, by the bichromate method according to Turin (JDPZ, 1966). Soil classification according to availability of total N (Wohtlmann classification - JDPZ 1966). Determination of P_2O_5 spectrophotometrically (Spectrophotometers, Series 7000,

2004) and K_2O photometrically (Sherwood`s Flame Photometers, 420/425, 2004) (JDPZ, 1966).

Results and Disscusion

The values obtained from the examined parameters are displayed in Table 2 while the grouping of soils according to the values of examined fertility elements are displayed in Table 3. Results from the Table 2 shows that: Vlasenica location has soil that is weakly alkaline to alkaline reaction, carbon-free, rich in humus, N and P_2O_5. The content of K_2O was very high for this filed. The resuts from the Rogatica location showed that soil is weakly alkaline reaction, carbonate, rich in humus and N. The content of P_2O_5 and K_2O was very high in this field. The location Šekovići present the soil was a neutral reaction, weakly carbonated, rich in nitrogen, well supplied with P_2O_5 and well supplied with K_2O. The pH of the soil from the Gacko location was neutral to slightly alkaline.

Table 2. The results of chemical analysis for soil fertility in the selected location

Location	pH H_2O	pH 1M KCl	$CaCO_3$ %	Humus %	N %	P_2O_5 mg/100g soil	K_2O mg/100g soil
Vlasenica	8.42	7.30	<1	4.64	0.30	>40	78.01
Rogatica	7.89	6.98	7.78	5.70	0.37	>40	83.33
Šekovići	7.94	7.07	3.76	14.10	0.70	17.78	18.53
Gacko	7.64	6.76	<1	7.78	0.51	>40	98.92
Foča	7.68	6.90	<1	7.85	0.52	>40	158.66
Bileća	8.29	7.50	6.78	6.51	0.42	37.90	127.03
Vojkovići	6.5	5.20	2.56	3.94	0.26	5.54	23.98
Trebinje	7.35	7.07	< 1	14.51	0.95	> 40.00	35.36
Nevesinje	5.43	6.58	1.19	3.51	0.23	8.96	22.21
Ilidža	7.63	6.58	<1	6.53	0.43	5.06	57.69

The content of K_2O was very high for this filed in the same location (Gacko). For the location Foča results showed that the soil belongs to the neutral reaction, poorly carbonate, very humus soils, well supplied with N and P_2O_5, and with excessive content of K_2O. The studied soil in the location Bileća belongs to the group of neutral to slightly alkaline soils, carbonate, rich in humus and N, P_2O_5, and with very high content of K_2O. The location Vojkovići has slightly acidic to acidic soil reaction, it is medium carbonate, rich in humus and N, but poor in P_2O_5 and optimally provided with K_2O. The soil from the location Trebinje belongs to neutral reaction, free carbonate, very rich in rich in humus, N, P_2O_5 and K_2O. The location Nevesinje has soil that belongs to the group of acidic soils with a low $CaCO_3$ content, but with a high humus and N content, an optimal level of K_2O and a low level of P_2O_5. The studied from the location Ilidža belongs to the group of neutral to slightly alkaline soils, carbon-free, rich in humus and N and K_2O, but poor in P_2O_5.

Table 3. Grouping of soils according to the values of examined fertility elements

Elements of fertility	pH (H_2O)	pH (KCl)	$CaCO_3$ (%)	Humus (%)	N (%)	P_2O_5 (mg 100 g^{-1})	K_2O (mg 100 g^{-1})
Distribution	< 4.5	< 4.5	0	< 1.00	>0.3	>20	>20
	4.5-5.5	4.51-5.50	0.01-2.00	1.01-3.00	0.30-0.20	10-20	10-20
	5.5-6.5	5.51-6.50	2.01-5.00	3.01-5.00	0.20-0.10	<10	<10
	6.5-7.2	6.51-7.20	5.01-10.00	5.01-10.00	0.10-0.06		
	> 7.2	7.21-8.20	>10	>10	0.06-0.03		
		>8.20			0.03-0.02		

Literature data indicates that the most commonlyused indicators for soil quality evaluation around theworld are: the pH reaction of the soil and the quantity of organic materia (Boshevska and Jankuloski) following by available nitrogen, phosphorus and potassium (Bunemann *et al*., 2018). Acid pH reaction was found in Vojkovići and Nevesinje, alkale in Vlasenica and Bileća and netural in Rogatica,Šekovići, Gacko, Foča, Trebinje and Ilidža. The acidic pH in Vojkovići and Nevesinje is likely a result of a combination of factors including the high rainfall and leaching, decomposition of organic matter, use of acid-forming fertilizers, and local vegetation types. Alkale pH could be in the soil that contain high content of $CaCO_3$ (Bileća) or other alkaline minerals such as sodium carbonate (soda ash) or magnesium carbonate (since in Vlasenica $CaCO_3$ is <1). The lowest content of humus was found in location Nevesinje (3.94) and Vojkovići (3.94). One of the possible reason is lower pH values as well as content of N and K_2O.Studies shown that pH affects microbial activity and the stability of humus (Aciego and Brookes, 2008), N is essential for microbial activity (Michael *et al*., 2000), and K supports microbial health and activity (Pramod *et al*., 2019). Content of calcium carbonate ($CaCO_3$) in agricultural soils depends on several factors, including soil pH, crop requirements, and local agricultural practices. (Wan, 2018). Generally, the aim is to achieve a soil pH that is optimal for the specific crops being grown, usually between 6.0 and 7.0 for most crops. In our study the location Bileća had the highest content of $CaCO_3$ (6.78). It can be due to the pH. High content of $CaCO_3$ contributes to the high pH and alkalinity of the soil. $CaCO_3$ acts as a liming agent, increasing soil pH and creating an alkaline environment. However, over-application of $CaCO_3$ can lead to soil alkalinity, which may negatively impact certain crops and soil health. The locations Vojkovići, Nevesinje and Ilidža have poor level of P_2O_5. The availability of phosphorus in the soil is influenced by a number of factors (Johan *et al*., 2021). The pH value has a big influence (Whitelaw 2000; Hocking, 2001). At lower pH values, phosphorus solubility decreases due to increased ionization of hydroxides and active groups of Al and Fe silicates (Gypser *et al*., 2021). It is desirable to apply an adequate fertilizer that, by injecting into the rhizosphere layer, will directly increase the content of easily accessible phosphates and create a more suitable environment for the development of plant cultures. The soils of the Foča and Bileća are rich in potassium content, which is the result of the richness of the parent substrate with this element. Inadequate application of mineral fertilizers, which does not take into account the presence of potassium inthe soil, led to a significant increase in the content of this element (Stupar *et al*., 2024).

Conclusion

Agrochemical analysis of the soil is crucial for farmers as it provides important information about the nutrient content, pH and overall fertility of the soil. By monitoring soil fertility, we have obtained results that reflect the state of the productive capacity of the soil in the eastern part of the Republic of Srpska (Bosnia and Hecegovina). From our study, it can be concluded that the locations Vojkovići, Nevesinje, Ilidža need an additional supply of fertilizers P_2O_5, and for the locations Foča, Bileća the application of K_2O fertilizers should be avoid. The rest of the investigated sites (Vlasenica, Rogatica, Šekovići, Gacko, Trebinje) contain optimal amounts of nutrients. The results of the presented study met expectations, as they clearly show the current state of soil fertility in the selected area. However, in order to gain a better insight into soil fertility in the selected area, further research is needed at more sites to provide more accurate information.

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ASSESSMENT OF POTENTIAL TOXIC ELEMENTS IN SOILS OF THREE URBAN AREAS AROUND VOLOS (GREECE) INDUSTRIAL ZONE

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Abstract

Over the last few years, the urban zone around the city of Volos is facing threats from PTEs contamination caused by the industrialization. The scope of this study is to determine the contamination levels of 10 Potential Toxic Elements (PTEs) in three urban areas which are located near the Industrial zone in the city of Volos. For this purpose, a total of 30 soil samples from parks, playgrounds and roadside were collected from Agios Georgios, Velestino and Rizomilos areas (central Greece). The sampling was conducted in June 2022 and the concentrations of chromium (Cr), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), Cobalt (Co) and zinc (Zn) were measured. Furthermore, the level of soil pollution was assessed by using Contamination Factor (CF) and Pollution Load Index (PLI). According to the results, Fe, Mn and Cr elements revealed the highest concentrations. Specifically, in Agios Georgios area the mean concentration of PTEs was the following: Fe>Mn>Cr>Ni>Zn>Cu>As>Co>Pb>Cd, in Velestino area the order was Fe>Mn>Cr>Zn>Ni>As>Pb>Cu>Co>Cd and in Rizomilos the concentration of the studied elements was Fe>Mn>Cr>Ni>Zn>Cu>Co>Pb>As>Cd. Moreover, the measurement of the Contamination Factor indicated that most of the studied soil samples belong to the low and moderate degree of contamination. The Pollution Load Index (PLI) showed that all the three urban areas are moderately polluted. Through this research a more in-depth study should be conducted in the urban areas around the industrial zone of Volos.

Keywords: *toxic elements, contamination, urban areas, industrial, Greece.*

Introduction

The presence of Potential Toxic Elements (PTEs) in soils originated from both natural and anthropogenic sources (Yu et al., 2021). PTEs are recognized as harmful when they remain in local ecosystems for extended periods (Jing et al., 2018). The anthropogenic inputs of PTEs include use of agricultural practices, mining operations, waste disposal and industrial activities (Cachara et al., 2013). The steel industry is a major contributor to the release of PTEs through the emission of pollutants in the form of dust and gases (khudhur et al., 2018). Although steel production is economically important, it also poses a significant threat to the environment. The process generates substantial quantities of dust which can be transported over wide areas by wind and rain and ultimately settling and accumulating in soils (Birat, 2020). The contamination of urban soils close to steel industrial plants by PTEs is a growing global concern. This soil is subject to elevated levels of pollutants due to emissions and waste from steel production. Over time, PTEs can accumulate in the soil, increasing the risk of their transfer to local water sources, crops and ultimately to the food chain. This situation not only degrades the quality of soil and harms ecosystems but also poses significant health risks to nearby residents (Igalavithana et al., 2015; Tardani et al., 2023; Nieder and Benbi, 2024. Human exposure to PTEs through skin contact, ingestion and inhalation can result in various medical problems (Tardani et al., 2023). The Magnesia is a Prefecture with approximately 200.000 inhabitants. In the area around the city of Volos, the capital of Magnesia, several industrials existed such as the steel and cement factories. The aim of present work to determine the levels of PTEs in soils of parks, playgrounds and roadside in three urban areas, located near to the industrial area of city of Volos (Central Greece).

Material and Methods

The research was carried out in three urban areas named Agios Georgios, Velestino and Rizomilos These areas are in a distance of 6.5, 7.0 and 10 kilometers of the industrial area of the city of Volos (Central Creece), respectively. The whole area characterized by Mediterranean climate with hot, dry summers and cool, humid winters.
A total of thirty surface (0-10 cm) were collected from the three studied surrounding areas of steel industry which located in prefecture of Magnesia. The soil samples (10 samples of each area), consisting of three different sub-samples each were collected from parks, playgrounds and roadside from the studied urban areas in June 2022 (Figure 1).

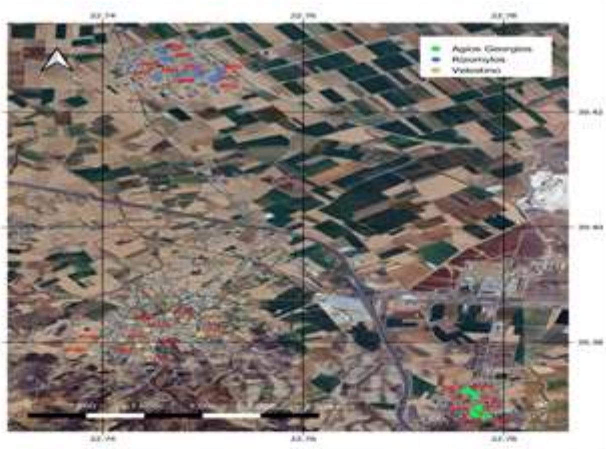

Figure 1. Location map of the samples collected from the three urban areas.

The samples, before analyzed, were air-dried in 60^{o}C until constant weight, sieved through a 63 μm sieve and placed in plastic containers. Ten PTEs were analyzed: chromium (Cr), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), Cobalt (Co) and zinc (Zn). The determination of the concentration of 10 PTEs was carried out by digestion with HNO_3 and H_2O_2 and their concentration was performed using ICP-MS.
The Contamination Factor (CF) (Hakanson, 1980) and Pollution Load Index (PLI) (Tomlinson et al., 1980) were assessed using the following equations:

$$CF = C_{sample} / C_{Background} \qquad (1)$$

where C_{sample} is the concentration of the PTE in soil and $C_{Background}$ is the concentration of PTE background (mg kg^{-1}, the values derived from Kabatas-Pendias, 2011).

This index classifies as follows: class I: low contamination (CF < 1), class II: moderate contamination (1 ≤CF < 3), class III: significant contamination (3 ≤ CF < 6) and class IV: very high contamination (CF ≥ 6).

$PLI = (CF_1 \text{ X } CF_2 \text{ X} \ldots\ldots\ldots \text{X } CF_n)^{1/n}$ (2)

where CF is the contamination factor, CF_n is the contamination factor for nth element and n is the number of the studied PTEs.

The values of Pollution Load Index (PLI) are: PLI < 0.7 = unpolluted; 0.7 < PLI < 1 = slightly polluted; 1 < PLI < 2 = moderately polluted; 2 < PLI < 3 = severely polluted; PLI > 3 = heavily polluted.

Results and Discussion

The maximum, minimum and mean concentrations of the measured potentially toxic elements (PTE) in the studied urban areas are presented in Table 1. The highest mean content of chromium (Cr) (102.1 mg kg^{-1}), manganese (Mn) (610.3 mg kg^{-1}), iron (Fe) (21797,7 mg kg^{-1}), Cobalt (Co) (15.4 mg kg^{-1}), nickel (Ni) (66.8 mg kg^{-1}), copper (Cu) (24.1 mg kg^{-1}), arsenic (As) (19.7 mg kg^{-1}) and lead (Pb) (14.0 mg kg^{-1}) was measured in Agios Georgios urban area and content of zinc (Zn) (62.6 mg kg^{-1}) and cadmium (Cd) (0.276 mg $kg^{-}1$) in Velestino. In Agios Georgios five of the ten mean concentration of potentially toxic elements (PTE) were elevated beyond the background levels: for Cr was 102.1 mg kg^{-1} vs background level 59.5 mg kg^{-1}, for Mn was 610.3 vs 480, for Co was 15.4 vs 11.3, for Ni 66.8 vs 29 and for As 19.7 vs 6.83. In Velestino area three of ten PTE was higher compared to the background levels: Cr was 75.9 mg kg^{-1} vs background level 59.5 mg kg^{-1}, for Ni 47.3 vs 29 and for As 13.3 vs 6.83. Additionally, in Rizomilos three of the ten measured PTE had higher content in comparison to background levels: for Cr was 93.0 mg kg^{-1} vs background level 59.5 mg kg^{-1}, for Ni was 65.9 vs 29 and for As was 7.6 vs 6.83.

Table 1. Maximum, minimum and mean pseudo – total concentrations of the 10 studied potentially toxic elements (mg kg^{-1}) in the three urban areas.

		Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Pb
						mg kg^{-1}					
Soil	**Maximum**	139.6	954.2	32111.2	21.5	92.6	39.7	76.8	38.0	0.432	17.3
(Agios Georgios)	**Minimum**	48.7	345.6	19532.6	9.0	47.6	10.2	40.2	7.3	0.044	9.5
	Mean	102.1	610.3	21797.7	15.4	66.8	24.1	54.0	19.7	0.234	14.0
Soil	**Maximum**	88.8	714.2	25780.7	11.8	56.7	22.0	90.5	43.5	0.522	28.6
(Velestino)	**Minimum**	62.4	275.4	10229.2	6.9	32.1	12.1	25.4	4.2	0.132	7.6
	Mean	75.9	387.9	18201.8	9.4	47.3	18.0	62.6	13.3	0.276	13.2
Soil	**Maximum**	151.1	699.5	21598.2	15.8	120.8	23.0	81.8	10.5	0.538	15.3
(Rizomilos)	**Minimum**	80.3	262.7	12761.0	7.0	26.1	9.4	32.7	3.2	0.070	8.3
	Mean	93.0	445.6	18090.0	11.0	65.9	15.6	56.7	7.6	0.241	10.6
EU		**-**	**-**	**-**	**-**	**75**	**140**	**300**	**-**	**3**	**300**
BG		**59.5**	**480**	**-**	**11.3**	**29**	**38.9**	**70**	**6.83**	**0.41**	**27**

EU=Limits according to the European Union Directive (CEC, 1986)
BG=Background as reported by Kabata-Pendias (2011)

The maximum, minimum and mean values of Contamination Factor (CF) of the studied potentially toxic elements (PTE) in the three urban areas is illustrated in Table 2 as follows: Cr (0.82-2.54), Mn (0.55-1.61), Co (0.55-1.05), Ni (0.90-4.17), Cu (0.24-1.02), Zn (0.36-1.29), As (0.47-6.37), Cd (0.11-1.31) and Pb (0.31-1.06). Among the measured content of PTE, 27 values indicated low degree of contamination (CF < 1), 24 values showed moderate degree of contamination (1 ≤CF < 3), 2 significant contamination (3 ≤ CF < 6) and 1 very high contamination (CF≥6).

Table 2. Maximum, minimum and mean values of Contamination Factor (CF) of the potentially toxic elements in the three urban areas.

		Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb
						mg kg^{-1}				
Soil (Agios Georgios)	**Maximum**	2.35	1.61	1.90	2.84	1.02	1.10	5.01	1.05	0.64
	Minimum	0.82	0.57	0.80	1.64	0.26	0.57	1.07	0.11	0.35
	Mean	**1.70**	**1.00**	**1.40**	**2.30**	**0.60**	**0.80**	**2.90**	**0.60**	**0.50**
Soil (Velestino)	**Maximum**	1.49	1.49	1.05	1.96	0.57	1.29	6.37	1.27	1.06
	Minimum	1.05	0.61	0.61	1.11	0.31	0.36	0.61	0.32	0.31
	Mean	**1.28**	**0.81**	**0.83**	**1.63**	**0.46**	**0.89**	**1.95**	**0.67**	**0.49**
Soil (Rizomilos)	**Maximum**	2.54	1.46	1.40	4.17	0.59	1.17	1.54	1.31	0.31
	Minimum	0.94	0.55	0.62	0.90	0.24	0.47	0.47	0.20	0.57
	Mean	**1.56**	**0.93**	**0.97**	**2.27**	**0.40**	**0.81**	**1.11**	**0.59**	**0.39**

Legend				
CF	**Class I CF<1**	**Class II 1≤CF<3**	**Class III 3≤CF<6**	**Class IV CF≥6**

The Pollution Load Index (PLI) for the three studied areas presented in Table 3. The values of PLI ranged from 1.276 to 1.315, suggesting moderately polluted for the areas. Although all regions belong to the second class of PLI (moderately polluted), Agios Georgios area is more polluted according to the results in Table 3.

Table 3. Pollution Load Index (PLI) for the three urban areas.

Area	PLI			Index	
Agios Georgios	1.315			moderately polluted	
Velestino	1.276			moderately polluted	
Rizomilos	1.277			moderately polluted	
Legend					
PLI	**Indice I PLI < 0.7**	**Indice II 0.7 < PLI < 1**	**Indice III 1 < PLI < 2**	**Indice IV 2 < PLI < 3**	**Indice V PLI > 3**

Conclusion

In this study the determination of the content of 10 PTEs from parks, playgrounds and roadside from Agios Georgios, Velestino and Rizomilos areas (central Greece) was conducted. From the measured PTEs, Fe, Mn and Cr elements revealed the highest concentrations. The Pollution Load index indicated that all three urban areas are moderately polluted. Based on these findings, further in – depth research is recommended for the urban regions surrounding the industrial zone of Volos.

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CIRCULAR ECONOMY MODEL IN AQUACULTURE SECTOR IN LATVIA

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Abstract

The development of aquaculture in Latvia is a topical issue affecting both consumers and fish farmers, and many Latvian ponds which would like to apply new products to aquaculture, as well as improve the situation of aquaculture in Latvia as a whole. The production of fish and possibly certain niche products for the internal and external market is an important potential for aquaculture in Latvia. Nowadays, more people want to live more in environmentally friendly surrounding with consume quality redecoration. Around 30% of the world fish stocks are fished unsustainably. This means that aquaculture needs to develop sustainability, as food consumption, including fish, will also increase as the population grows. It is necessary to carry out research in various directions, from growing to research on the environmental and economic aspects the aquaculture sector in Latvia. Currently, the aquaculture industry faces growing environmental challenges and support is needed to develop a sustainable management approach, including research into the production of economically viable and environmentally friendly nutrients. Many different sustainable production approaches have been developed to meet industrial requirements while meeting environmental protection measures at the same time. Currently, one of the most applied models is the “circular economy,” which is defined as "an economic system that replaces the concept of “end of life” with material reduction, alternative reuse, recycling and regeneration in production/distribution and consumption processes, with the aim of achieving sustainable development, thereby creating environmental quality, economic well-being and social equality for the benefit of current and future generations”.

Key words: *aquaculture, circular economy, by-products, fish farmers.*

Introduction

Aquaculture can be considered as an agricultural sector by the nature of the production process, since the methods used to propagate and reproduce fish are similar to those used to reproduce livestock (Subasinghe et.al.,2009). In order to successfully develop the aquaculture sector in Latvia, it is necessary to carry out research in various directions, from growing to research on the environmental and economic aspects of aquaculture. Currently, the aquaculture industry faces growing environmental challenges and support is needed to develop a sustainable management approach, including research into the production of economically viable and environmentally friendly nutrients. In the aquaculture production process, the final product is fish which, when processed into the food industry processes, leaves behind waste fish which can be used effectively in the production of fishmeal and oil by further application and production of feed from farmed animals or aquaculture animals. This is very important in the context of the bioeconomy and the circular economy, as residues of raw material (fish) from production are efficiently used without leaving waste behind, which in turn reduces the environmental footprint in nature. Aquaculture is fully part of the bioeconomy sector as a whole. According to the Strategic Association for Bioeconomy Research, Latvia is one of the most bioresource-rich countries in EU and has several advantages in the development of bioeconomy compared to other European countries. Today,

many different sustainable production approaches have been developed to meet industrial requirements while meeting environmental protection measures. Currently, one of the most applied models is the “circular economy,” which is defined as "an economic system that replaces the concept of “end of life” with material reduction, alternative reuse, recycling and regeneration in production/distribution and consumption processes, with the aim of achieving sustainable development, thereby creating environmental quality, economic well-being and social equality for the benefit of current and future generations. It comes from new business models and responsible consumers "(Kirchherr, Reiki and Hekkert, 2017). This concept was recently updated to include the term “bioeconomy,” which in itself does not include circulation and efficiency (D' Adamo, Falcone and Morone, 2020). The European Commission defined it as follows: “the bio-economy covers all primary manufacturing, economic and industrial sectors that use, produce or process biological resources (agriculture, forestry, fisheries and aquaculture) to produce food, feed, bio-products, energy and services.”The circular economy and the bio-economy converge on a number of points, in particular the use of biological resources, and in particular where this biomass is a by-product, which is an investment in another industry. These two strategies therefore tend to overlap as they are both based on the use of biological resources and have strong synergies (D' Adamo, etc., 2020; Kardung, etc., 2021).
The aim of the present paper is to provide an overview of circular economy importance in aquaculture.

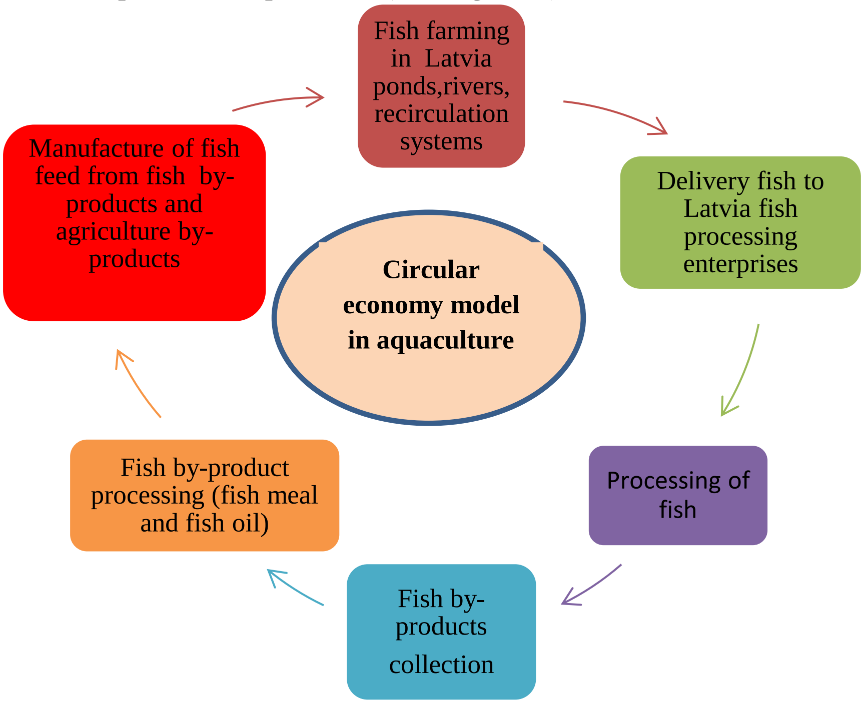
Material and Methods

In this research was used European Commision Bioeconomy strategy, Action programme for fisheries 2021-2027. Analysis synthesis, the logical construction method was used for aquaculture model implementation in Latvia, based on the operating principles of aquaculture enterprises (2024), the framework of the Fisheries Action program 2021-2027 (https://www.zm.gov.lv/lv/programma-zivsaimniecibas-attistibai-2021-2027gadama) and the principles of the circular economy strategy for Latvia (https://www.varam.gov.lv/sites/varam/files/content/files/varamzin_200120_aestrategija.pdf). Scientific literature review was used as well. In this research was analysed the main digitalisation support programmes in Latvia, which are implemented by the European Maritime and Fisheries Fund (https://oceans-and-fisheries.ec.europa.eu/funding/european-maritime-and-fisheries-fund-emff_en), the Investment and support Agency of Latvia (https://www.liaa.gov.lv/en), Rural support Service(https://www.lad.gov.lv/en) and the European Regional Development Fund(https://ec.europa.eu/regional_policy/funding/erdf_en).

Results and Discussion

Many different sustainable production approaches have been developed to meet industrial requirements while meeting environmental protection measures. Currently, one of the most applied models is the “circular economy,” which is defined as "an economic system that replaces the concept of “end of life” with material reduction, alternative reuse, recycling and regeneration in production/distribution and consumption processes, with the aim of achieving sustainable development, thereby creating environmental quality, economic well-being and social equality for the benefit of current and future generations. It comes from new business models and responsible consumers "(Kirchherr, Reiki and Hekkert, 2017). This concept was recently updated to include the term “bioeconomy,” which in itself does not include circulation and efficiency (D' Adamo, Falcone and Morone, 2020). The European Commission defined it as follows: *“the bio-economy covers all primary manufacturing, economic and industrial sectors that use, produce or process biological resources*

(agriculture, forestry, fisheries and aquaculture) to produce food, feed, bio-products, energy and services.”
The circular economy and the bio-economy converge on a number of points, in particular the use of biological resources, and in particular where this biomass is a by-product, which is an investment in another industry. These two strategies therefore tend to overlap as they are both based on the use of biological resources and have strong synergies (D' Adamo, etc., 2020; Kardung, etc., 2021). In fact, in recent years, the European Commission and several industrial associations have used the term “circular bioeconomy” and support their integration (Kardung u.c, 2021).
The circular economy aims at reducing pollution through the use of less raw materials, generating less waste and CO2, longer use of resources/products, ensuring the re-use of resources where waste from one process becomes another for process raw materials, or/and natural product renewal. Aquaculture production also uses a number of basic principles used in the lifecycle of fish production products (see Figure 1).



***Source: Author’s elaboration**

Figure 1. Circular economy model in aquaculture in Latvia

In a circular economy, the value of products and materials is maintained for as long as possible, waste generation and resource use are reduced, and once a product reaches the end of its life cycle, resources remain in an economy where they are used over and over again to generate added value. This model can help create permanent jobs in the aquaculture sector in Latvia, promote innovations that offer competitive advantages, and ensure a good level of protection for people and the environment, while also providing consumers with longer-to-use and innovative products that help save money and improve quality of life. There are 6 areas of activity are identified for aquaculture in the circular economy:
1) Regeneration - the use of materials (fish and marine resources) is applied in aquaculture enterprises. for the preservation and restoration of coastal and inland aquatic ecosystems.
2) The scope of division is also used in aquaculture - provision of services of fishery producer organisations and associations to members of organisations.
3) Optimisation is also widely used - aquaculture enterprises improve their activities in the production of existing products (fish species).

4) Internal circulation – re-use of by-products of fish processing for the manufacture of animal feed.
5) Virtualization - provision of online purchase possibilities for manufactured products - aquaculture enterprises also offer to purchase fish by ordering them through the website of the undertaking and then supply them to customers.
6) Exchange in aquaculture enterprises – commencement of production of a new fish species, trials, experiments, etc.

The bioeconomy and the circular economy have contact points. The concept of circular bioeconomy (circular bioeconomy) is used in research literature. The bio-economy uses the principles of the circular economy, whereas the circular economy uses biobased products. The circular economy principles can be applied to all sectors, but in different ways to each. Aquaculture as a bioeconomy sector is no exception in this context. The circular economy is more to do with environmental conservation and restoration as well as waste recycling, while aquaculture, like any manufacturing industry, is to be associated as a waste producer. Aquaculture has grown significantly in recent decades to meet global demand for animal protein. However, this current production model may become unsustainable. This sector needs large volumes of water. Waste decomposition means direct environmental impacts that can be reduced by their continued use (Dauda, Ajadi, Tola-Fabunmi and Akinwole, 2019).

This legal framework and concept also applies to aquaculture. Therefore, according to the above definition, aquaculture, like any other production system that requires the use of inputs to produce a product, produces secondary unconsumed inputs in the form of waste or by-products (Dauda et al., 2019). Concerns about aquaculture waste and by-products are reflected in the growing body of scientific work in this field (Morris, Backeljau, & Chapelle, 2019). Various quantifications, definitions and classifications of aquaculture products, wastes and by-products are provided in the literature. Quantitatively, the proportions of primary products, by-products and wastes generated in aquaculture depend on the type chosen. Primary products obtained by processing methods achieve low to medium efficiency in fish.

An estimated 45% of fish raised in aquaculture facilities is converted directly, with the remaining 55% considered a by-product. For finfish, this by-product percentage can mainly be explained by the sum of the percentages of the head and skeleton, which includes the bony skeleton and associated meat, 20%; viscera, 12.5% (consisting mainly of intestines); skin and Abdominal skin flaps accounted for a total of 5%, while skin fragments and blood accounted for 4% (Stevens, Newton, Tlusty, & Little, 2018).

In order to reduce the environmental impact of the aquaculture sector, different solutions can be developed, such as the use of green sources for energy supply and the application of circular bioeconomy strategies. Waste is a sustainable source of biomolecules with potential applications in cosmetics, pharmacology, agriculture (such as fertilisers or biodiesel) or the development of an aquaculture system. All these approaches would reassess aquaculture waste and by-products. The introduction of a circular bioeconomy model will ensure more sustainable production systems and allow for a responsible aquaculture certification and labelling programme (Osmundsen, etc., 2020).

In Latvia, support programmes for fisheries are also important for the importance of aquaculture, which are designed to enable fish processing and aquaculture enterprises to become more developed by introducing different production facilities, freezers for fish, modernising and arranging fishing vessels, establishing a single fishery system, as well as developing aquaculture enterprises and promoting diversity of fish species. The role of public support, which can ensure a balanced transition in order to achieve the objectives set, is important for the sector to be sustainable (Staffas et.al., 2017).

In Latvia, the main digitalisation support programmes are implemented by the European Maritime and Fisheries Fund, the Investment and support Agency of Latvia, Rural support

Service and the European Regional Development Fund. Support is provided both for the purchase of new production facilities, for the modernisation of fishing vessels and their berths, for the installation of freezers, and even for the construction and establishment of new fish processing plants. Aquaculture, on the other hand, supports the equipping of ponds with sensors, the expansion of ponds, the creation of new ponds and the diversification of fish species. Thanks to the financing of EU funds, it is possible to develop and modernise Latvian fish processing enterprises in order to make them more competitive not only in Latvia, but also in Europe and the world.

Conclusions

Aquaculture continues to grow faster than other major food production sectors, and with most fisheries resources expected to be unsustainably harvested or overfished for at least the next decade, aquaculture must fill the aquatic food supply with the demands of a growing and affluent world The growing gap between populations with high demand for animal protein, Aquaculture fulfils the functions of political (support for sustainability of aquaculture, promotion of the use of aquaculture by-products), economics (food security, reduction of expenditure on food purchases, income generation, poverty reduction), social (social cohesion development, promotion of public health and education, maintenance and preservation of values and traditions), technology (promotion of technological innovation in aquaculture enterprises) and environment (preservation and improvement of the environment).

Acknowledgements

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RESEARCH OF ARSENIC (As) CONTENT IN AGRICULTURAL LAND

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Abstract

Arsenic is a solid, brittle nonmetal, gray in color, with a metallic luster. It is found in the soil in the form of various minerals, it appears as trivalent and pentavalent, and mostly in the form of oxide, As_2O_3. This element has been used for years to make insecticides, herbicides, wood preservatives, etc. Plants do not accumulate it to a greater extent, even with its greater presence in the soil. The presence of arsenic in soil originates from different materials. These can be agricultural materials such as pesticides, desiccants, phosphorus fertilizers, manure, etc. Using agrochemical analysis, Agriculture Extension Service “Sombor” from Serbia, examined the percentage representation of the agricultural land samples in relation to the arsenic content in the layer from 0 to 30 cm. Determination of arsenic content present in the soil in exposing the soil to concentrated nitric acid (cc NHO_3) with the addition of hydrogen peroxide (H_2O_2), and then a reading is made on an atomic absorption spectrophotometer (AAS). According to the analyes of agricultural land in the area of the city of Sombor regarding the content of arsenic, it was determined that all the tested samples contained arsenic and that its content in the layer from 0 to 30 cm ranged from 2.91 mg/kg to 18.02 mg/kg.

Keywords: *arsenic, soil, content, concentration, dose.*

Introduction

Arsenic is a solid, brittle nonmetal, gray in color, with a metallic luster. It is found in the soil in the form of various minerals, it appears as trivalent and pentavalent, and mostly in the form of oxide, As2O3. This element has been used for years to make insecticides, herbicides, wood preservatives, etc. In some places, organic arsenic compounds are used as stimulants, as well as in medicine for making some medicines (Kabata-Pendias,2011).

Plants do not accumulate it to a greater extent, even with its greater presence in the soil. On the surface of fruit that has been sprayed with arsenic-based pesticides, there may be more of this element than 0.5 μg/g, but these residues are easily removed by washing. However, the presence of arsenic in seawater significantly increases its content in fish, shellfish, oysters, etc. (Kastori, 1997).

After lead, arsenic represents the greatest toxicological risk for domestic animals. Inorganic arsenic is quickly resorbed through the gastro-intestinal tract (with food) and is highly toxic (Petrović,1991).Poisoning in humans can occur accidentally or intentionally.

The lethal dose for humans is 0.1 to 0.15 grams of arsenic trioxide (As2O3). It must not be more than 0.25 mg/m3 in the air. This is how the frequency of kidney, bone and colon cancer increases (Antosiewicz,1992).

The presence of arsenic in soil originates from different materials. These can be agricultural materials such as pesticides, desiccants, phosphoric fertilizers, manure, etc. Arsenic can reach the soil and from the atmosphere. The volatility of its compounds allows arsenic to be found in the air. This especially applies to areas near copper smelters, which represent as much as 40% of the total anthropogenic pollution by this element (Barcelo,1990).

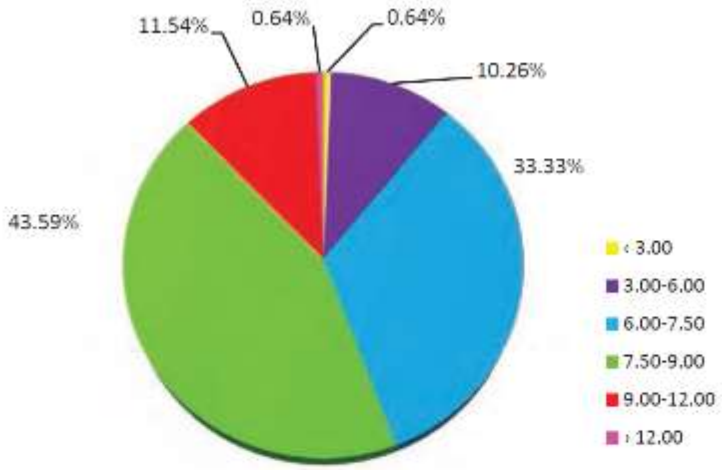
In second place is the burning of coal. Waste sludge can thus be a source of soil pollution with arsenic. Their application is possible only if they contain less than 8 mg/kg of dry sludge matter. The level of arsenic can also be increased by the use of surface or underground water to irrigate plants, if there is more of this element in them.
The European Union has accepted the regulation that the maximum allowed amount of arsenic in agricultural land can be up to 20 mg/kg. Larger content is allowed in parks, playgrounds and open spaces (Sabadoš, 2019).

Material and Methods

Determining the arsenic content in the soil consists in exposing the soil to concentrated nitric acid (cc NHO3) with the addition of hydrogen peroxide (H2O2), and then reading it on an atomic absorption spectrophotometer (AAS). The analyzes were done at the Faculty of Agriculture in Novi Sad in the laboratory for agrochemical analysis.

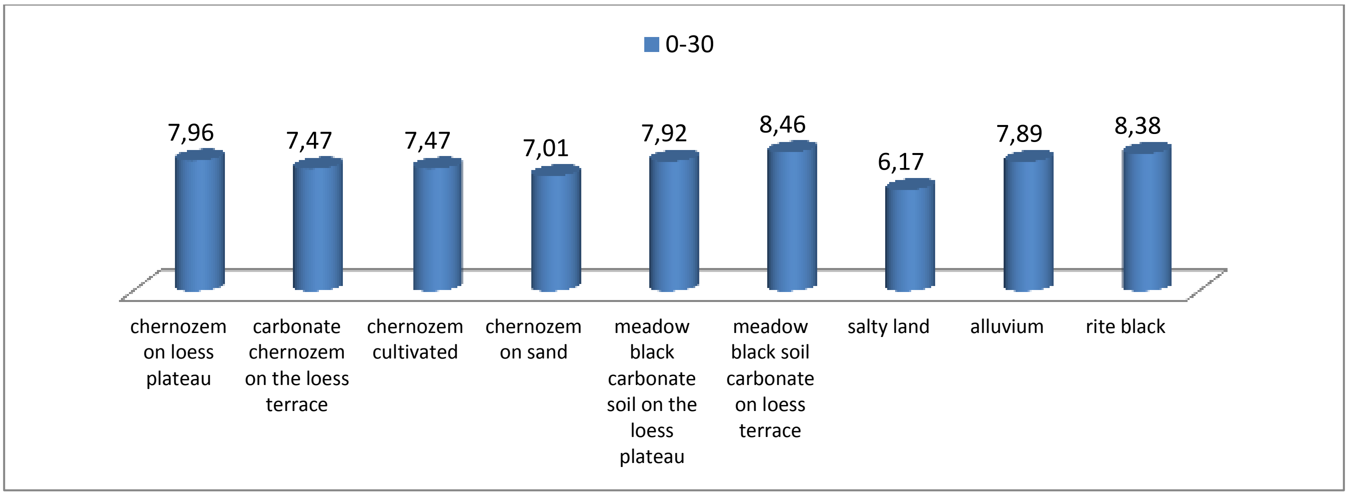
Results and Discussion

According to the analysis of agricultural land in the area of the city of Sombor regarding the content of arsenic, it was determined that all the tested samples contain arsenic and that its content in the layer from 0 to 30 cm ranges from 2.91 mg/kg (point number 1 in the atar of the inhabited place Riđica), up to 18.02 mg/kg.



Graph 1. Percentage representation of examined samples of agricultural land in relation to arsenic content in the layer from 0 to 30 cm

The highest percentage of arsenic is 43.59% and that is 7.50-9.0 values. In second place is 6.0-7.5 with 33% representation. What is satisfactory is that the larger amounts of arsenic that were found were found on only 0.64% of the surfaces.



Graph 2. Average arsenic content mg/kg by land type in the area of the city of Sombor

This graph shows the content of arsenic in mg/kg by land type in the area of the city of Sombor. The highest amounts, 8.46 mg/kg, were found on the carbonate meadow holm oak on the loess terrace, as well as the amount of 8.38 mg/kg on the mountain holm oak. The quantities belong to the permitted quantities, but stand out due to the higher amount of arsenic compared to other types of land. Some results show that compared to other types of land, the amount of arsenic is increased by 1.50 mg/kg.

Picture 1. Atar of the settlement of Riđica with the lowest content of arsenic in the soil in the area of Sombor

The picture shows the vineyard where we found the largest amount of arsenic, which can be connected to the plant protection agents used in viticulture. However, these are only assumptions, it must be investigated.

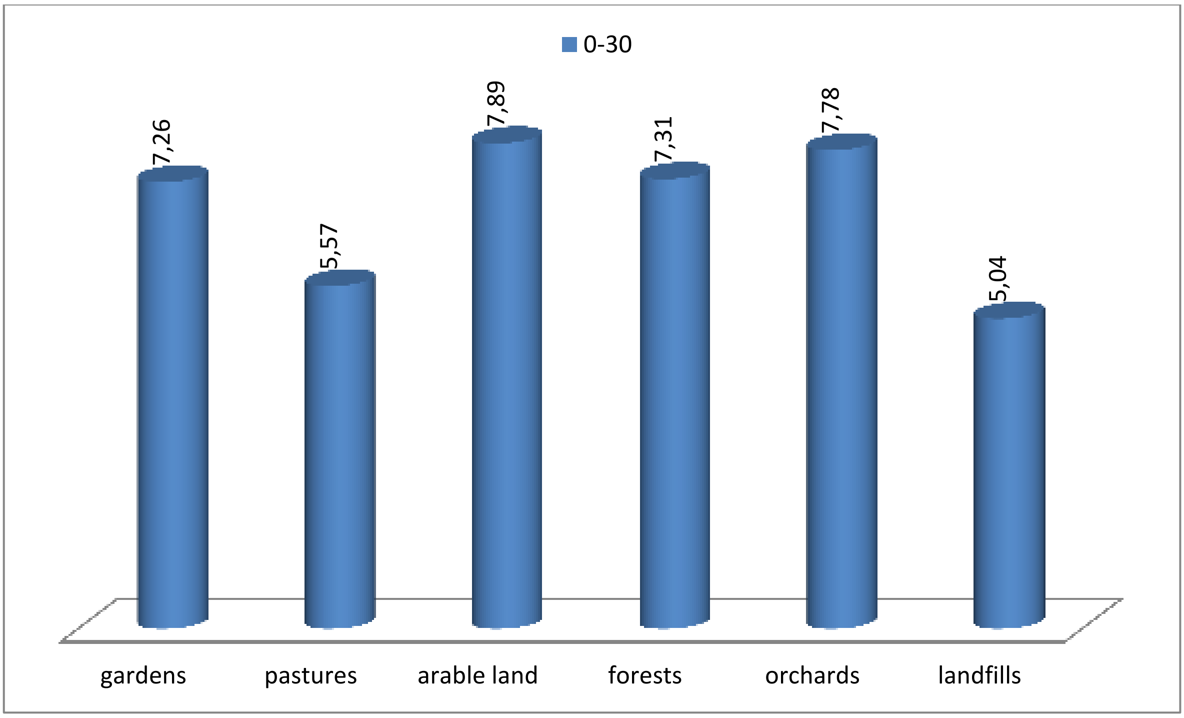
Most of the tested samples have an arsenic content in the range of 6.00 to 9.00 mg/kg, i.e. 76.92%, while none of the tested soil samples has an arsenic content higher than 20 mg/kg, which is the maximum allowed amount in soil according to European directives. of the Union. The highest average content was recorded for the land type meadow blackberry on the loess terrace (8.46 mg/kg), while the lowest average content of arsenic was on salt marshes (6.17 mg/kg).

Table 1. Arsenic content in agricultural land in the city of Sombor (by land type)

deph t	min max aver	chernozem on loess plateau on loess plateau	carbonate chernozem on the loess terrace	chern ozem culti vated	chern ozem on sand	meadow black carbonate soil on the loess plateau	meadow black soil carbonate on loess terrace	salty land	alluvium	rite black
0-30	min	6.28	5.59	4.85	2.91	6.59	6.52	3.15	5.04	7.10
0-30	max	9.46	7.47	10.4	11.1	9.07	18.02	9.16	10.25	10.79
0-30	medium	7.96	9.07	7.47	7.01	7.92	8.46	6.17	7.89	8.38

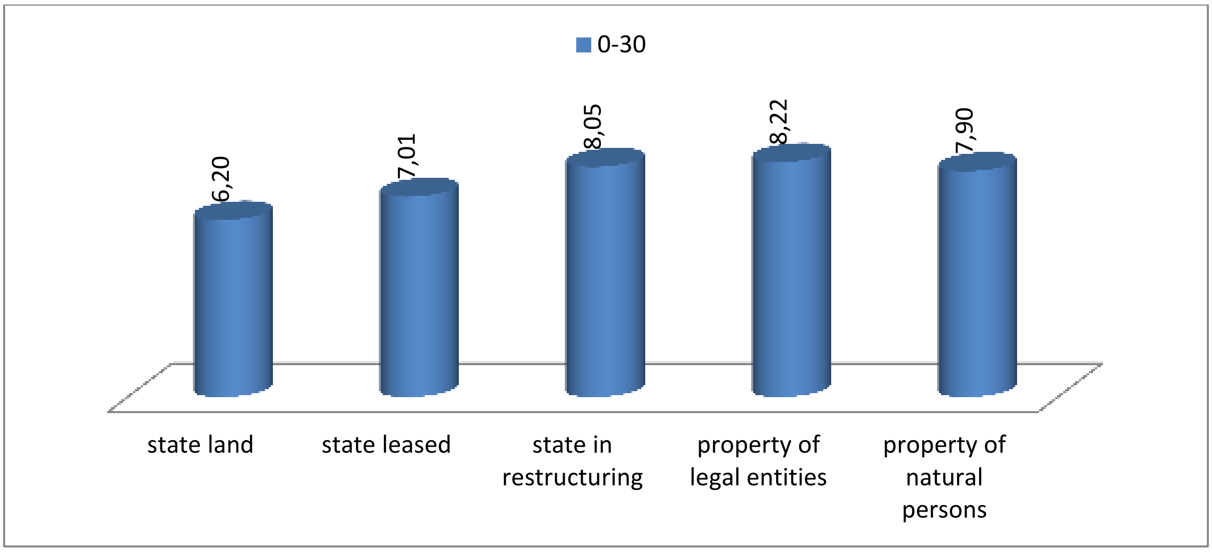
In this table we can see that the content of arsenic varies from the minimum amount of 3.15 mg/kg to 18.02 mg/kg depending on the type of land. Minimum, maximum and mean values are presented depending on the type of land.

In the land in the area of the city of Sombor, according to the method of use, the highest content of arsenic in the land is in arable land and orchards, and the lowest content is in current and former garbage dumps as well as in land used as pastures.



Graph 3. Average arsenic content (mg/kg) in the layer from 0 to 30 cm of agricultural land in the city of Sombor by land use method.

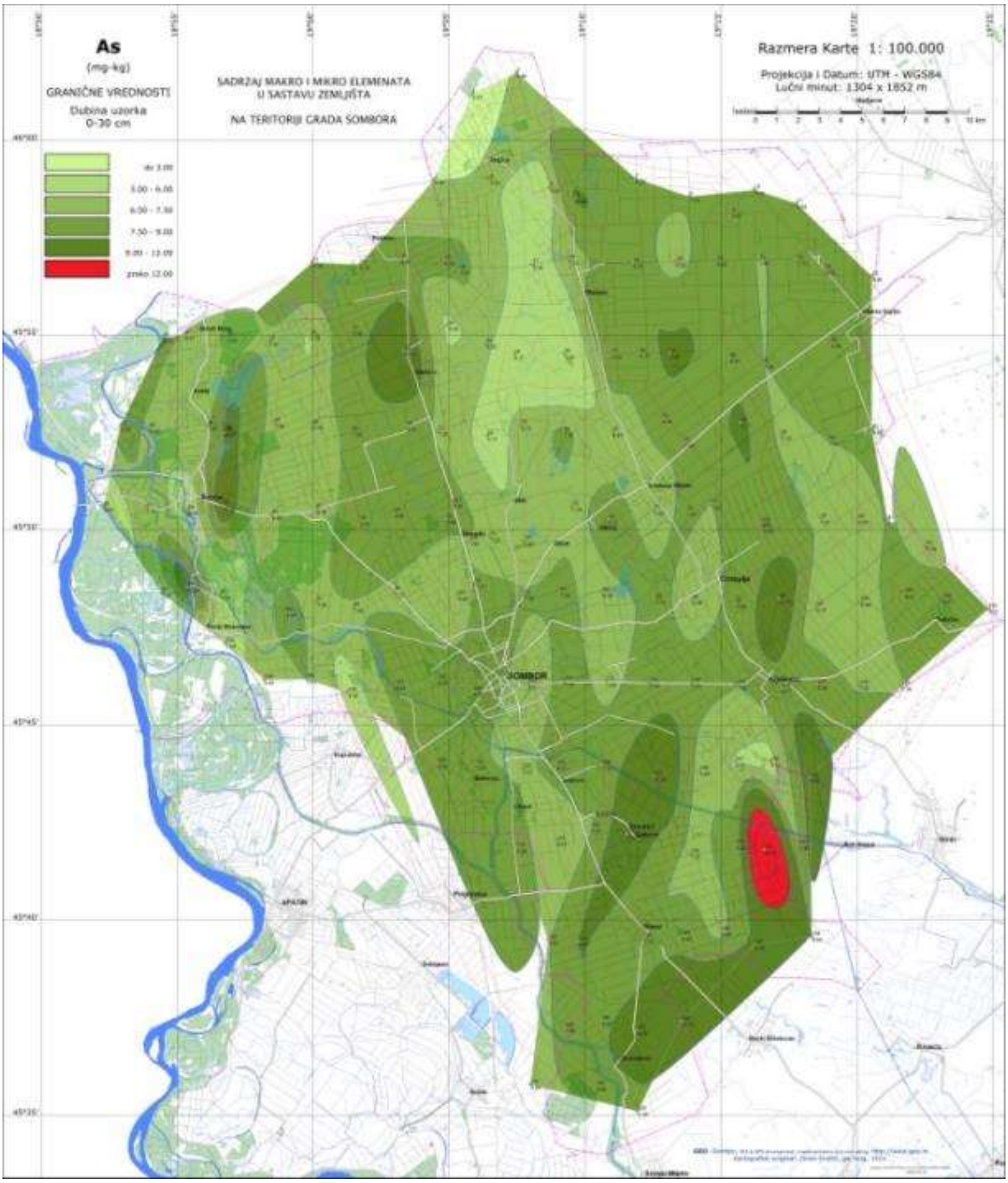
On the graph, we can see the average arsenic content (mg/kg) in the layer from 0 to 30 cm of the agricultural land of the city of Sombor by way of use, where we see that the results show the permitted amounts of arsenic in the soil. Also, among these categories, we can find that the highest amount of arsenic was found on arable land (7.89 mg/kg) and the lowest amount on pastures (5.57 mg/kg) and landfills (5.04 mg/kg).



Graph 4. Average arsenic content (mg/kg) in the layer from 0 to 30 cm of agricultural land in the area of the city of Sombor, by ownership

In the samples of agricultural land in relation to ownership, the highest content of arsenic can be noted on land owned by legal entities and cooperatives, and the lowest content on state land. On th map, we can find the arsenic content at a depht of 0-30 cm.

Map number 1. Arsenic content in the territory of the municipality of Sombor



Conclusions

Through this research, we have come to positive results, where the amount of arsenic on the agricultural land of the city of Sombor is within the permitted amounts. At less than 1%, we have an increased amount of arsenic. Larger amounts were observed on arable land, which is a consequence of the application of increased amounts of pesticides and mineral fertilizers, which if this trend continues in the future, we could have a problem with an increased amount of heavy metals in the soil.

Accumulation, and therefore the toxicity of this element, is greater on acidic soils, especially if the pH value of the soil is less than 5. On heavier soils, its toxic effect occurs less often than on sandy soils, because in the former, arsenic binds better. The sensitivity of plants to high concentrations of arsenic is different. The most sensitive species include beans, alfalfa and legumes in general, while the tolerant species are potatoes, tomatoes and carrots. In nature, the phytotoxic effect of high concentrations of arsenic or its adverse effect on plant yield can very rarely be observed. It is recommended to use pesticides correctly and use mineral fertilizers rationally.

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ANIMAL HUSBANDRY

THE EFFECTS OF HEAT STRESS ON THE EXISTENCE OF *STAPHYLOCOCCI* IN MILK PRODUCED BY DAIRY COWS

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Abstract

Mastitis is an inflammation of the mammary gland affecting the dairy cattle. This disease is prevalent in our farms at a frequency estimated at 20% of the clinical pathology in dairy cows. Mastitis causes considerable economic losses due to the decrease in quantity and the quality of milk produced. This must include the cost of therapies and reforms. The germs responsible for mastitis are very numerous; *Staphylococci* are the most important pathogens causing subclinical mastitis. This study tends to assess the heat stress experienced by lactating cows and its effect on *Staphylococcal* mastitis. Therefore, we observed 15 milking dairy cows with an average age of 3.5 years after calving. Temperature-humidity index (THI) was calculated after recording temperatures and humidity, to test the effects of three levels of THI (<68; (72-79); >80) on *Staphylococcal* mastitis. The results indicated that the number of coagulase-positive *Staphylococcus aureus* was higher during the winter and spring seasons at values of 1.03×10^2 and 1.19×10^2, respectively, compared to the summer season at value of 0.75×10^2. The result of the antibiogram showed that *Staphylococcus aureus* was resistant to Lyncomicin and oxacilin. In conclusion, heat stress causes respiratory alkalosis that has a bacteriostatic effect on *Staphylococcus aureus* growth, and the best timing for the treaty is the summer season using one of the following antibiotics (Amoxicilin, Rifampicin, Levofloxacin, Erythromicin, Chloronphennicol).

Keywords: *Antimicrobial use*, *Heat stress*, *Lactation cow*, *Mastitis*, *Staphylococcus aureus.*

Introduction

The dairy industry is a strategic sector for the economies of countries (Lahari, 2023). It is a national priority. In Algeria, as in most countries, bovine mastitis is the main disease of dairy farms. Accurate knowledge of the frequency of mastitis-causing bacteria in dairy cows is essential to determine and adapt mastitis control programs to different epidemiological situations. An average incidence of subclinical mastitis for 25 percentage of cows was identified in central Algeria (Saidi et al., 2012). *Staphylococci* and *Sreptococci* were the most commonly identified germs in subclinical mastitis (Saidi et al., 2012). *Staphylococcus aureus* is spherical in shape. It measures 1 μm of diameter, it is immobile, it produces an enzyme called coagulase and it may cause udder infection (Sinha et al., 2010). *Staphylococcus aureus* can form a biofilm that can cause a less severe form of mastitis, but it exhibits greater resistance to antibiotics. (Cucarella et al., 2004). Heat stress can affect productivity and animal welfare (Collier et al., 2006 ; Nikkhah and Alimirzaei, 2023). The thermal discomfort occurs when the temperature–humidity index (THI) exceeds 68. (Attia et al., 2022).

Bertocchi et al. (2014), showed that the temperature-humidity index (THI) correlates positively with the somatic cell score (SCS). The objective of present study was to evluate the effect of THI on *Staphylococcal* mastitis in lactating cows.

Materials and methods

This study was conducted on the farm «SARL El Asnam» level in Chlef city, Algeria, during the period of December 2022 to July 2023, on a population of 15 dairy cows of the breed Prim Holstein, Montbéliard and Fleckvieh, with an average age of 3.5 years. These cows were followed just after calving. Cows udders studies did not present any visible inflammation or clinical signs.

Three hundred samples were collected for each season, with an average of 60 samples per visit for four quarters of the 15 udder. The analyses were carried out at the laboratory of the University of Hassiba Ben bouali, ouled Fares, Chlef, Algeria. The use of test paper confirmed that the 15 cows had subclinical mastitis. The samples were taken at the four quarters of each udder just before milking. The milk is collected in a sterile test tube after disinfection of the teats, and elimination of the first jet. Milk samples are immediately placed at +4°C. The temperature and humidity measurement was performed using an Operation manual for temperature and humidity meter (HTC-1 Digital Thermometer Hygrometer Clock, Fujian, China)

Isolation and identification of *Staphylococcus aureus*

The bacterial culture was carried out on Chapman medium (Pasteur Institute, Algeria). *Staphylococcus aureus* was identified using catalase and coagulase tests,along with gram staining, (HIMedia Laboratory, India)

The diffusion method with Mueller-Hinton agar was utilized to investigate antibiotic susceptibility.

According to the Europran committee (2023) on antimicrobial susceptibility testing, the recommended dose of 8 antibiotic discs (Bio-Rad, France) for S. aureus has been set for erythromycin (15 µg), oxacillin (5 µg), gentamicin (30 µg), lincomycin (15 µg), chloramphenicol (30 µg), rifampicine (30 µg), Amoxiciline (25 µg) and levoflaxine (5 µg). Accordingly ,After 24 hours of incubation at 37°C, the inhibition diameters were measured, allowing the interpretation in categories S (sensitive), I (intermediate), R (resistant) .(EUCAST, 2023).

Statistical analysis

Data analysis was performed by SPSS program.The significant level was set at $p < 0.05$. The results of the antibiogram were expressed as percentages.

Results and discussion

It was referred to in the Table 1 that the number of *Staphylococcus* is higher during the winter and spring season at (1.03×10^2 ; 1.19×10^2), respectively than during the summer season at a value of (0.75×10^2) with $p<0.05$. Knowing that the number of *staphylococci* has reached the microbiological infective limit (10^2- 10^3) ufc/g (JORA, 2017). In a cold season, the frequency of mastitis is higher when animals are confined with inadequate housingconditions (Bouhroum et al., 2022). Moreover, the periparturient period is marked by an immune deficiency that causes the udder to be vulnerable to environmental and contagious pathogens. (Derakhshani et al., 2018).*Staphylococcus* mastitis is responsible for 80% of the infections in the herd. In subclinical mastitis, *S. aureus* and *S. agalactiae* are the most common bacteria. (Elhaig and Selim, 2015). The indirect impact of heat stress on milk *Staphylococcus* counts during summer season arises from the blood pH becoming alkaline because of pulmonary hyperventilation, a crucial apparatus for removing excess body heat.. Respiratory alkalosis is always coupled with urinary bicarbonate excretion (Bonnefoy et al., 2011). The temperature humidity index is an indicator that can be used to assess the level of heat stress in dairy cows (Bohmanova et al., 2007 ; Attia et al., 2022). Table 2 shows that when the THI has a high value of 81.66 exceeding the thermal comfort zone (severe stress) the number of *Staphylococcus* drops to a value of 0.75 x 102 cfu/g ($p < 0.05$). *Staphylococci* can not be developped due to metabolic alcalosis, as demonstrated earlier. Because the pH of their growth is in an interval of (6-7) (Mehwish et al., 2014). The isolates of *Staphylococci aureus* are sensitive to Erythromicin, Levofloxacin, Rifampicin, Chloronphenicol, gentamicin and Amoxicilin (Table 3). *Staphylococci* have developed resistance to oxacillin through the production of penicillin-binding protein (PLP), for example, PLP2a (Daurel et al., 2008). Peptidoglycan is an important structure of bacteria, it is synthesized by PLP, β-lactam is able to bind itself to PLP by covalent binding and inhibit the activity of bacteria. This bond causes a rupture of balance between lysis and peptidoglycan synthesis, bacteria become unable to resist the osmotic pressure exerted on their plasma membrane and die by osmotic lysis. A change in the antibiotic target leads to its inactivity. The acquired resistance of *Staphylococcus aureus*, is characterized by the possession of a new PLP, PLP2a by having very little affinity for β-lactams (Quincampoix et al., 2001). The resistance of *Staphylococci* can also be explained by the hyperproduction of penicillinase, an enzyme that hydrolyses β-lactamines (oxacillin, dicloxacillin, meticillin). Lincomycin antibiotics have only bacteriostatic activity on staphylococci (Quincampoix et al., 2001). The extension of antibiotic resistance leads to difficulties in the management of infections due to Staphylococci aureus in dairy cow during winter and spring seasons.

Table 1. Evolution *of Staphylococci aureus* by season in dairy cows of Chlef city, Algeria

Microbiological limits	*Staphylococcus aureus*	Season
(10^2- 10^3) ufc/g	M=1.03x 10^2	Winter T=16.9 °C H= 47.75%
	M=1.19 x 10^2	Spring T=27.62°C H=46%
	M=0.75 x 10^{2***}	Summer T=33.1*** °C H= 47

M= mean ; T= temperature ; H= humidity ; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2. Evolution of *Staphylococci* as a function of temperature–humidity index in dairy cows in Chlef city, Algeria

Season	Winter	Spring	Summer
Number Staphylococci (ufc/g)	M=1.03x 10^2	M=1.19 x 10^2	M=0.75 x 10^{2***}
Temperature humidity index	61.11	74.57	81.66***
Critical interval	<68 Thermal comfort zone	(72- 79) moderate to severe stress	> 80 severe stress

M= mean ; *$p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 3. The result of the antibiotic susceptibility of the *Staphylococcus aureus* strain in infected dairy cows in Chlef city, Algeria

Antibiotic	Inhibition diameter (mm)	Critical diameter (mm)	Result
Erythromicin	35.66	23 - 29	S
Levofloxacin	24.25	23-29	S
Lincomycin	19.2	27 - 32	R
Gentamicin	14.66	6	S
Oxacillin	30.75	30-38	I
Amoxicilin	22	14-21	S
Rifampicin	37.75	30-36	S
Chloronphenicol	28.33	>=23	S

S= sensitive, R= resistant I= intermediate

Conclusion

In conclusion, the role of thermal stress on *Staphylococcal* mastitis is bacteriostatic. Therefore the treatment of mastitis with one of the following antibiotics such as : Erythromycin, Rifampicin, Chloramphenicol, Amoxicillin, Gentamicin, and Levofloxacin during the summer season will give better results for the health of the udder in infected cows.

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MICROBIOLOGICAL SAFETY OF FEED IN 2023

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Abstract

The microbiological safety of feed directly affects the health status of animals and their production results and depends on numerous factors, the most important of which are the quality of feed, climatic conditions and the technological production process. The aim of the study was to determine the microbiological safety of feed in the year 2023 in Republic of Srpska (Bosnia and Herzegovina), during which 245 samples were tested. Samples were tested for *Salmonella* spp., coagulase positive staphylococci and *Staphylococcus aureus*, *Clostridium perfringens*, total number of microorganisms and total number of yeasts and molds, by test methods BAS EN ISO 6579-1, BAS EN ISO 6888-1, BAS EN ISO 7937, BAS EN ISO 4833-1 and BAS ISO 21527-2. Samples of feed were tested within self-control (94.29%) and official controls (5.71%). In relation to the Rulebook on microbiological criteria in feed, 20% of unsatisfactory samples were identified, of which 93.88% were from self-control and 6.12% from official controls. The highest percentage of unsatisfactory samples of feed was due to the increase in total yeasts and molds (17.55%), then due to increase in total number of microorganisms (15.51%). The presence of pathogenic microorganisms *Salmonella* spp., *Clostridium perfringens* and coagulase-positive staphylococci and *Staphylococcus aureus* was not determined in the tested samples. The cause of unsatisfactory feed samples was an increase in the number of saprophytic microorganisms, whereby this risk was constantly present and mainly led to indirect losses in livestock production.

Keywords: *feed, safety, microbiology, criteria, risk.*

Introduction

Ultimate goal with regard to microbial load in feed is not giving sterile feed but to give feed with safe contamination level. Evaluation of feed safety for microbiological contamination and establishing a safer level for allowing entry into feeding is needed (Gopi *et al.*, 2017).
In recent years, we have witnessed events at the global level: hunger in underdeveloped countries, increased demand and production of food of animal origin, consequently the increased production of feed, financial crises, wars, climate change with extreme variations and weather events (heavy rains followed by dry periods) (Golić and Nedić, 2023). All this leads to a shortage of feed on the world market, often of very poor quality, especially in terms of microbiological criteria, whereby subjects in the feed business do not have much choice when choosing raw materials for the production of feed mixtures.
When it comes to feed, contamination with yeasts and molds can occur at all stages in the food chain, it can occur before harvest and during storage. Contamination by yeasts, especially molds, carries with it, in addition to changes in the appearance, taste and quality of food, the danger of contamination with mycotoxins. Mycotoxins are products of several types of toxigenic molds. Conditions for the development of mold are not always conditions for the production of mycotoxins, but an increased number of molds is always an indication of the possible presence of mycotoxins. High temperatures and dry periods are conducive to the reproduction of molds and the formation of mycotoxins. In recent years, on the global level,

there have been significant climate disturbances, accompanied by extremely high or low temperatures, the appearance of large and extensive rains with floods and major droughts. All these influences have contributed to the increase of contamination by yeasts and molds, primarily cereals, but also other nutrients (Tournas, 2005; Moos, 2008; Barth, 2009).
Nedeljkovic *et al*. (1994) observed a significant increase, not only in the number of contaminated feed samples, but also in the number of unsatisfactory samples, especially piglet feed mixtures, which are associated with climatic conditions. Given that they are mainly isolated genera of molds that contaminate nutrients and mixtures in warehouses, it is clear that such a large number of unsatisfactory mixtures is caused by errors made during storage and handling, both nutrients as components of mixtures and finished mixtures, as well as poor ambient conditions in which they are stored (Leeson *et al*., 1995).
Salmonella has been the pathogens of greatest focus and concern with respect to animal feed due to the organisms' ability to infect food producing animals and thereby pose a potential risk in human foodborne disease (Ge *et al*., 2013; Jackson *et al*., 2013; Molla *et al*., 2010). *Salmonella* spp., one of the most important bacterial zoonotic agents, is an essential bacterium in the assessment of microbiological quality of feed (EFSA, 2006).
By far, the most important bacterial pathogen in feed is *Salmonella* which frequently occurs in a large number of feed ingredients of animal or plant origin and also in compounded feed. Other pathogenic bacteria with relevance for animal and human health and where feed might be a vector for the dissemination of the pathogen is limited to a few other species such is *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Clostridium* spp. (EFSA, 2008).
However, comparing the results from the years 2009-2012 to the years 2003-2010, a slight decline in the number of samples contaminated with *Salmonella* spp. is permanently noticeable (Kwiatek *et al*., 2008; Kukier and Kwiatek, 2011).
Presence of sulphite reducing clostridia in analyzed samples is an indication of rare, but fairly continuous contamination of animal feed whit sulphite reducing clostridia (Nedeljković *et al.*, 1994). Most of the tested mixtures for pig contained the permitted number of clostridia (97%), and in a small number of samples, mostly piglets, an impermissible number of clostridia was found, which ranged up to 1,700 CFU/g (Mašić *et al.*, 2002). Presence of sulphite reducing clostridia is not necessarily etiologically related to health disorders, and feeds in which their presence is not detected can contain their toxins. Therefore, the presence of sulphite reducing clostridia can be considered to be etiologically related to health disorders only when their numb is verified by confirmation of toxin-producing bacteria, i.e. toxin in the feed.
Excessive number of molds in feed mixtures for young animals was 43.5-48.1%, and in feed mixtures for older animals 14.1-17.2% (Mašić *et al*., 2002).
In the examination of samples of food for domestic animals and pets, the presence of fungi was not detected in only 12.2% of samples (Kubizna *et al*., 2011). However, determining the number of fungi is not enough to assess the quality of feed mixtures, but for this it is necessary to determine the concentration of mycotoxins.
The aim of the study was to determine the microbiological safety of feed in 2023 in Republic of Srpska (Bosnia and Herzegovina).

Material and methods

The test material was 245 samples of feed, originating from the territory of the Republic of Srpska (Bosnia and Herzegovina). The survey was conducted in 2023, throughout the year, independently of the season, in the Public Institution Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka. Samples were submitted as part of self-control and official control. In relation to the method of sample delivery, the structure of samples is shown in Table 1.

Table 1. Structure of samples in relation to the method of delivery

Self-control	Official control
%	%
94.29	5.71

The following standard test methods were used for microbiological testing samples of feed:

1. BAS EN ISO 6888-1 (ISBIH, 2022) for the enumeration of coagulase-positive staphylococci, *Staphylococcus aureus* and other species,
2. BAS EN ISO 7937 (ISBIH, 2005) for enumeration of *Clostridium perfringens*,
3. BAS EN ISO 6579-1 (ISBIH, 2018) for the detection of *Salmonella* spp.,
4. BAS ISO 21527-2 (ISBIH, 2009) for the enumeration of yeasts and moulds,
5. BAS EN ISO 4833-1 (ISBIH, 2014) for the enumeration of microorganisms.

The results of our research are presented in tables as percentage values.

Results and discussion

Results of microbiological testing of feed are shown in Table 2.

Table 2. Results of microbiological testing of feed

Satisfactory samples	Unsatisfactory samples
%	%
20	80

The obtained results indicate a much more favorable microbiological status of feed than the research of other authors (Golić and Nedić 2017; Golić *et al*., 2020). By observing all these results over a number of years, it is concluded that they vary considerably in terms of microbiological correctness. In addition to modern production and all measures within the framework of good hygiene and good production practice, undertaken by subjects in the feed business, it is obvious that other factors also affect the microbiological status of feed.

In relation to the method of sample delivery, the structure of unsatisfactory samples of feed is shown in Table 3.

Table 3. Structure of unsatisfactory samples of feed in relation to the method of delivery

Self-control	Official control
%	%
93.88	6.12

Observing the structure of unsatisfactory samples of feed in relation to the method of delivery, it can be concluded that the self-control plans are adequate and that the awareness of producers regarding the production of safe animal feed is very developed.

Results of microbiological testing of feed according to the examined parameters from the Rulebook on microbiological criteria in feed (2012), are shown in Table 4.

Table 4. Results of microbiological testing of feed according to the examined parameters

Type of testing	Satisfactory samples	Unsatisfactory samples
	%	%
Number of microorganisms	84.49	15.51
Number of yeasts and molds	82.45	17.55

The percentage of feed samples that did not meet the microbiological criteria in the period 2017-2019 averaged 18.86% for the number of microorganisms, and for the number of yeasts and molds 16.95% (Golić *et al.*, 2020). In the period 2014-2016, Golić and Nedić (2017) have determined 27.22% unsatisfactory samples due to the number of microorganisms and 21.75% unsatisfactory samples due to the number of yeasts and molds. The results obtained in our research have a significantly higher percentage of unsatisfactory samples due to the increased number of microorganisms and the increased number of yeasts and molds.
The presence of pathogenic microorganisms *Salmonella* spp., *Clostridium perfringens* and coagulase-positive staphylococci and *Staphylococcus aureus* was not determined in the tested samples. The obtained results are in accordance with the results Čabarkapa *et al.* (2009). When it comes to coagulase-positive staphylococci in feed, there is not much data in the available literature. EFSA (2008) does not consider the pathogenicity of coagulase-positive staphylococci present in feed at all. Golić and Nedić (2017) did not determine the presence of coagulase-positive staphylococci and *Staphylococcus aureus* in feed samples in the period 2014-2016. In connection with this, the question of the justification of the obligatory laboratory testing of feed on this parameter is raised. A special argument in support of the doubt about the justification of mandatory testing for this parameter, which should be taken into account, is the scientifically based, generally accepted fact, at which values of coagulase-positive staphylococci will be performed production of the enterotoxin ($>10^5$CFU/g). Certainly, in cases of suspected animal poisoning, testing of feed samples should be done in the presence of staphylococcal enterotoxins. For the period 2014-2016, the percentage of unsatisfactory feed samples for *Clostridium perfringens* was on average 0.30% (Golić *et al.*, 2020), and for the period 2017-2019, the percentage of unsatisfactory feed samples for *Clostridium perfringens* was on average 0.28% (Golić and Nedić, 2017). In view of the common isolation of *Clostridium perfringens* from the environment and from the intestinal tracts of livestock (75% to 95% of broilers) (van Immerseel, 2004), and the fact that *Clostridium perfringens*-associated diseases appear to need initiators in addition to the presence of the organism (Songer, 1996; Craven, 2000; van Immerseel, 2004), the significance of feed contamination by this bacterium is open to question. In the examination of components for feed for the period 2007-2010, the percentage of samples in which it is isolated *Salmonella* spp. ranged from 1 to 3.6%, average 2.15% (Kukier and Kwiatek, 2011), and in the study of complete feed mixtures for poultry, pigs and cattle for the period 2007-2010, the percentage of samples in which it is isolated *Salmonella* spp. ranged from 0 to 3.5% (Kukier *et al*., 2012). From current literature, the apparent prevalence of *Salmonella* (0.3-1.0%) in finished animal feed in New Zealand is similar to recent prevalence figures reported internationally (Cressey *et al*., 2011). Golić and Nedić (2017) found 0.74% of unsatisfactory feed samples due to the presence of *Salmonella* in the period 2014-2016. In the period 2009-2012, in Poland, the percentage of feed materials contamination by *Salmonella* spp. ranged from 0.84% to 3.58% with an average value of 1.83% (Kukier *et al*., 2013). The industry-based data from 2005 and 2006 reports an incidence between 0 and 0.8% of *Salmonella* contaminated samples in compounded feed to different food animal species (poultry, swine and cattle) (EFSA, 2008), which is in accordance with the obtained results of our research.

Conclusion

The presence of pathogenic microorganisms *Salmonella* spp., *Clostridium perfringens* and coagulase-positive staphylococci and *Staphylococcus aureus* was not determined in the tested samples, so, the risk of the presence of pathogens in feed is negligible. The cause of unsatisfactory feed samples was an increase in the number of saprophytic microorganisms, whereby this risk was constantly present and mainly led to indirect losses in livestock production.

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THE IMPACT OF AGE ON REPRODUCTIVE TRAITS OF SIMMENTAL CATTLE

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Abstract

The reproductive traits of Simmental cattle were observed and analyzed at the TC "Zona" farm in Podromanija, Sokolac, Republika Srpska, Bosnia and Herzegovina. A sample included a total of 78 cows, each of which calved three times. Of the examined traits, special attention was given to the age of heifers at first insemination and the impact of age on other monitored reproductive and productive traits (service period duration, duration of pregnancy, and intercalving interval). Special emphasis was placed on determining the strength and significance of the correlation relationship by calculating the correlation coefficient (r) between the age of heifers (cows) at first, second, and third fertilization, on the duration of the service period, intercalving interval, calf weight, and pregnancy duration. Based on the provided information, it can be said that the TC "Zona" farm in Podromanija, Sokolac, achieved satisfactory results in reproductive traits during the analyzed period.

Key words: *age, reproductive traits, Simmental breed cattle, correlation.*

Introduction

Reproduction of cattle represents the most significant phase of production. In this phase, animals are provided for herd replacement, herd expansion, milk production, fattening, and meat production. This master's thesis aims to examine the influence of heifer age on the reproductive traits of heifers (cows) of the Simmental breed. To achieve the best possible reproductive results, all phases of reproduction must be well organized. The study's main objective was to determine the age of heifers (cows) at first, second, and third fertilization (pregnancy), the duration of pregnancy, the duration of the service period, and the duration of the intercalving interval. Special emphasis was placed on determining the strength and significance of the correlation by calculating the correlation coefficient (rp) between the age of heifers (cows) at first, second, and third fertilization and the duration of pregnancy, the duration of the service period, and the intercalving interval.

Materials and Methods

The research on the reproductive traits of Simmental breed cows was conducted on the farm "TC Zona", Podromanija, Republic of Srpska, Bosnia and Herzegovina. Cows are inseminated artificially on the TC "Zona" Podromanija Sokolac farm. Considering that only 78 cows had complete, regular, and proper records of reproductive and production traits, they were selected for the research. All cows calved three times. During the analysis of the examined traits, special attention was paid to the age of the heifers at first fertilization and the impact of age on other examined reproductive traits during the analyzed period (2023). Based on the data from the herd and production records, the following were determined: the age of the heifers (A.H.), i.e., cows (A.C.) at first, second, and third fertilization; the duration of the first, second, and third pregnancies (D.P.); the duration of the service period (D.S.P.); and the intercalving intervals (I.I.). During data processing and testing the significance of differences between the examined indicators, common descriptive statistical methods were applied. The t-test was used to test the means of samples with equal variances, and the calculation of the phenotypic correlation coefficient between the determined parameters was performed according to the formula for the appropriate sample (*Latinovic, 1996*). The determined correlation coefficients were defined according to the Roemer-Orphalova classification for the strength of trait associations (*Latinovic, 1996*).

Results and Discussion

Analyzing the obtained results, it is found that out of the total number, the highest number of heifers were fertilized at the age between 15 and 16 months (49 heifers, 62.82%), while the least fertilized were at the age of 19 or 20 months (10 heifers, 7.8%). The average age of heifers at first fertilization was 16.54 months.

Table 1. Average values and variability of age of heifers (cows) at fertilization

Fertilization	N	$\bar{x}$	C.V.%	Min	Max
First	78	16,54	8,46	15,01	20,04
Second	78	29,57	8,08	27,23	33,90
Third	78	43,12	5,68	41,04	46,88

Based on the data, we conclude that the average age of cows at second fertilization was 29.57 months, and at third fertilization, it was 43.12 months (Table 1). *Petrovic* (2000.) in their study reported that heifers were fertilized slightly earlier for the first time, at around 17 months of age, which is consistent with findings by *Pantelic et al.* (2005.; 2008.). On the other hand, *Djurdjevic* (2001.) found the average age at first calving to be 27.73 months.

Table 2 shows the average values, absolute, and relative measures of variation regarding the duration of the first, second, and third pregnancies, including calf sex.

Table 2. Average values and variability of pregnancy duration (days)

Pregnancy	Sex	N	$\bar{x}$	C.V.%	Min	Max
First	♂	37	286,65	0,85	280	295
	♀	41	286,49	1,01	280	296
	Both sexes	78	286,56	0,93	280	295
Second	♂	43	286,58	0,78	280	290
	♀	35	285,60	0,94	285	291
	Both sexes	78	286,17	0,87	279	291
Third	♂	35	287,34	0,57	285	291
	♀	43	287,81	0,78	285	297
	Both sexes	78	287,60	0,69	285	297

Guided by the fact that the duration of gestation in cows is a biological constant, the duration of pregnancy in the first three calvings was approximately similar, with the pregnancy in cows giving birth to male calves lasting slightly longer than in those giving birth to female calves.

Regardless of the calf's sex, the longest pregnancy duration was observed during the third calving (287.81 days), while the shortest was during the second calving (285.60 days). Based on the data, it's noted that pregnancies tended to last longer when male calves were involved, except in the case of the third pregnancy (Table 2). For Simmental breed cows, *Petrovic et al*. (2007) found that the gestation length was 284.14 days, while *Pantelic et al.* (2005) determined it to be 285.51 days, which is a few days shorter than in this study.
The service period represents the period between calving and successful fertilization. Table 3 shows the average values and variability of the service period between the first calving and second fertilization, second calving, and third fertilization, and third calving and third fertilization.

Table 3. Average values and variability of the service period duration (days)

Service period	N	$\bar{x}$	C.V.%	Min	Max
First	78	116,53	12,26	90	139
Second	78	120,79	9,09	98	143
Third	78	111,49	9,61	90	132

The second service period lasted the longest (120.79 days), while the third service period was the shortest (111.49 days). *Caput et al.* (1989) reported that the service period lasted approximately similar to this study, with an average of 125 days.
The average values and variability of the intercalving interval between the first-second and second-third calvings are shown in Table 4. The third intercalving interval is not shown because the analyzed cows have not yet calved for the fourth time.

Table 4. Average values and variability of the intercalving interval (days)

Intercalving interval	N	$\bar{x}$	C.V.%	Min	Max
First	78	402,78	3,59	375	445
Second	78	408,39	2,82	387	430

The first intercalving period averaged 402.78 days, while the second was 5.61 days longer (Table 4). *Petrujkic et al.* (2011) suggest that the intercalving interval should ideally be around 380 days. If it is shorter than 12 months, it indicates that lactations may have been shortened or dry periods reduced, which can negatively affect the reproductive properties of the herd. In Table 5, the significance of differences between the analyzed reproductive traits is presented.

Table 5. Significance of differences in reproductive traits

Indicators	N	Average values	Differences	t-test	Significance
D.P.$_1$- D.P.$_2$	78 -78	286,56 – 286,17	0,39	1,050	^{ns}
D.P.$_1$- D.P.$_3$	78 -78	286,56 – 287,60	-1,04	-2,977	***
D.P.$_2$- D.P.$_3$	78 -78	286,17 – 287,60	-1,43	-3,972	***
D.S.P.$_1$- D.S.P.$_2$	78 -78	116,53 – 120,79	-4,26	-5,731	***
D.S.P.$_1$- D.S.P.$_3$	78 -78	116,53 – 111,49	5,04	4,249	***
D.S.P.$_2$- D.S.P.$_3$	78 -78	120,79 – 111,49	9,30	10,035	***
I.I.$_1$ – I.I.$_2$	78 -78	402,78 – 408,39	-5,61	-7,000	***

***P<0.001; **P<0.01; ^{ns}P>0.05. D.P.1, 2, 3 - duration of the first, second, third pregnancy, days; D.S.P.1, 2, 3 - duration of the first, second, third service period; I.I. 1, 2 - first and second intercalving interval.

The calculated differences in the duration of pregnancies (D.P.1 – D.P.2; D.P.1 – D.P.3; D.P.2 – D.P.3) showed varying levels of significance. The differences between D.P.1–D.P.3 and D.P.2–D.P.3 showed very high significance ($P<0.001$), while the difference between D.P.1–D.P.2 was statistically significant ($P>0.05$) (Table 5). The differences observed in the duration of the first (-4.26), second (5.07), and third (9.30) service periods were highly statistically significant ($P<0.001$) (Table 5). Similarly, the calculated difference in the length of the intercalving interval (-5.61) was statistically confirmed at the level of $P<0.001$ (Table 5).
In Tables 6, 7, and 8, coefficients of phenotypic correlation and strength of association of reproductive traits are shown concerning the age of heifers at first, second, and third conception.

Table 6. Phenotypic correlation coefficients and strength of association of reproductive traits (in relation to the age of heifers at first conception)

Traits	r_{xy}	$t_{exp.}$	Strength of association
A.H.F.F.-D.S.P.$_1$	$0{,}109^{***}$	1,04	Complete
A.H.F.F.-D.S.P.$_2$	$0{,}144^{***}$	1,41	Complete
A.H.F.F.-I.I.$_1$	$0{,}072^{**}$	0,64	Strong
A.H.F.F.-I.I.$_2$	$0{,}136^{***}$	1,24	Complete
A.H.F.F-D.P.$_1$	$-0{,}138^{***}$	1,26	Complete
A.H.F.F.-D.P.$_2$	$-0{,}215^{***}$	1,97	Complete
A.H.F.F.-D.P.$_3$	$-0{,}005^{ns}$	0,04	Absent

***$P<0.001$; **$P<0.01$; $^{ns}P>0.05$. A.H.F.F. - age of heifers at first fertilization, D.S.P. 1, 2 - duration of the first, second service period; I.I. 1, 2 - first and second intercalving interval; I.I. 1, 2, 3 - calf mass, first, second, third calving; D.P. 1, 2, 3 - duration of the first, second, third pregnancy.

Based on the data presented in Table 6, it can be seen that the calculated coefficients of phenotypic correlation between the age at first conception, duration of the first and second service periods, second intercalving interval, and duration of the first and second pregnancy showed very high significance ($P<0.001$). According to the Roemer-Orphal scale, the strength of the association in the mentioned cases was complete, with the calculated correlation coefficients between the age at first fertilization and the duration of the first, second, and third pregnancies being negative in direction. A high correlation ($P<0.001$) was found for the calculated correlation coefficient between the age at first fertilization and the first intercalving interval (rxy=0.072**).

Table 7. Phenotypic correlation coefficients and strength of association of reproductive traits (in relation to the age of cows at second fertilization)

Traits	r_{xy}	$t_{exp.}$	Strength of association
A.C.S.F.-D.S.P.$_2$	$0{,}262^{**}$	0,62	Strong
A.C.S.F.-D.S.P.$_3$	$0{,}153^{***}$	1,40	Complete
A.C.S.F.-I.I.$_2$	$0{,}260^{***}$	2,38	Complete
A.C.S.F.-D.P.$_2$	$-0{,}130^{***}$	1,19	Complete
A.C.S.F.-D.P.$_3$	$0{,}061^{**}$	0,53	Strong

***$P<0.001$; **$P<0.01$; $^{ns}P>0.05$. A.C.S.F. - age of cows at second fertilization, D.S.P. 2, 3 - duration of the second and third service periods, I.I.2 - second intercalving interval, D.P. 2, 3 - duration of the second and third pregnancies.

Based on the data presented in Table 7, we can see that the calculated phenotypic correlation coefficients between the age of cows at second fertilization, the duration of the third service period, the second intercalving interval, and the duration of the second pregnancy showed very high significance (P<0.001), and high significance (P<0.01) between the age of cows at second fertilization and the duration of the second service period, and the third pregnancy. According to the Roemer-Orphal scale, the strength of the association in the mentioned cases was complete and strong.

Table 8. Phenotypic correlation coefficients and strength of association of reproductive traits (in relation to the age of cows at third fertilization)

Traits	r_{xy}	$t_{exp.}$	Strength of association
A.C.T.F.-D.S.P.$_3$	$0,221^{***}$	2,02	Complete
A.C.T.F.-D.P.$_3$	$0,081^{**}$	0,71	Strong

***P<0.001; **P<0.01; ^{ns}P>0.05. A.C.T.F. - age of cows at third fertilization, D.S.P. 3 - duration of the third service period, D.P... 3 - duration of the third pregnancy.

Based on the data presented in Table 8, the calculated phenotypic correlation coefficients between the age of cows at third fertilization and the duration of the third service period showed very high significance (P<0.001), and high significance (P<0.01) between the age of cows at third fertilization and the duration of the third pregnancy. According to the Roemer-Orphal scale, the strength of the association in these cases was complete and strong.

Conclusions

Based on the analyzed reproductive traits, the following can be observed: The average age of heifers (78) at first fertilization was 16.54 months, the age of cows at second fertilization was 29.57 months, while the age of cows at third fertilization was 43.12 months. The duration of pregnancy for the first, second, and third calvings averaged 286 days. Calculated differences in pregnancy duration (D.P.1 – D.P.2; D.P.1 – D.P.3; D.P.2 – D.P.3) showed varying significances, with D.P.1-D.P.3 and D.P.2-D.P.3 differences being highly significant (P<0.01), while the difference between D.P.1 and D.P.2 was statistically significant (P>0.05). The duration of the first service period was 116.53 days, the second was 120.79 days, and the third was 111.49 days. The calculated difference in the length of the intercalving period was statistically confirmed. Based on the presented data, it can be observed that with increasing age at first fertilization, the duration of the first and second service periods also increased, along with the intercalving intervals. Negative correlation coefficients were found between the age of heifers at first fertilization and the duration of all three pregnancies. The strength of association is either complete or absent. Based on the findings of this study, it is evident that the age of heifers at first fertilization is statistically significantly associated with the service period, the second intercalving interval, and the durations of the first and second pregnancies. Furthermore, the calculated phenotypic correlation coefficients between the age at second fertilization, the duration of the second and third service periods, the second intercalving interval, and the duration of the second and third pregnancies showed very high significance (P<0.001). The strength of association in these cases was complete and strong. Based on the presented data, it can be seen that the calculated phenotypic correlation coefficients between the age at third fertilization, the duration of the third service period, and the duration of the third pregnancy showed very high significance (P<0.001). According to the Roemer-Orphal scale, the strength of association in these cases was complete and strong.
Overall, it can be concluded that Farm TC "Zona", Podromanija - Sokolac, achieved satisfactory results in reproductive traits during the analyzed period.

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HISTOLOGICAL ANALYSIS OF STRIATED MUSCULATURE WITH THE PURPOSE OF ASSESSING MEAT QUALITY

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Abstract

Skeletal muscles are made of striated muscle tissue the cells of which have the capacities to contract. Dimensions of muscle fiber differ depending on the type of animal, breed, sex, or individual muscles of the same organism. In our research, we compared the thickness of the longitudinal of muscle fibers of sample A – a muscle of unknown anatomic topography and sample B -Musculus longissimus dorsi. In sample A, the thickness of muscle fibers is greater than in sample B. Also, more nuclei are present along the cellular sarcolemma in sample A compared to sample B. The presence of the elements of connective tissue varies in both samples (the presence of fat tissue, collagen, elastic fibers). Our research finds that there is a slight difference in the thickness of the muscle fibers (medium value) in both muscles. Understanding the histological characteristics of muscle tissue is necessary for successful meat production. Meat is a product that is created through various biochemical processes following rigor mortis of the skeletal muscles of animals. The amount and quality of meat greatly depend on the characteristics of muscle tissue, i.e., the structure, number, diameter, and representation of certain types of muscle tissue, and the selection and nutrition played a decisive role, i.e., the structure of muscle tissue depends on multiple endogenous and exogenous factors, physical activity, especially in pig and poultry production.

Keywords: *histology, muscles, meat quality, bovine.*

Introduction

The striated muscle tissue is part of the skeletal musculature, which is innervated by cerebrospinal nerves and is subject to the influence of the will. It is also found in organs innervated by the vegetative nervous system when not subject to the influence of the will (esophagus, intestines of fish) (Katica et al. 2010). The basic structural component is striated muscle fibers - *Myofibra,* in the form of elongated prisms or cylinders. The thickness of muscle fibers varies from 10-100 micrometers or more, depending on the activity of the muscle. Through our study, we compared the width of longitudinal sections of muscle fibers of samples A-muscle of unknown anatomical topography and samples B-muscle-steak-*Musculus longissimus dorsi*, (out of category meat in the market) (Ivanković et al. 2013). Authors observed a slight difference in the width of muscle fibers, the presence of connective tissue and its arborization in the studied samples. The fiber is surrounded by membrane-sarcolemma. Inside the fiber is the sarcoplasm in which the contractile formations-myofibrils are immersed. A muscle fiber-cell *(Myocyte)* contains a large number of nuclei; the position of the nuclei in the fibers of adult organisms is along the edge, directly next to the sarcolemma. The nuclei are oval and contain fine-grained chromatin and between 1-2 nucleoli. Sarcoplasm is specially organized in fiber. Myofibrils are mesh-like protein structures that make up the contractile apparatus of muscles. The presence and arrangement of the connective tissue ultimately affects the complete and proper marbleization of the muscles, which, it should be emphasized, is desirable.

Material and methods

The striated musculature was taken from a fresh steak muscle (B) and striated muscle (A) of unknown topographic location, of bovine.
The samples were stored in plastic vials with a screw that contained 10% formalin, until the moment of making histological preparations, i.e. molding in paraffin blocks.
The samples were placed in 70% alcohol for two days, then in 96% alcohol for one day and finally in 100% alcohol for one day. After this procedure, the samples were transferred to a mixture of 100% alcohol and toluene for two hours, and then only to toluene for four hours.
The prepared musculature samples were placed in paraffin I for five hours and paraffin II for twelve hours. This completed the molding process in paraffin blocks. Sample processing from fixation to paraffin embedding was performed on a MICRON model STP-120 rotary tissue processor. After the completion of the molding process, the musculature was cut with a LEICA RM 2145 digital microtome in several serial cuts with a thickness of 0.5 to 1.5 microns. The sections obtained in this way were placed on slides, stained with hematoxylin-eosin (HXE), covered with a coverslip and glued with Canada balsam.
Histological preparations were analyzed with a MOTIC TYPE 120M binocular light microscope, under magnification 100, 200, 400 times and immersion;
Microscopic elaboration and measurements were performed with a special program, Motic Image Plus 2.0ML.

Results and discussion

The striated muscles basically have a unique structure. Numerous factors influence the dimensions of muscle fibers, and the dimensions differ depending on the species of animal, sex, category, and there are differences between muscles of the same organism (Cheng et al. 2015).
Through microscopic, i.e. histological analysis of the muscle fiber samples (A, B), on the longitudinal section we found in sample B the uniformity of the longitudinal sections of the muscle fibers, and on the inside of the sarcolemma the presence of numerous spindle-shaped, basophilic colored nuclei, along the muscle fibers (Figure 1).

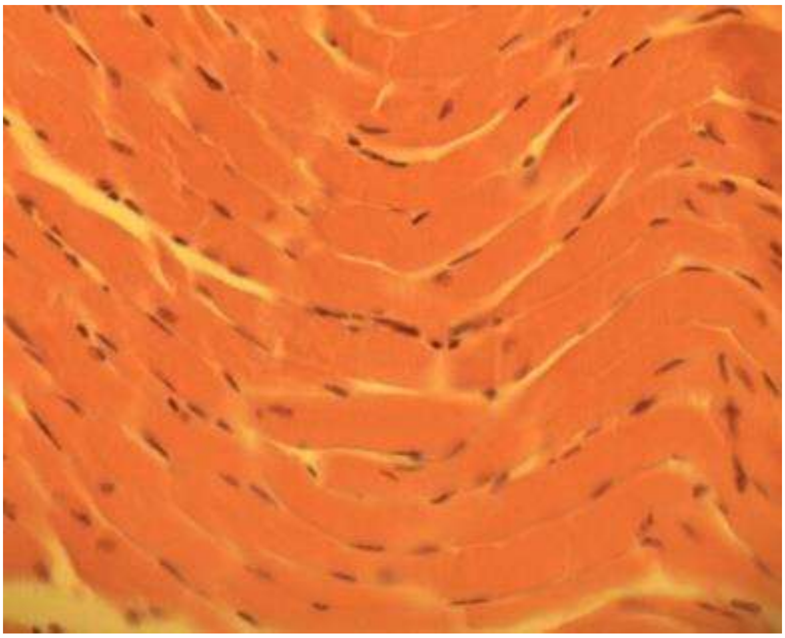

Figure 1: Sample B-longitudinal section of the musculature, (HXEx400)

On the cross-section of sample B are visible uniform bundles and bundles of elastic and collagen fibers with cross-sections of blood vessels (Figure 2).

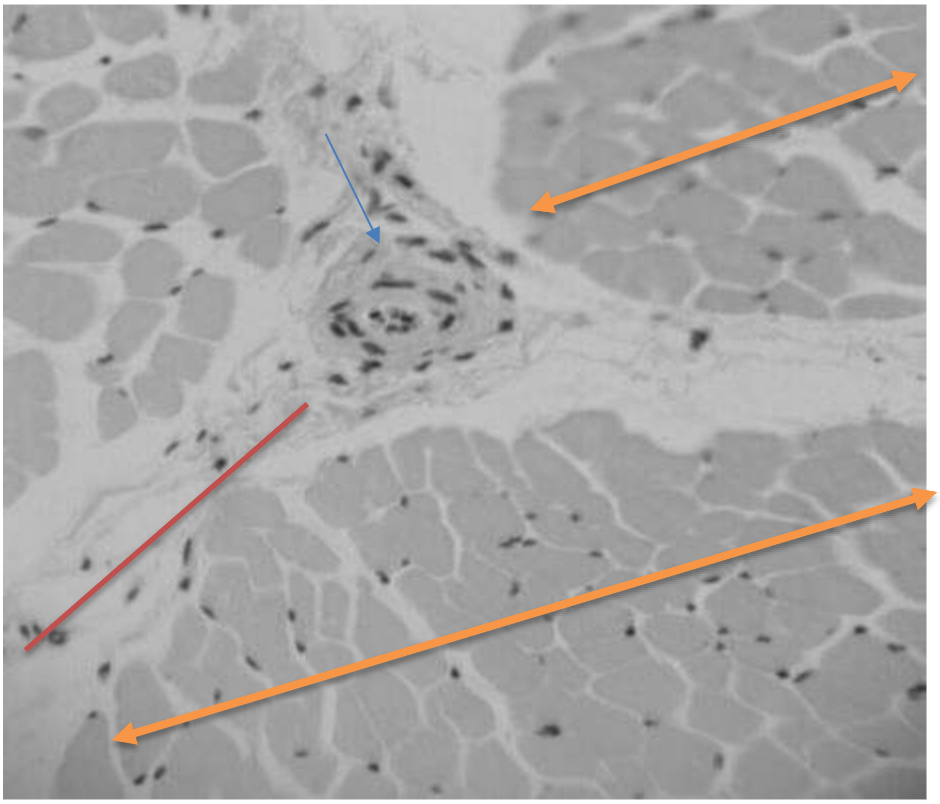

Figure 2: Sample B-musculature cross-section, blue arrow-blood vessel; orange line-binder; green arrows -bundles and muscle bundles (HXEx400)

Collagen in muscles is a protein; with age, its properties change, fibril diameters become larger, collagen becomes firmer (Lawrie, 1985). The relative relationship between the connective tissue (membrane of the connective tissue of each muscle, that is, individual muscle bundles and ultimately each muscle fiber) and muscle fibers in individual muscles is different and affects the quality of the meat (juiciness). On the cross-section of sample B are visible uniform bundles and bundles of densely packed muscle fibers. The presence of elastic and collagen fibers with a cross section of a blood vessel is visible between the bundles (Figure 2), which is in accordance with the statements of Brooks et al. (2011).

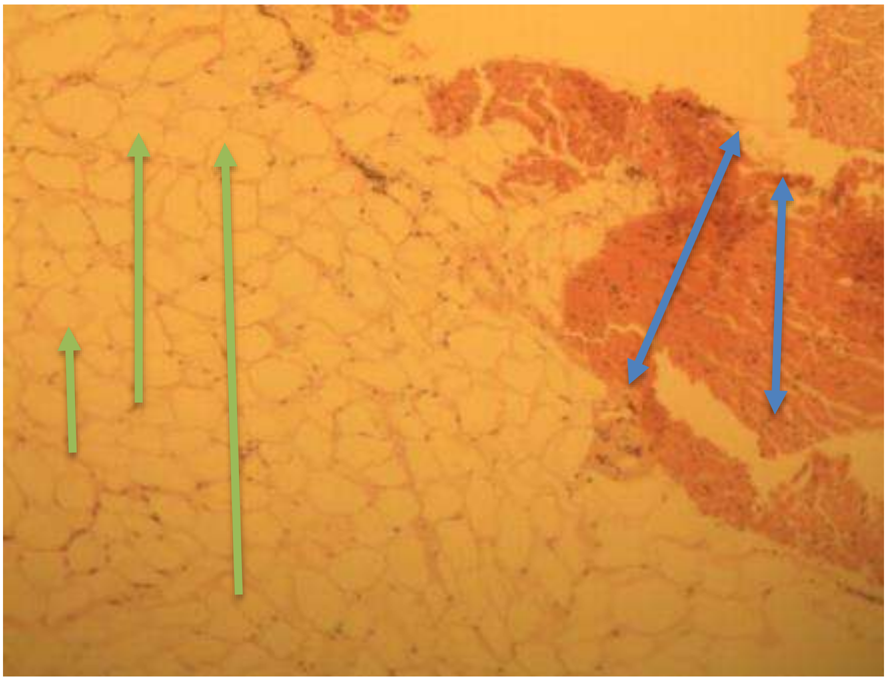

Figure 3: Sample B-adipocytes, connective tissue, musculature, gray arrows adipocytes; blue arrows myocytes (HXEx200)

In Figure 3, lobules of adipocytes and part of the musculature can be seen. Also, islands of myocytes surrounded by adipocytes can be seen, which indicates that fat tissue, in some way, isolates the muscle. Muscle atrophy is expressed when the sheaths are completely surrounded by adipocytes, as established by Valenzuela et al. (2020).

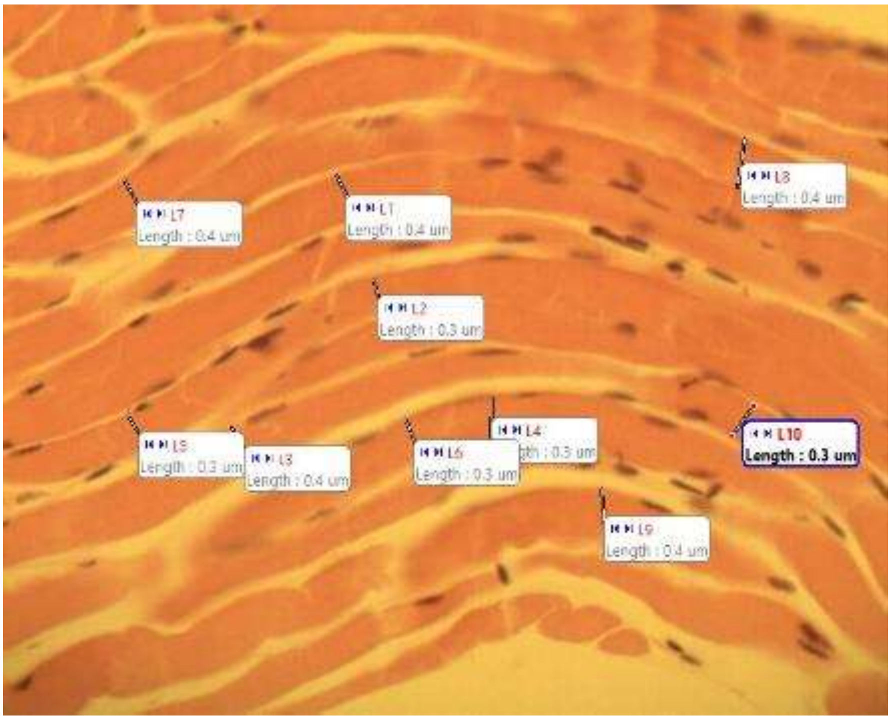

Figure 4: Sample B-width of striated fibers in micrometers (µm) (HXEx400)

By measuring the width of the longitudinal cross-sections of the muscle fibers of sample B, we found a mean value of 0.35 µm.

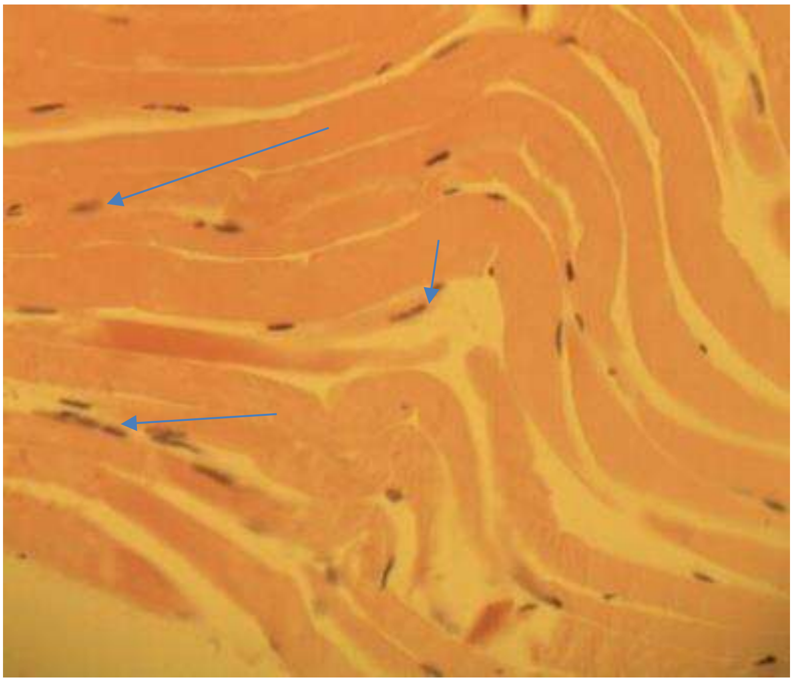

Figure 5. Longitudinal section of musculature, Sample A, arrowheads indicate myocyte nuclei (HXEx400)

Figure 5 shows a longitudinal section of sample A with sparsely represented basophilic nuclei next to the sarcolemma, with a wider band of sarcoplasm, unlike sample B (steak) where the number of nuclei-nuclei next to the sarcolemma was numerically higher.

Our studies revealed a slight difference in the width of muscle fibers and the representation of connective tissue elements in one and the other sample (B and A).

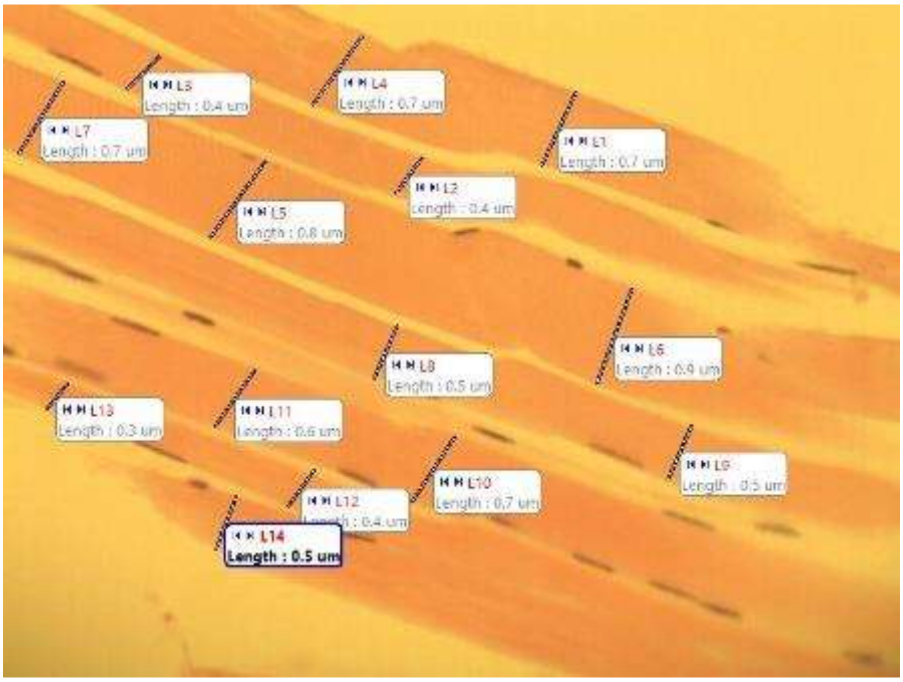

Figure 6. Sample A-width of cross-striped fibers in micrometers (µm) (HXEx400)

By measuring the width of the longitudinal sections of the muscle fibers of sample A, the mean value of the width was calculated, which was 0.57 µm.

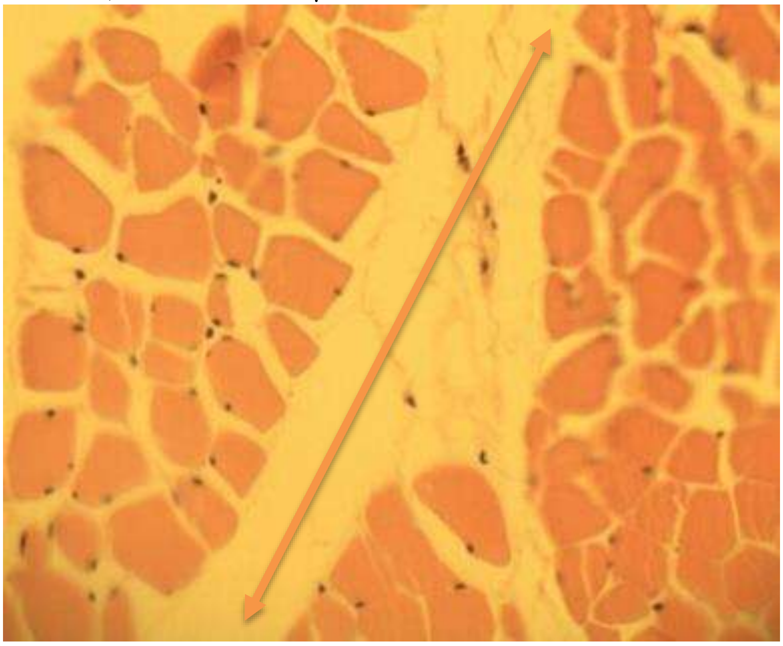

Figure 7. Cross-section of the musculature of sample A, green arrow-connective tissue (HXEx400)

Cross-section of the muscle (Figure 7) – bundled (entwined) muscle fibers with an almost regular square appearance, not densely packed with scarce nuclei and the presence of some connective tissue. Figures 7 and 8 clearly show the arborization of connective tissue with the presence of blood vessels, connective fibers and perimysial fat tissue that participates in the marbling process. The main contribution to intramuscular adipose tissue is the presence of perimysial adipocytes, which agrees with the statements of the authors Carvalho and Smith, (2018) and Asa et al. (2017).

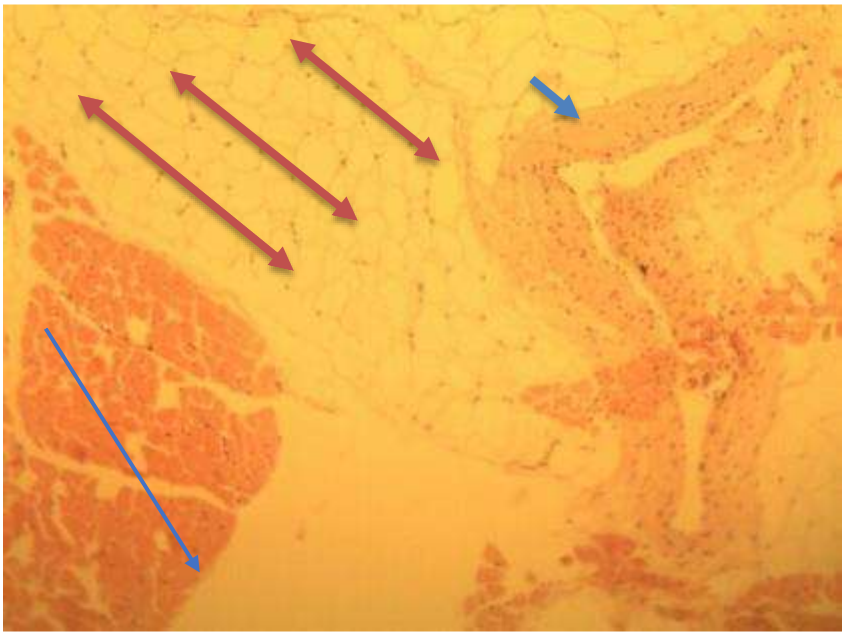

Figure 8. Sample A- Presence of white adipose tissue and connective tissue (orange arrows) with cross-section of venous blood vessel (blue arrow) and part of striated muscle, (HXEx200)

Marbling is a hyperplastic and hypertrophic process of intramuscular fat development.

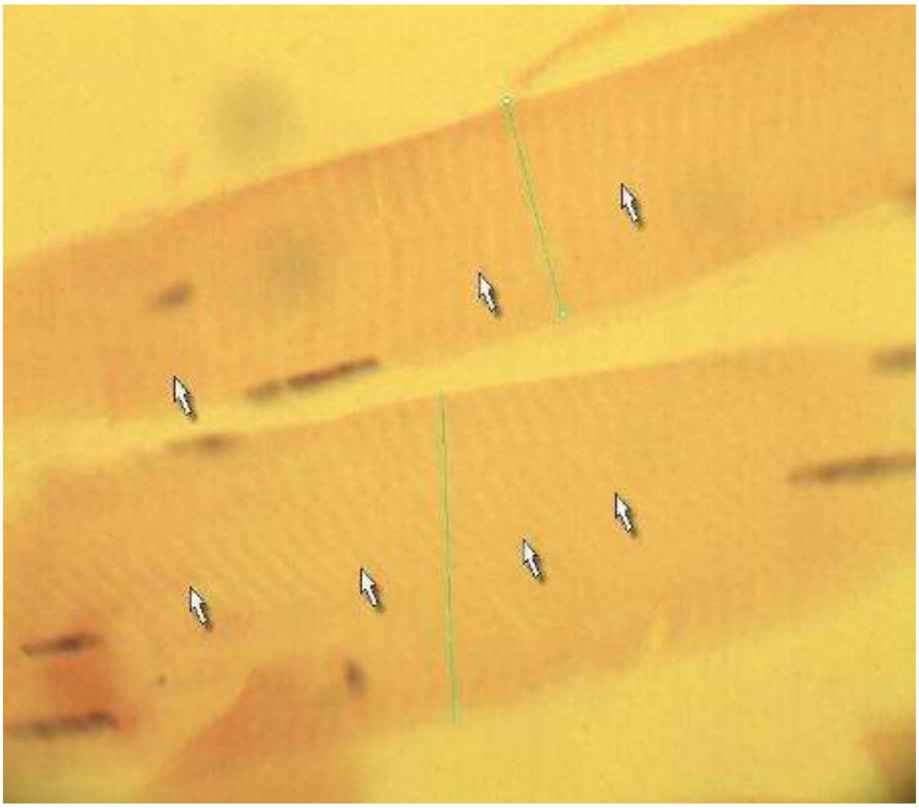

Figure 9. Sample A, white arrows indicate clearly expressed transverse striation (light and dark fields), and the green line indicates the width of the muscle fiber (IMMERSION).

When observed with a light microscope, under immersion, (Figure 9), bright and dark fields are clearly visible. Darker stripes are anisotropic, i.e. A-stripes, which double refract light, and light stripes are isotropic and single refract light (Mlaćo et al. 2017).
That is, the transverse striation of myofibrils is a consequence of the regular arrangement of actin and myosin myofilaments within myofibrils, (Žikić et al., 2016).

Conclusions

Through our histological study of striated musculature, of known and unknown samples, (B, A), we wanted to distinguish the structure, level, and arrangement of the presence of connective tissue, especially adipose tissue, in order to better understand the arborization and marbling of muscle-meat for the purpose of easier recognition, classification and putting into use.
The mean value of the width of the longitudinal sections of the muscle fibers of sample A is 0,57µm, and of sample B 0,35 µm.
A slight difference in the width of muscle fibers and the representation of connective tissue elements was observed in samples A and B.

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INVESTIGATION OF THE EFFECTS OF DOMESTIC WATER BUFFALO, HUNGARIAN RACKA SHEEP AND HUNGARIAN GREY CATTLE GRAZING ON DIFFERENT WOOD-PASTURES IN HUNGARY

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Abstract

Nowadays, the role of nature conservation and animal husbandry has increased drastically due to the effects of climate change. Formerly, wood-pastures were a characteristic farming type in the Pannonian region. In the current work, we studied wood-pastures in the North Hungarian Mountain Range and in the Transdanubian Central Mountain of Hungary. Our purpose was to find out the nature conservation values and grassland management values of wood-pastures grazed by different animals. Among the sample areas of North Hungarian Mountain Range, the sample area of Cserépfalu was grazed by Hungarian Grey Cattle, while the Szurdokpüspöki was grazed by domestic water buffalo. The sample areas of Transdanubian Central Mountain were grazed by water buffalo and Hungarian Racka Sheep. Coenological surveys were between 1994 and 2024 in the main vegetation period according to the method of Braun-Blanquet. We applied the diversity and grassland management value to evaluate the state of the vegetation. Grazing of the studied livestock produced different results among the areas. Based on the results, cattle grazing resulted in a variable, mosaic-like, shrubby area with high cover values, sheep grazing resulted in vegetation with higher grassland management values and buffalo grazing resulted in a variable and nutrient rich area with optimal diversity and grassland management values. Hence, grazing by cattle provides adequate solution to create and conserve wood-pasture habitats. However, grazing by sheep forms valuable grassland with better grassland management values. Nevertheless, grazing by buffalo is probably proper for nature conservation and grassland management values as well.

Keywords: *grazing, nature conservation, Pignatti life form, ruminant.*

Introduction

Wood pastures are one of the oldest land-use types in Europe, where livestock graze in mosaic habitats characterised by grasslands with different tree and shrub species. Over the centuries, wood pastures have been important traditional elements in Carpathian-basin as one of the dominant farming types of the country (Moreno *et al*., 2018; Burgess and Rosati, 2018) (Figure 1). The importance of wood-pasture habitats has increased significantly because of the current global climate change issues, as shrinking grasslands lead to potential for animal husbandry in areas that were formerly not considered of high management relevance.

Öllerer *et al.* (2019) provided a complex review of the effects of domestic livestock grazing on temperate forest vegetation. They concluded that successful wood-pasture conservation depends on the choice of grazing animal species and that the lack of grazing can negatively affect biodiversity and forest management. It needs to be taken into account when treating wood-pastures as semi-natural habitats (Bernes *et al.*, 2018; Burrascano *et al.*, 2013).

Figure 1. Location of Hungary (Carpathian-basin) in the map of Europe

Large herbivores have essential role in the formation of forests, shrublands and grasslands (Mitchell, 2005). Therefore, grazing livestock can contribute to the current forest-grassland mosaic (Varga *et al.*, 2020). Different native and introduced livestock such as cattle, sheep and horses can provide a substitute for wildlife activity (Póti *et al.*, 2007). The grazing type influences the vegetation structure and the yield of grassland (Naveh, and Whittaker, 1980, Török *et al.*, 2018). There were also some investigations on the effects of grazing animal choice in Hungary. Most of the research confirms the traditional nature conservation management that cattle grazing is suitable for habitat conservation (Török *et al.*, 2014; Turcsányi-Járdi *et al.*, 2022; Penksza *et al.*, 2022). Penksza *et al.* (2024) confirmed that grey cattle grazing is a suitable practice for wood-pasture conservation. However, in this study, it was also reported that sheep also have similar grazing characteristics in mountain wood-pasture conditions. In addition, Fűrész *et al.* (2023) studied a domestic water buffalo grazed shrubland in a mountain with a similar feature to wood-pasture. In this study found that the domestic water buffalo could be a more effective animal for habitat formation and conservation than the grey cattle because it has better digestibility (Mihailou and Massaro, 2021; Escarcha *et al.*, 2018; Warriach *et al.*, 2015). In the current work, our aim was to study a wood-pasture from Transdanubian Central Mountain (Balatoncsicsó), which is grazed by sheep and domestic water buffalo.

Materials and methods

Data collection and surveyed areas

In the present study, authors took into account two previous studies conducted in the North Hungarian Mountain Range, which focused on the effects of domestic water buffalo, grey cattle and sheep grazing (Fűrész *et al.*, 2023; Penksza *et al.*, 2024). The current work was carried out in Balatoncsicsó (Figure 2) which belongs to the Transdanubian Central Mountain of Hungary where grazing is by domestic water buffalo and sheep. Authors conducted ten

Figure 2. Wood-pasture sample area in Balatoncsicsó

coenological surveys of the woody sample areas, ten in the grassland sample areas in 1994, 2009 and 2024 and ten in the control, untreated grassland sample area in 2024 in the vegetation period (May and June) based on the Braun-Blanquet method (1964) in 2 × 2 m quadrats. The coverage was estimated by percentage for each present species. The name of the species was recorded based on the nomenclature of Király (2009).

Statistical analysis

To evaluate data, the non-parametric statistical method was used to analyze the cover values of species of different sample areas, as these variables were not normally distributed according to the Shapiro–Wilk test ($p < 0.05$). Accordingly, the non-parametric Kruskal–Wallis test (= 0.05) was used, and the non-parametric Dunn's test with Bonferroni correction was used for multiple pairwise comparisons (Addinsoft XSTAT, 2016).

Results and discussion

Based on the results (Figure 3), it can be seen that the wood-pasture sample areas in Balatoncsicsó have different grazing impacts in terms of changes in cover values.
Compared to the cover results of the control, untreated area, no significant results were found for several sample areas. The results of the control area were significantly different only from the data of the grassland coverage of the survey in 2009. The results of woody areas clearly show that there has been no significant change over 30 years, although the records from 1994 show less similarity with the other records. The results of grassland areas unequivocally show that there has been no significant change over 30 years, however, the records from 2009 show more similarity with the other data. The results of the woody sample area survey from 1994 were statistically significantly different from the mean cover values of grassland. Whereas the mean cover value from 2009 grassland area was significantly different from the cover values of the control area and the woody sample area. The mean cover of the control area was most similar to the data from under the trees of 2009 and 2024.

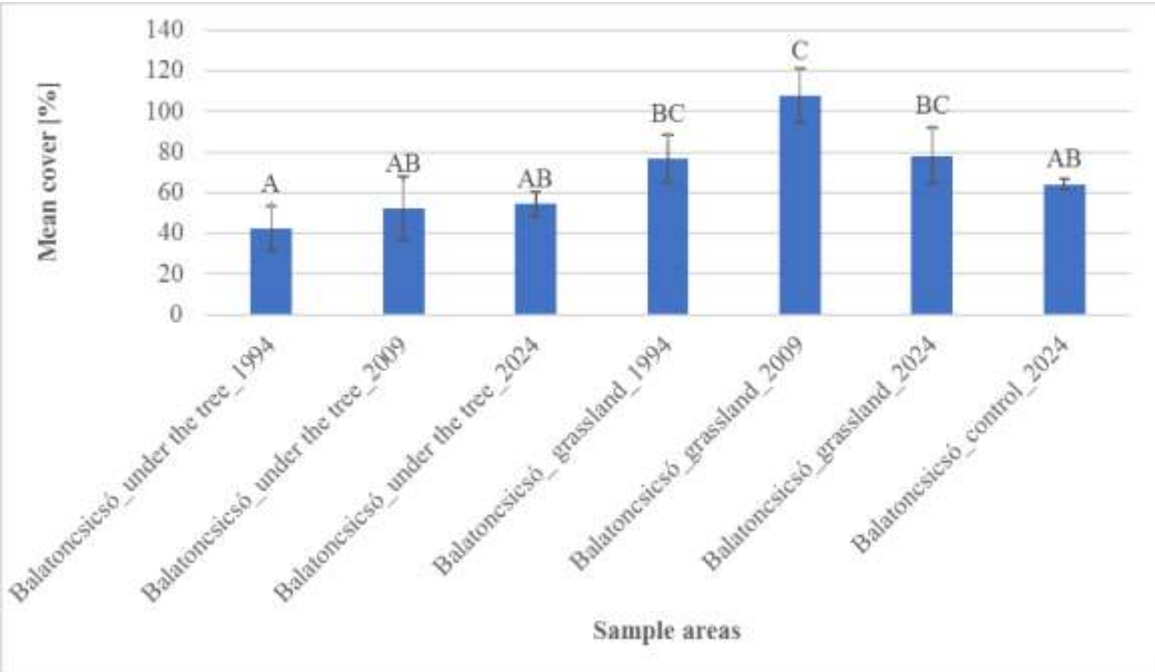

Figure 3. Cover (mean, standard deviation) in the sample areas. Based on the Kruskal–Wallis and Dunn's post hoc tests with Bonferroni correction. Bonferroni-corrected significance level: 0.005. Stand-alone letters indicate homogeneous groups (A or B or C) that significantly differ from each other. When heterogeneous groups have at least two different letters, they are not significantly different from groups with a common letter.

Conclusion

The literature review highlights the outstanding nature conservation and farming values of wood-pastures. To sustain wood-pasture, grazing is essential, but it requires the adequate choice of animal. Preliminary studies have confirmed that cattle are suitable for wood-pasture habitat conservation and biodiversity maintenance, while sheep grazing produces similar vegetation with more beneficial grassland management value. In addition, domestic water buffalo grazing, which has not been practiced in dry grasslands before, may be a more appropriate method to conserve habitat, maintain biodiversity and increase the proportion of plants with high grassland management value.

Consequently, based on the literature reviewed and the results described, it can be concluded clearly that cattle, sheep and domestic water buffalo are capable of sustaining and conserving wood-pasture biodiversity.
Nevertheless, the present work has only investigated the mean cover values, and in the future, it would be useful to carry out a Pignatti life-form data processing and the grassland management values of this sample area to obtain a more complex overview of the impact of grazing.

Acknowledgments

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INBREEDING IN LATVIAN DARKHEADED SHEEP BREED BY PEDIGREE DATA

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Abstract

The aim of this study is to evaluate the effective population size and the level of inbreeding in genetic resources of Latvian Darkheaded (LT) sheep breed population. For the analysis, 549 genetic resources sheep (ram and ewes) pedigree data was used, which were alive in 2020. The pedigree data started from 1983 with a total number of animals 2295 – 1817 (407 rams and 1410 ewes) with and 478 without progeny. The average age of breeding rams and ewes by birth year of offspring was 3.1 and 3.5 years, respectively. During the evaluation period, the average generation interval based on the age of the ram and ewes was 3.4 years with the generation interval from the sire to the sire 3.2, the sire to the daughter 3.1, the dam to the son 3.9 and the dam to daughter 3.7 years. Ewes and rams with the higher number of progeny in the population were defined and the highest number of progeny from ewes was 9 and for rams 34 lambs. The number of inbreed animals was 1177 with an average inbreeding coefficient of 2.75%. Starting from 2008 the level of inbreeding in LT genetic resources population has been related to genetic resources programme realisation in Latvia, which was accepted in 2004. Breeding organization and the breeders on farm level have to monitor the rams and ewes mating and control the level of inbreeding.

Keywords: *genetic resources, Latvian local sheep, pedigree completeness, pedigree analysis*.

Introduction

Latvian Darkheaded (LT) sheep breed belong to short-tailed sheep breed and is economically important for Latvian conditions.

The Latvian blackhead was created in the 1920s and 1930s by crossing local coarse-wool sheep with Shropshire and Oxfordshire sheep imported from Sweden and England. 1937 the import of improving breeds was stopped and Latvian blackhead was accepted as a breed (Cjuksa, 1951; Grigaliūnaitė *et al.*, 2003). Up until the 1970s, local breeding material was used for breed selection. In the 1970s, Finnish landraces were used to increase the fertility of ewes. Due to the decrease in the population, as well as to exclude the inbreeding and to improve the meat quality, at the end of the 20th century, German Blackhead breeding rams were used, and at the beginning of the 21st century, the mating of LT with Estonian Blackhead x Oxford Down crossbreed is accepted (Breeding programme, 2023).

LT breed are characterised by a strong body structure and produce 1.6 – 1.7 lambs per ewe (Barzdina & Kairisa, 2023); live weight is 55 – 65 kg for females and 100 – 120 kg for males.

The implementation of the LT breed conservation program started in 2004 and the animals were evaluated according to their phenotype. At that time, as genetic resources were accepted 46.2% LT purebred animals. Starting from 2015, only purebred animals with 100% of LT were accepted into the old type LT sheep breed conservation program. In 2022, in the conservation programme 487 ewes and 41 rams was included (Breeding programme, 2023). During that time the number of sheep under conservation program is decreased. In small, local populations the level of inbreeding should be controlled, because it can be resulted in reducing of the genetic diversity of native breeds, inbreeding depression and appearance of the lethal genes in the population (Barczak, *et al.*, 2009; Gizaw *et al.*, 2013; Sonesson & Meuwissen, 2001).

The level of inbreeding in the LT population was not evaluated before, thus the purpose of the research is to evaluate genetic diversity in the local LT sheep breed by the level of inbreeding based on pedigree data.

Materials and methods

Pedigree data

For the analysis, 549 genetic resources sheep (rams and ewes) pedigree data was used, which were alive in 2020. The pedigree data started from 1976 with a total number of animals 2295 – 1791 (284 rams and 1507 ewes) with and 478 without progeny (Table1). Animals with no pedigree information were defined as founders and the number of founders in the pedigree was 241.

Table 1. Structure of the pedigree

Group	Number of animals	Number of inbred	Number of founders	Number of animals with known both parents	Number of animals with no progeny
Ewes	1856	1018	150	1507	446
Rams	439	159	91	284	32
Total	2295	1177	241	1791	478

Methods and data analysis

The calculation in the current study was done using the POPREP (Groeneveld *et al.*, 2009) and the CFC (Sargolzaei *et al.*, 2006) software.

Coefficients of inbreeding (F) were calculated for each sheep in the pedigree according to Colleau (2002) algorithm using the CFC software. Pedigree completeness was calculated based on the MacCluer et al. (1983) algorithm using the POPREP software.

Results and discussion

Pedigree completeness and ancestral path

The completeness of the pedigree in different years is shown in Figure 1. The improvement of the pedigree completeness is started from 2007, when the completeness of the 5th and 6th generation started to increase and in the 2020 reached around 85%. The first- and second-generation completeness in 2020 was around 100%.

In the data the ancestral path in the pedigree varied from 1 to 15 and 66% of the animals had the path for more than 6 generations (Figure 2). 241 animals had no information about their ancestors, and they are founders in the pedigree.

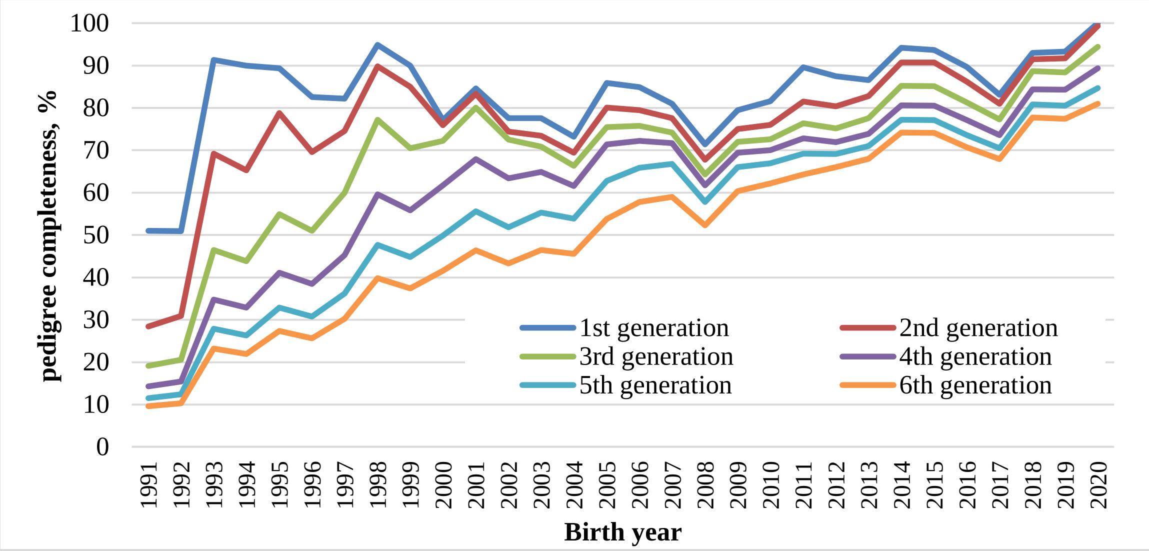

Figure 1. The average pedigree completeness, % by birth year of offspring.

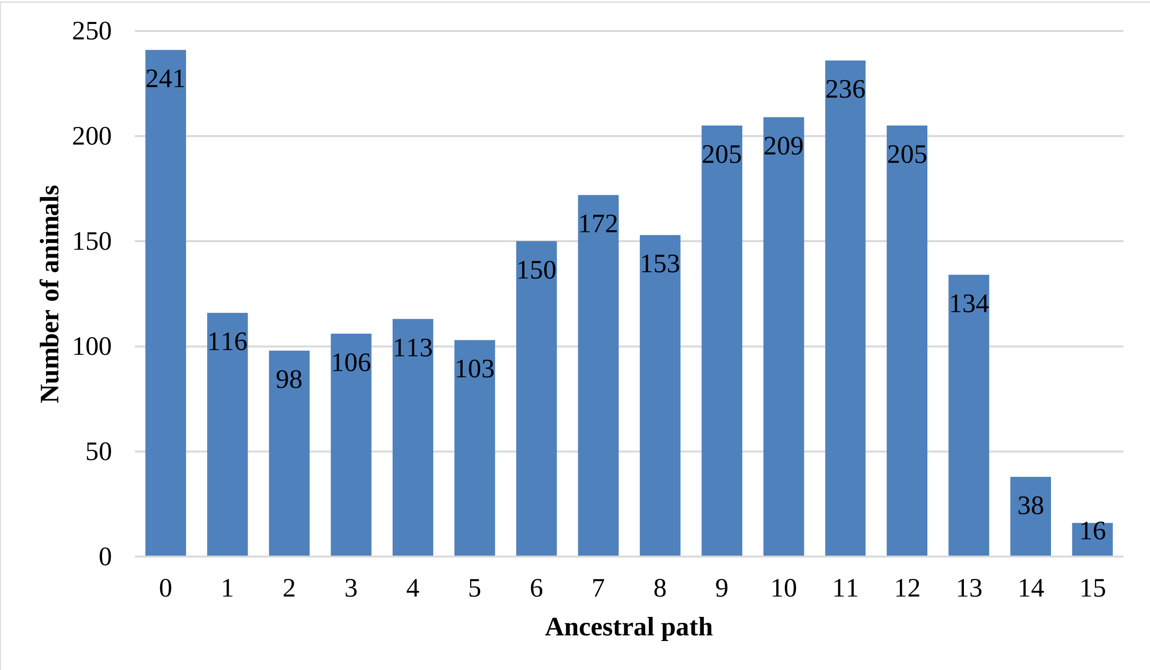

Figure 2. Ancestral path in the pedigree.

Analysis of inbreeding

The average level of inbreeding in the pedigree was 1.4% with maximum level 37.5% (Table2). The average and maximum coefficient of inbreeding was higher for ewes compare to the rams.

Table 2. Minimum, maximum and average inbreeding, % in the pedigree

Group	Number of animals	Average F, %	Average F, % in the inbred	Maximum F, %	Minimum F, %
Dams	1856	1.5	2.70	37.5	0.012
Sires	439	1.0	2.80	25.0	0.025
Total	2295	1.4	2.75	37.5	0.012

Table 3. Distribution of inbreeding coefficients (*F*) for inbred animals (n=1177).

Inbreeding coefficients (*F*), %	Number of animals (n=1177)	Animals, %
0 < F <= 5	1057	89.8
5 < F <= 10	69	5.9
10 < F <= 15	29	2.5
15 < F <= 20	3	0.3
20 < F <= 25	14	1.2
25 < F <= 30	1	0.1
30 < F <= 35	-	-
35 < F <= 40	4	0.3

The number of inbreed animals was 1177 with an average inbreeding coefficient of 2.75% and the distribution of the animals according to the level of inbreeding is given in Table 3. Around 90% of animals were with level of inbreeding until 5%, however, there were animals with level of inbreeding more than 10%.
The distribution of the animals according to the level of inbreeding by year of birth is given in Figure 3. Hire is the tendency the number of animals with higher level of inbreeding is decreasing during the last ten year. Sheep, which were born after 2015, are with coefficient of inbreeding less than 10% and more than 90% of animals are with level of inbreeding less than 5%.

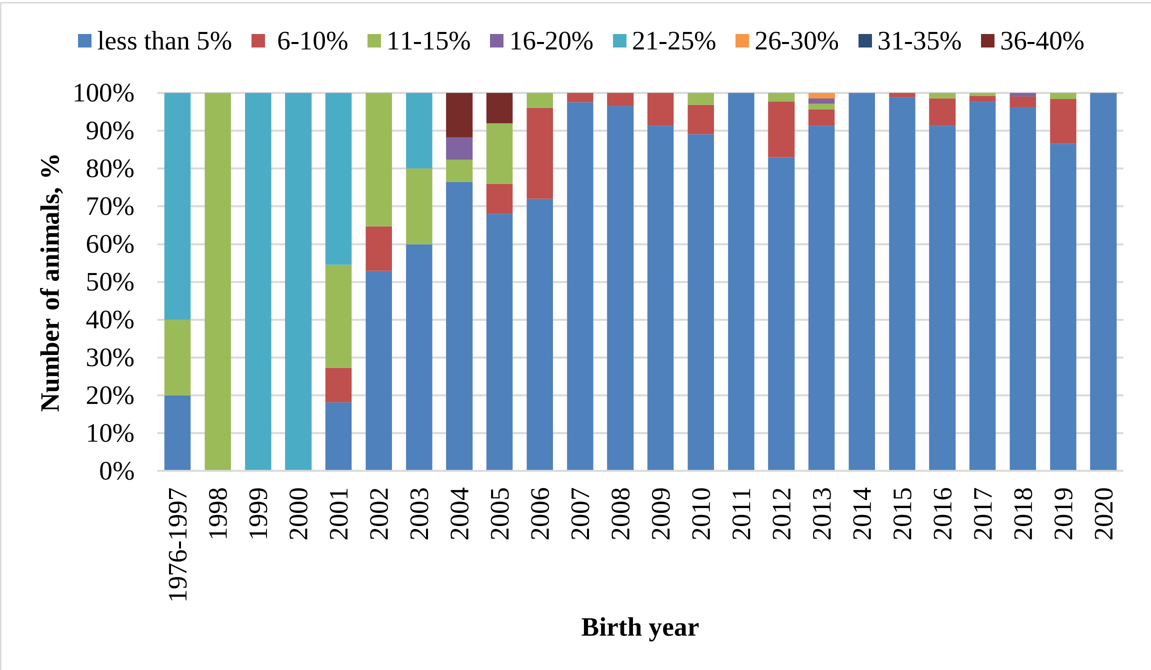

Figure 3. Distribution of inbred animals by year of birth.

In different countries, the level of inbreeding in local sheep populations was studied using pedigree information. Investigation in local sheep breed in Germany showed the increase in inbreeding rates approximately 1% per year during period from 2007 and in 2015 reached around 10% (Schäler et al., 2018). The inbreeding of four Italian local dairy sheep breeds was analysed (Cortellari et al., 2022). In this study, the average coefficients of inbreeding were higher compared to our population and ranged from 1.8% to 9.3% (9.3% for the Sarda breed (n=1053), 6.0% for Delle Langhe (n=104), 1.8% Comisana (n=529), 3.9 % for Massese (n=371)) and only in the Sarda population were sheep with inbreeding level 20 – 35%

(Cortellari et al., 2022). In the Olkuska local sheep breed with pedigree information from 11,344 animals, the level of inbreeding was 6.9% with the average number of complete generation 5.66 $\pm$ 0.85 and the individual coefficient of inbreeding was in the range from 0.8% to 18.8% (Sobieraj-Kmiecik et al., 2020). Compared to the LT population, the Olkuska population did not have animals with an inbreeding level higher than 18%. The results of Sweden Gute sheep, which is a local breed, showed that the average inbreeding is low – 3.8% and the authors concluded that the breeding programme for Swedish Gute is successful and can be used as an example in other local populations (Rochus et al., 2017). According to the distribution of the level of inbreeding there are sheep with inbreeding above 10%. In a Finnsheep population, the proportion of highly inbred animals with inbreeding greater than 6.25% was 14.1% (Li et al., 2011). In local LT population the average level of inbreeding is lower compare to other local population, however there are highly inbreed animals and it's mean the farmer should monitor the level of inbreeding during sheep mating.

Conclusion

The level of inbreeding in LT breed population was not evaluated before. Calculation is based on pedigree of 2295 animals and the number of inbreed animals was 1177 with an average inbreeding coefficient 2.75%. Starting from 2015 the level of inbreeding in LT genetic resources population is around 2-3% which is higher than the recommended inbreeding level (0.5%-1%). However, in the LT genetic resources population are sheep with coefficient of inbreeding higher than 5%. Breeding organization and the breeders on farm level have to monitor the rams and ewes mating and control the level of inbreeding to successful conservation of genetic diversity in LT population.

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COMPARATIVE EFFICACY OF DIFFERENT ACARICIDAL COMPOUNDS AGAINST TICKS INFESTING BOVINE IN AND AROUND RAWALAKOT AZAD KASHMIR, PAKISTAN

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Abstract

To measure the effectiveness of four important types of acaricides such as ivermectin, doramectin, trichlorfon and deltamethrin under field conditions a study was conducted. Randomly total 50 bovines were selected and carefully examined for presence of ticks. Firstly, five groups of animals were made having ten animals in each group. Group A animals were treated with Ivermectin (Ivomec) at 200µg/kg body weight and Group B were treated with Doramectin (Dactomax) at 200µg/kg body weight subcutaneously. Group C was treated with deltamethrin spray, ICI Pakistan (2% solution) at dorsal side of animal body. Group D was treated with sponging of trichlorfon, saguvan (1% solution) on animal body. Group E was given no treatment and kept as a positive control. At 7th, 14th, 21st and 28th days post treatment, the efficacy of the drugs was calculated and at day 7 ivermectin, doramectin and trichlorfon was 75%, 85%, 68% percent effective and deltamethrin was 65% effective while at day 14 these drugs were 57%, 66%, 28% and 49% effective and at day 21 these were 34%, 54%, 17% and 28% percent effective while at 28th day 25%, 37%, 12% and 19% effective that depicts the re-infestation of ticks in all treatment groups from 14th day of treatment. The drugs and the dose interaction showed significant difference among each other with maximum efficacy of drugs at 7th day post treatment. Doramectin was found to be much effective and long lasting drug at single dose application against tick infestation as compared to other drugs. This study exposed at single dose doramectin is comparatively better acaricidal drug for tick control. Improving the hygienic conditions of the surrounding environment associated with treatment of animals after every 14th days is necessary for proper tick control.

Key Words: *Buffalo, Cattle, Ivermectin, Deltamethrin, Husbandry.*

Introduction

Pakistan is eminent farming nation; in the total country's gross domestic product the agriculture sector of Pakistan is contributing 18.2%. The animals are contributing about 11.5% in the GDP and 60% in agriculture. In satisfying the constantly increasing demand of milk, meat, animal protein and also providing draft power for agriculture in the country these animals are playing an energetic role. In Pakistan economy by gross value addition of 3%

during the year 2019-2020 (Rs.1,505 billion from Rs. 1,461 billion) livestock sector played a central role (Pakistan Economic Survey 2020-2021).
In the establishment of livestock population and industry parasitic problems are consider to be one of important hurdle. (Durrani and Shakoori, 2009). Parasitic infestation particularly ecto-parasites (Ticks, mites and lice) are the most important veterinary problem in many developing and under developed countries. The key problem among ecto parasites is tick infestation. The tick infestation cause severe health issues among various ectoparasitic diseases (Ramzan et al., 2008). Economically tick infestation is thought to be an important issue in domestic animals. The annual global economic losses have been expected US$14000-18000 million due to tick infestation, and in livestock of Pakistan and India the price of management of Tick borne diseases is as high as US$ 498.7 million per annum (Minjauw and McLeod, 2003).
Usually two important types of losses are associated with ticks. The more burdens of ticks, anemia/ blood loss, udder and hides damage, infection associated with toxins are categories as direct losses. The diseases spread by ticks can led to weakness or even death these are called indirect losses related with the ticks (Nady et al., 2014). In the world tick and their related diseases badly affect livestock health status. In livestock of Pakistan variety of ticks are found and diverse diseases are spread by ticks. Different factors play role in boosting up the severity of ticks and their related diseases, some important factors among those are: Area/locality, type and population of hosts, the animal species, and status of farmers both social and local and the acceptance and adaptation of technologies against ticks for their control (Jabbar et al., 2015). Both ticks and tick borne diseases together effect the dairy industry of Pakistan by affecting milk production and damaging the hide quality hence impacting leather industry (Nady et al., 2014). For tick control the most common method used in small and large ruminants is the periodic application of acaricides i.e. macrocyclic lactones, trichlorfon and cypermethrin etc. (Iqbal et al., 2017). Given the significance of ecto-parasites and the economic losses they cause, this study was conducted to evaluate the efficacy of various drugs against ticks. The efficacy of ivermecin, doramectin, deltamethrin and trichlorfon was assessed against ticks on 7th, 14th, 21st and 28th day post treatment.

Material and methods

Study area

In District Poonch Azad Kashmir, this experimental study was conducted to estimate the effectiveness of ivermectin, doramectin, deltamethrin and trichlorfon against ticks.

Examination of animals

Positive animals were used in treatment trial. Fifty animals that were heavily infested with ticks were divided into 4 groups as A, B, C, D and E. In every group 10 animals were placed. Group A was given treatment of Ivermectin 200µg /kg as subcutaneous injection and Group B was treated with doramectin 200µg/ kg as a subcutaneous injection , Group C was treated with Deltamethrin spray, ICI Pakistan (2% solution) applied at dorsal side of animal body. In Group D sponging of Trichlorfon, Saguvan (1% solution) on animal body was done and Group E was kept as control. Efficacy of drugs was calculated at different days’ post treatment on the basis of reduction in tick count (Table, 1).

Table 1. Experimental design for acaricidal treatment of tick infested animals

Groups	**Acaricide used**	**Dose**	**Efficacy (Days post treatment)**
A	Ivermectin	Single 200µg / kg	7^{th} , 14^{th} , 21^{st} ,28^{th}
B	Doramectin	Single 200µg/ kg	7^{th} , 14^{th} ,21^{st} , 28^{th}
C	Deltamethrin	Spray (2%)	7^{th} , 14^{th} , 21^{st} , 28^{th}
D	Trichorfon	Sponging (1%)	7^{th} , 14^{th} , 21^{st} , 28^{th}
E	---------	------	7^{th} , 14^{th} , 21^{st} , 28^{th}

After treatment tick score s was performed for acaricidal efficacy using 1-4 scale (Table, 2) (Arias et al., 2015).

Table 2. Ticks scoring for acaricides efficacy after treatment

Score	**Tick burden**	**Estimated number of Ticks**
1	Heavy	> 25
2	Moderate	15-25
3	Low	<15

By using the below formula of (Malik et al., 2021). The efficacy of the drugs was calculated
% efficacy = C –T/C X 100
Where: C = in the control group the mean number of ticks per animal and T = in the treatment group the mean number of ticks per animal.
Two way ANOVA and Turkey's test was used to analyze treatment trial where ticks were dependent variables and drugs and days were independent variables. Statistical package for social sciences (SPSS) version 20 software was used.

Results and discussion

Efficacy of Drugs

Animals selected for drug trial had heavy burden of ticks present on their body at first day of treatment. It was observed that ticks started dropping off the host just on applying drugs at recommended dose rate.The reaction of ivermectin, doramectin and trichlorfon was quick and spontaneous at 7^{th} day post treatment.
Animals treated with ivermectin, doramectin and trichlorfon were almost free from ticks. while deltamethrin treated animals have more tick burden as compared to other groups. At 14^{th} day of treatment re-infestation was observed. Animals treated with ivermectin and deltamethrin were showing moderate tick burden, doramectin treated animals were showing low and trichlorfon treated animals were showing heavy tick burden at day 14 post treatment. Ivermectin, deltamethrin and trichlorfon treated animals were showing heavy tick burden but doramectin treated animals were showing moderate tick burden at day 21. Animals in all treatment groups showed 100% re-infestation at day 28 post treatment, while control group remain positive and showed an increased intensity of tick with the passage of time as shown in (Figure 1).

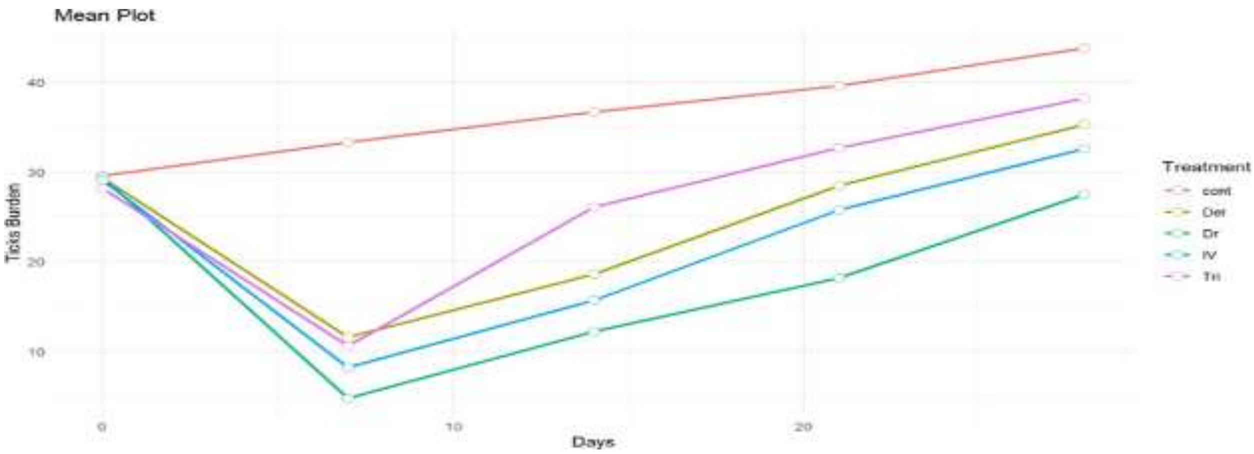

Figure 1. Effect of diverse drugs at different days against ticks on tick infested animals.

Efficacy Percentage

The efficacy of the drugs was calculated and at day 7 post treatment ivermectin, doramectin and trichlorfon was 75%, 85%, 68% percent effective and deltamethrin was 65% effective, while at day 14 these drugs were 57%, 66%, 28% and 49 % effective and at day 21 these were 34%,54%, 17% and 28% percent effective while at 28th day 25%, 37%, 12% and 19% effective that depicts the re-infestation of ticks in all treatment groups from 14th day of treatment.

The calculated efficacy of the drugs at day 7 post treatment for ivermectin, doramectin and trichlorfon was 75%, 85%, 68% percent effective and deltamethrin was 65% effective, while at day 14 these drugs were 57%, 66%, 28% and 49 % effective and at day 21 these were 34%,54%, 17% and 28% percent effective while at 28th day 25%, 37%, 12% and 19% effective that depicts the re-infestation of ticks in all treatment groups from 14th day of treatment.

Related to doramectin efficacy the results of present study are almost similar to the one reported by (Gokbulut et al., 2010). According to (George and Davey, 2004) efficacy of the doramectin injection is very good as compared to other drugs but the efficacy percentage start reducing if treatment is not repeated after two weeks. Similarly, (Caproni et al., 1998) also reported that as compared to ivermectin and other acaricides efficacy of doramectin is higher.

Contradictory to our findings (Ramzan et al., 2008) reported that ivermectin subcutaneous injection give hundred percent cure against ticks, (Davey et al., 2005) also claimed that to obtain control against tick ivermectin is more effective as compared to moxidectin, (Empel and Koanacki, 1990) and (Kutzer et al., 1990) further recorded that for the complete cure against ecto-parasites a single subcutaneous injection of ivermectin was sufficient.

Ivermectin and doramectin both belongs to class of Avermectins and are Glutamategated chloride channel allosteric modulators. When considered the pharmacokinetics, persistence of doramectin is significantly longer than ivermectin, doramectin is also newly introduced and less common in practice as compared to ivermectin this may have a positive effect on its efficacy.

Because of repeated use resistance can develop against anti-parasitic drugs. Moreover, after application of two doses of ivermectin better results can be obtained whereas after single administration of doramectin longer and persistence efficacy is obtained. It is generally accepted that from many years ivermectin is in use against ectoparasites and a large number of treatments results in an earlier development of resistance this can also be a reason for its less efficacy (Gokbulut et al., 2010).

Likewise (Ravichandran, 2018; Asmma et al., 2014; Haque et al. 2011) also reported same efficacy of deltamethrin when it is compared with other acaricides. Deltamethrin belongs to group of pyrethroids that are sodium channel modulators. In different states of Brazil (Furlong et al., 2007; Pereira, 2006) also reported similar efficacy of deltamethrin as observed in our study.
A higher efficacy of deltamethrin was reported by (Ofukwu and Akwuobu, 2010) which is totally contrast to our findings. However, when compared with other drugs prolonged effect of deltamethrin is not observed after 14 days of treatment the reason could be its topical application as topical acaricides work rapidly but are not long lasting.
Trichlorfon is member of organophosphate group and is cholinesterase inhibitor. Like current study (Jariko et al., 2020; Seddiek et al., 2016) reported almost same results for trichlorfon at single dose against effective tick control in cattle.
Similarly (Sarchahi, 2005; Adamo et al., 2001) also reported the same results, and according to their findings trichlorfon is an effective and safe treatment for ectoparasites but treatment should be repeated at intervals to achieve better results.
According to (Bianchi et al., 2003) the differences observed in efficacy of different acaricides can based on the way they are used, including methods of application such as inadequate spraying, under dosage or over dosage etc. (Furlong and Martins, 2000) also concluded that high frequency of acaricidal use cause the development of resistant.
In present study at initial days of treatment efficacy of topical antiparasitic drugs was high and it decline very fast with the passage of time. The reason behind this can be these drugs are only absorbed in skin due to topical administration but don’t go to the blood directly so, their efficacy is much lower than injectables as injections reach in the blood first and show long lasting effects.
The efficacy of drugs reaches at their maximum at 7 days post treatment in our study. Reinfestation occurs at 14 days post treatment in more than 50% animals in this district. The reason behind re-occurrence are: mixed animals housing, animal houses were not tick free zone because cracks and crevices found in the wall and floor and ticks hide in these particular areas. Grazing area of animals is also not tick free; animals went out for grazing can become infested again with ticks and infested animals spread ticks to non-infested ones when they come in contact with each other.

Conclusions

All acaricidal drugs used in the study were showing maximum efficacy 7th day post treatment but Doramectin showed prolonged effect as compared to ivermectin, deltamethrin and trichlorfon when used against ticks, therefore, it appears that doramectin is better acaricidal compound. The decline in drug efficacy can be due to increased resistance due to widely used acaricides. Frequent use of these compounds with increased dose rate led to an increase in tick population. This study was made for single application of acaricides without any further treatment repetition; this is the reason why ticks are returning back in short time. Progress of sanitary measures with unadorned treatment of diseased animals at 14 days’ intervals and also maintaining hygiene of surrounding environment are advisable for tick control.

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Author’s Role: MUH, AR and AS scheduled and planned the research experiment. AM completed the research trials and wrote the primary draft of document. MAASH scrutinized the data.

Conflict of Interest Declaration:

The authors declare there was no conflict of interest.

Ethical Report:

Earlier to treatment trial on tick infested bovine former approval was obtained from the owners.

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THE FUNCTIONAL CHARACTERISTICS OF STARCH IN GROWING PIG NUTRITION

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Abstract

This research aimed to analyse the effect of amylose to amylopectin ratio on the enzymatic digestion of starch, the starch availability for microbial fermentation, and on productive performances of growing pigs. Swine diets are rich in carbohydrates, especially starch, which is the main energy source. Starch from different feedstuffs ranges from low to high amylose, which makes it rapidly digestible in the proximal parts of the intestine to poorly digestible, but fermentable in the lower gut. Starch with different digestion characteristics in diets for growing pigs affects intestinal morphology, dietary energy concentration, growth performances, feed efficiency, and meat quality. Starch usually contains 70-80% of amylopectin and 20-30% of amylose, while waxy starch contains less than 1% of amylose, and in legume grains starch, amylose content varies between 14-88%. Greater slowly digestible starch levels may provide a better gain to feed ratio in growing pigs. Dietary starch with a higher content of amylose and with lower digestibility provides a favorable environment for microbial growth in the distal gut, short-chain fatty acids (SCFA) production, and population of *Bifidobacterium* spp. and *Lactobacillus* spp. Amylose content in starch ≥40% is required to have beneficial effects of dietary starch on bifidobacteria and butyrate intestinal production in growing pigs, but growth rate and feed intake are likely to be lower on these rations. Diets with higher amylose to amylopectin ratio could improve the intestinal digestive and absorptive capability.

Keywords: *Swine, Amylose, Amylopectin, Growth performance, Intestinal fermentation.*

Introduction

Starch is an important nutrient for swine where on average accounting for 55% of the diet, whereby his analyses are mostly related to quantity, but starch also has a range of specific and functional characteristics.

The main part of the α-linked carbohydrates is degraded to monosaccharides by the endogenous enzymes (Stojanović, 2020). Particularly the carbohydrate fractions that are not degraded by endogenous enzymes have an impact on the physiology of the gut due to their interactions with the microbiota and the mucosa of the gastrointestinal tract, which is considered important in intestinal health (Bach Knudsen et al., 2012).

The rate and extent of enzymatic starch digestion depends on starch chemical characteristics, starch granule structure, feed particle size, and processing method (Stojanović et al., 2005). An amylose content is a significant factor affecting starch digestibility. Unlike highly branched amylopectin, amylose polymers have less surface area and more intramolecular hydrogen bonds (Stojanović, 2021). Therefore, amylose is digested at a slower rate and extent than amylopectin due to decreased accessibility for α-amylase.

Resistant starch is a fraction that is not digested in the small intestine but passes to the large intestine where is fermented (Higgins et al., 2004). Starch with high amylose content and a slower rate and extent of digestion decreases glucose absorption and increases short-chain fatty acids (SCFA) absorption in the portal vein, similar to fiber (Regmi et al., 2011).
Different starch types affect the utilization of energy and the metabolisms of protein and lipids in pigs (Yang et al., 2015). Starch from different feedstuffs ranging from low to high amylose, which makes it rapidly digestible in the proximal parts of the intestine to poorly digestible but fermentable in the lower gut, changing consumed starch from a source of glucose to SCFA (primarily acetate, propionate, and butyrate) source, similar to fiber. Enzymatic digestion of starch in the small intestine produces glucose as a final product for absorption. Non-degraded starch can be fermented in the large intestine yielding SCFA, which pigs can use as an energy source (Adamović et al., 2005). Also, may have a beneficial effect by selectively stimulating the growth and activating the metabolism of health-promoting bacteria in the intestinal tract thus improving the swine intestinal physiology. This review will address different aspects of starch fractions' role and functional characteristics in swine diets.

Functional characteristics of dietary starch

Starch usually contains 70-80% of amylopectin and 20-30% of amylose, while waxy starch contains less than 1% of amylose (waxy potatoes, waxy corn, waxy rice), and high amylose starch contains more than 70% of amylose (high amylose corn 46-55%), (Martens et al., 2018). In legume grains starch, amylose content varies between 14 and 88%.

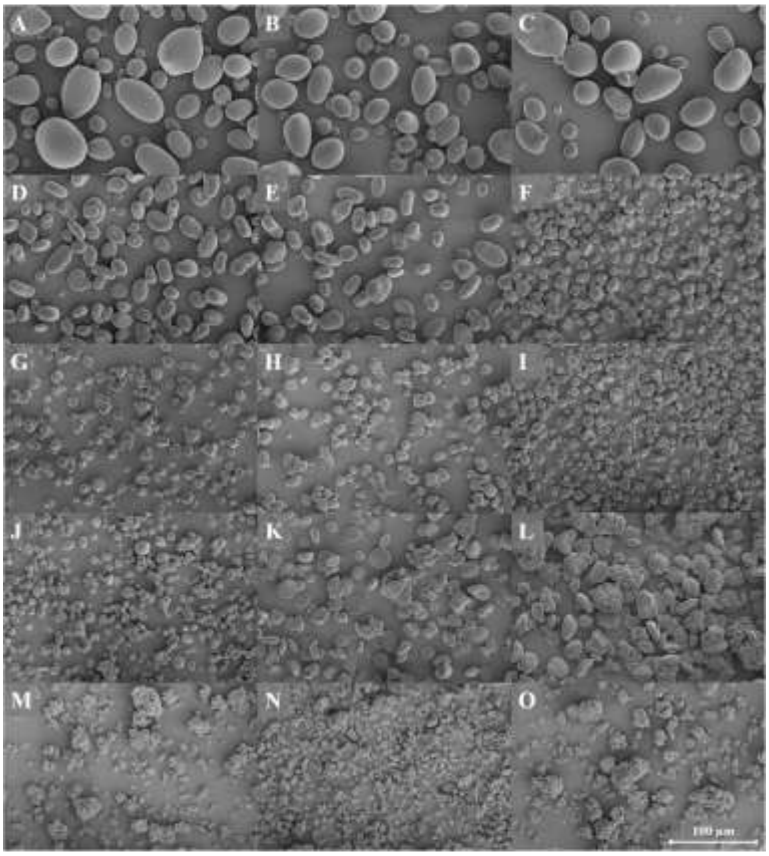

Figure 1. The granular structure of starch from different sources: A-potato, B-waxy potato, C-heat moisture treated potato, D-pea A, E-pea B, F-waxy corn, G-corn A, H-corn B, I-high amylose corn A, J-high amylose corn B, K-barley, L-wheat, M-waxy rice, N-rice A, O-rice B (Martens et al., 2018).

Amylose to amylopectin ratio is the main factor affecting starch digestion. According to its molecular configuration and structure, amylose is not easy to digest in the small intestine, while amylopectin is readily digestible, which may lead to a rapid increase in postprandial blood glucose and insulin levels. Unlike highly branched amylopectin, amylose has less surface area and more intra-molecular hydrogen bonds that result in less accessibility to α-amylase and therefore is digested at a lower rate and to a lesser extent than amylopectin (Singh et al., 2010).

A diet with amylose to amylopectin ratio of 0.40-0.60 could improve the intestinal digestive and absorptive capability by affecting small intestinal morphology, digestive enzymes, and nutrients absorption in weaned piglets (21-50 days), (Wang et al., 2022). But, on the other hand, these diets could reduce the daily gain and feed conversion rate of piglets.

Feeding the weaned piglets with a diet containing 160 g/kg potato starch (source of resistant starch) increases the villi length at the mid-small intestine, which was positively correlated with average daily gain. The colon crypt depth was increased with a higher dietary content of resistant starch. The concentration of SCFA increased in the large intestine with increasing amounts of resistant starch in diets (80 and 160 g/kg), whereas the proportion of acetic acid decreased, while the proportion of butyric acid increased. The increased SCFA production, especially of butyrate, may explain the positive effect on intestinal morphology (Hedemann and Knudsen, 2007).

According to Telles et al. (2023) the rapidly digestible starch fraction is greater in corn grain (31.1%) than in broken rice (28.5%), or wheat grain (26.1%), while the greatest slowly digestible starch content can be found in broken rice (39.5%), followed by corn grain (17.3%), and wheat grain (8.4%), whereby resistant starch fraction is greater in corn grain (21.5%), followed by wheat grain (15.0%), and broken rice (11.4%).

The weaned piglets (21-59 days) fed diet with 25% replacement of corn grain by broken rice had a greater average daily gain than pigs on corn grain based diet, or diet with 50% replacement of corn grain by broken rice (25%) and wheat grain (25%), (280, 234, and 233 g/day, respectively), and also better gain to feed ratio compared with animals that received corn-based diet (0.92 and 0.85), (Telles et al., 2023). The authors concluded that the growth performance of piglets was influenced by diets with moderately greater slowly digestible starch levels, although the starch profile in feed ingredients did not change serum levels of glucose, insulin, and urea.

At finishing pigs, a diet with the amylose/amylopectin ratio of 0.28 that contained pea starch compared with diets (amylose/amylopectin ratio 0.07 and 0.19) containing waxy maize starch and nonwaxy maize starch increased the average daily gain (1.04 kg/day vs. 0.92 and 0.98 kg/day) and the loin eye area (48.05 cm^2 vs. 46.20 and 46.75 cm^2), while decreased the feed to gain ratio (2.81 kg/kg relative to 3.02 and 3.13 kg/kg) and back fat (2.07 cm relative to 2.27 and 2.21 cm), (Li et al., 2017). This is likely due to the fact that after feeding, the digestion of amylopectin high starch results in a sharp but short increment in blood glucose and insulin, which favor nutrient partitioning to fat deposition. In contrast, a gradual increase in blood glucose from the digestion of slowly digestible starch provides a prolonged release of insulin in the blood, which might be more efficient for lean deposition. The energy cost of muscle tissue deposition is less than that of adipose tissue, and according to this lean deposition is more effective for weight gain.

Giuberti et al. (2012) found that amylopectin hydrolyzes faster and has a higher blood glycemic index in pigs. Gao et al. (2023) reported that lipid gain and lipid:protein gain in the young pig group fed high amylopectin diet was significantly higher than that in the high-amylose group. Feeding high amylose diets increases post-prandial lipid oxidation and therefor may reduce fat deposition in the long-term (Higgins et al., 2004).

Using a diet for finishing pigs (initial BW 75 kg) with a high amylose/amylopectin ratio (30:70) compared to a low amylose/amylopectin diet (12:88), decreased the triacylglycerol and cholesterol concentrations in plasma, reduced the lipid contents in liver, tended to reduce the intramuscular fat content, and increased the firmness and loin-eye area, with no significant effect on growth performance (Yang et al., 2015).
Like with dietary fiber, the similar effects of dietary fermentable starch on digestive physiology such as reduced digestibility of CP and increasing endogenous N losses, can be expected (Zijlstra et al., 2012). Compared with enzymatically digestible starch and glucose, the fermentable resistant starch is less efficient in converting into body energy (about 70%), (Noblet and van Milgen, 2004).
Compared with the high amylose diets (starch amylose to amylopectin ratio 3.09 and 1.47), the high amylopectin diets (starch amylose to amylopectin ratio 0.15 and 0.12) increased the average feed intake of weaned piglets (0.91 and 0.96 kg/day vs. 1.05 and 1.01 kg/day), and average daily weight gain (0.61 and 0.62 kg/day vs. 0.65 and 0.62 kg/day), but the feed to gain ratio was better for high amylose diets (1.49 and 1.56 kg feed/kg gain vs. 1.62 and 1.63 kg feed/kg gain), (Gao et al., 2023). The high amylopectin diets improved the nutrient digestibility of dry matter, CP, and energy, as well as energy retention (RE) and dietary NE concentration linearly increased with the higher content of dietary amylopectin. The equation for predicting the diet retained energy (RE) according to dietary amylose to amylopectin ratio (AM/AP) was established as RE (kJ/kg) = $(1235{,}243 - 48{,}298 \times AM/AP) \times 4.184$.

Table 1. Effects of dietary starch amylose/amylopectin ratio on protein, fat and energy deposition in pigs (Gao et al., 2023).

Item	Amylose/amylopectin ratio				
	3.09	1.47	0.25	0.15	0.12
Initial BW, kg	10.10	10.14	10.13	10.24	10.28
Final BW, kg	27.27	27.37	27.81	28.39	27.63
Protein gain, g/day	86.15	93.24	95.17	94.46	92.12
Lipid gain, g/day	52.46	65.37	67.97	85.89	72.81
Lipid:protein, g/g	0.61	0.70	0.72	0.91	0.79
Energy retention, MJ/day	4.18	4.73	4.81	5.57	5.10

Starch gelatinization and the disruption of the structure of starch granules (during the thermal treatment) increases the surface area and thereby the interactions between the starch and the digestive enzymes, which increases the ileal digestibility of starch and reduces resistant starch content (Stojanovic et al., 2023).
Diets containing different starch types can affect the enzymatic digestion of starch and starch availability for microbial fermentation in the gut. Dietary starch with a higher content of amylose and with lower digestibility increases digesta mass (provides a favorable environment for microbial growth in the distal gut), SCFA production, and population of *Bifidobacterium* spp. and *Lactobacillus* spp. in the gut (Bird et al., 2009). These bacteria establish an efficient barrier to the invasion and colonization of the gut by pathogenic bacteria. Butyrate improves immune surveillance and increases the growth and differentiation of enterocytes. After fermentation of undigested starch in the large intestine, the produced SCFA provides 60-70% of the energy for colonocytes.
Growing pigs fed a diet containing the highest amylose starch (63% vs. 28, 20, and <5%) had greater ileal digesta flow of DM and starch, postileal digestion of DM and starch, and fecal output of DM, starch, and CP, as well as the lower pH of ileal digesta (Regmi et al., 2011). The diet high in amylose content selectively promoted *Bifidobacterium* spp. in the large intestine. Authors also concluded that ≥40% amylose content in starch, is required to have

beneficial effects of dietary starch on bifidobacteria and butyrate production in the gut. However, growth rate and feed efficiency are lower when pigs consume a high amylose diet. The reduced enzymatic digestion and increased fermentation of starch probably contribute to lower energetic efficiency that results in lower feed efficiency of pigs fed diets containing high amylose starch.

Table 2. Effect of different amylose content in dietary starch on growing pig intestinal digestion, microbial fermentation, and performances (Regmi et al., 2011).

Item	Starch amylose content, %			
	<5	20	28	63
Growth				
Weight gain, g/day	718	760	762	624
Feed efficiency, kg gain/ kg feed	449	498	483	387
Ileal digesta				
DM flow, mg/g DM fed	24.5	25.6	35.6	291
Starch flow, mg/g DM fed	1.43	1.32	14.6	211
CP flow, mg/g DM fed	13.0	12.4	10.7	7.64
pH	7.53	7.50	7.04	6.62
Feces				
DM output, mg/g DM fed	64.9	64.0	62.9	107.0
Starch output, mg/g DM fed	0.32	0.32	0.34	2.49
CP output, mg/g DM fed	15.1	14.3	14.7	24.8
Postileal starch digestion, mg/g DM fed	1.11	1.03	14.3	209
Bacterial groups in feces, log10 16S rRNA gene copies/g wet weight				
Lactobacillus group	5.63	5.65	5.57	6.28
Bifidobacterium spp.	5.57	5.23	5.11	6.58

The high-amylose maize starch is very effective in increasing the colonic bifidobacteria numbers, comparable with established prebiotic oligosaccharides, such as the inulin-type fructans.

Weaned piglets fed a diet containing high-amylose maize starch (85%) compared to a diet with a highly digestible waxy starch (0% amylose) for 21 days, had lower ileal starch digestibility (87.8 and 96.2%), greater colon digesta wet weight (1000 and 440 g), longer colon length (3.07 and 2.58 m) a higher concentration of SCFA (by about two- to threefold), lower digesta pH (by about 1 unit), and fecal and proximal colonic lactobacilli and bifidobacteria numbers were significantly higher (Bird et al., 2007).

Conclusion

The inclusion of starch sources with different digestion characteristics in diets for growing pigs affects intestinal morphology, dietary energy concentration, growth performances, feed efficiency, and meat quality. High amylopectin content in starch positively correlates with the energy content of diets, average daily feed intake, and gain. The growth performance of pigs and the lipid:protein gain may be positively influenced by moderately greater slowly digestible starch levels in diets. High amylose starch can act as a versatile prebiotic. Resistant starch may interact with the digestive processes along the entire gastrointestinal tract, with the microbial community, and influence the structure and function of the intestine.

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EVALUATING THE INCLUSION OF PUMPKIN SEED CAKE IN DAIRY COWS DIET ON MILK PRODUCTION

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Abstract

Pumpkin seed cake, a byproduct of pumkin seed oil processing, is used as lactating cows feeds. Due to the high content of crude protein and high concentration of unsaturated fatty acids, pumpkin seed cake could be a good quality feedstuff for ruminants. There is an increased interest in finding alternative protein feeds that can be produced to substitute concentrate components of diets. This study was conducted to evaluate pumpkin seed cake as a substitute for the concentrate component in the diet of lactating cows based on milk production. Eight lactating cows were included in this study with 21 days period of investigation. The cows were divided into two groups: group one was in 60-90 days of lactation and group two was in 120-150 days of lactation. Milk production was measured for 7 consecutive days, from days 1 to 7, 8 to 14 and 15 to 21, respectively. Results of this study showed that pumpkin seed cake is a great source of protein (65.49% of DM) for ruminants, end especially for high producing cows. An increase in milk yield was recorded in this study by 6.68% in the first cows group, and by 7.92% in the second cows group where cow’s diet was supplemented with pumpkin seed cake.

***Keywords**: pumpkin seed cake, cows’ nutrition, milk yield.*

Introduction

One of the most important sources of protein for ruminants is soybean meal, which is characterized by a high protein content and a favorable amino acid profile. Due to its high price, alternatives are increasingly used in animal nutrition. One of them are pumpkin seed cakes, which are interesting because of their availability and nutritional value (Klir et al., 2017). As Vasta et al. (2008) indisated high and variable soya bean prices has led to total replacement of soya bean as protein source for ruminants with alternative feedstuffs. From this point of view Zdunczyk et al. (1999) concluded that pumpkin seed cake is a by-product with potential for ruminants feeding, with a higher crude protein concentration than soya bean meal.
Pumpkin (*Cucurbita spp.*) has been recognized as a pioneering crop, providing a balanced diet, and displaying better adaptation to less favorable soil and atmospheric conditions in comparison to other crops (Kokić et al., 2024). The different species of pumpkin are grown for their seeds and fruits, serving primarily as a nutritious food source that provides high-quality oil and valuable nutrients like protein, essential fatty acids, and dietary fiber (Hosen et al., 2021).
This by-product of pumpkin seed processing could serve as a valuable protein source for dairy farmers in the future. It has a relatively high content of crude proteins (598 g/kg) compared to

oilseeds, and is characterized by a high content of lysine (32 g/kg) and methionine (18 g/kg). In addition, it improves the palatability of concentrated feed for ruminants.
In research conducted by Chinese scientists from the University of Harbin (Li et al., 2023) cake can replace soybean meal in ruminant feed for lactating cows. They examined feed efficiency, rumen fermentation, antioxidant function and nitrogen distribution in a feeding experiment. Six multicalf cows were randomly divided into three treatment groups during the 27-day experiment, and the proportion of solid feed in all three groups was the same: the first group was fed soybean meal, the second group had soybeans replaced with 50 percent seed cake pumpkins and 50 percent of the dry residue from bioethanol production, the third group was given 100 percent cake. The result of the research showed that this cake can completely replace soybean meal without a negative impact on milk yield, rumen fermentation or digestibility. It is interesting that the antioxidant functions and blood parameters of the dairy cows in the second and third groups improved by changing the diet. It was concluded that this type of food is a useful source of protein for ruminants and that it did not affect rumen degradation, digestibility. The distribution of nitrogen between milk, faeces and urine did not differ in animals fed three meals.
The aim of this study was to evaluate the potential of replacing the concentrate component of dairy cow feed with pumpkin seed cake to enhance milk production in high-yielding dairy cows.

Materials and methods

The experiment was carried out at the Agricultural Combine “Zlatibor” and chemical analysis conducted at Institute for forage crops Kruševac in Serbia. Eight lactating Simmental dairy sows were used in this study. Days in milk ranged from 60 to 90 days (4 cows: RSXXXXXX-5416; RSXXXXXX-3698; RSXXXXXX-9032 and RSXXXXXX-4210) and from 120 to 150 days (4 cows: RSXXXXXX-4470; RSXXXXXX-0808; RSXXXXXX-0821 and RSXXXXXX-3206). The experiment last 28 days (7 days of treatment adaptation – control) and 21 days of data collection.
Cows were housed in individual tie-stalls fitted with rubber mattresses, bedded with straw and were fed for *ad libitum* intake. The first group of cows (60-90 days of lactation) was fed with 20 kg of maize silage, 5 kg of grass hay, 3 kg of alfalfa hay and 5 kg of concentrate. Another group of cows (120-150 days of lactation) was fed with 20 kg of maize silage, 5 kg of grass hay, 2 kg of alfalfa hay and 4 kg of concentrate. In the diets for both cows’ groups 10% of concentrate was substituted with pumpkin seed cake (PSC10). All cows were individually fed twice daily at 0830 and 1500h with approximately 70 and 30% of total daily feed allocation at each feeding, respectively. Cows had free access to water.
Cows were milked twice daily at 0600 and 1800 h. Milk production was recorded daily throughout the experiment. Cows were turned outside to a dry lot for exercise for at least 1 h daily in the morning after being milked.
The dry matter content was determined on a sample weighing 1 kg, by drying at a temperature of 60° C to a constant weight. The samples were ground on a mill with a sieve of 2 mm, and than on a laboratory cyclone mill with a diameter of 1 mm. The samples obtained in this way were dried at 105° C to a constant weight. All results related to chemical composition and amount of nutrients are expressed in absolute dry matter. All chemical analyzes were performed in duplicate.
Within the Weende analysis system, the content of crude ash, crude protein, crude fiber, crude fat and nitrogen free extracts were determined, as well as NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber). The content of crude ash was determined by dry burning the sample at 550° C and measuring the ash (AOAC, 942.05). The content of crude protein was calculated indirectly, through the amount of total nitrogen determined by the Kjeldahl method

on a Kjeltec Auto Analyzer (AOAC, 984.13). The content of crude fiber was determined by successive hydrolysis of the sample with a dilute solutions of H_2SO_4 and NaOH (AOAC, 978.10). The amount of crude fat was determined according to Soxhlet, a modification according to Ruškovski, with anhydrous ether (Đorđević et al., 2003). The content of nitrogen free extracts was determined by calculation, by subtracting the total amount of crude protein, crude fiber, crude fat and crude ash from 1000 g of dry matter. NDF (Neutral Detergent Fiber) – fiber insoluble in neutral detergent solution was determined without using Na_2SO_3 according to the metod by Van Soest and Robertson (1980). ADF (Acid Detergent Fiber) – is determined as the fraction of food insoluble in an acid detergent solution, and the amount of lignin by treating this insoluble residue with a 72% H_2SO_4 solution for 3h (AOAC, 973.18).
Data for 7-day milk production were analyzed with a model that included the effect of dietary treatment. Differences were considered significant at $P< 0.05$. Treatment means were compared using a ($P< 0.05$) Tukey᾽s test.

Results and discussion

The chemical composition of the component included in the experimental diet is presented in the Table 1.

Table 1. Chemical composition of the component included in the experimental diet, %

	Alfalfa hay	Grass hay	Maize silage	Concentrate	PSC	PSC10
Moisture	10.92	5.53	35.03	11.06	4.06	9.31
Dry Matter	89.08	94.47	64.97	88.94	95.94	90.69
C. ash	9.08	5.75	1.70	10.45	9.31	8.42
	(10.19)	(6.08)	(4.85)	(11.75)	(9.70)	(9.28)
C. protein	16.70	6.97	2.34	18.52	62.84	22.96
	(18.74)	(7.38)	(6.68)	(20.82)	(65.50)	(25.31)
C. fiber	27.81	42.12	9.23	7.11	5.57	6.40
	(31.22)	(44.58)	(26.34)	(7.99)	(5.80)	(7.06)
C. fat	2.04	1.84	0.78	2.89	13.62	4.66
	(2.29)	(1.95)	(2.22)	(3.25)	(14.19)	(5.14)
NFE	33.45	37.79	18.86	49.97	4.60	48.25
	(37.55)	(40.00)	(53.84)	(56.18)	(4.79)	(53.20)
NDF	48.24	62.08	19.84	42.07	30.76	40.86
	(54.15)	(65.71)	(56.63)	(47.30)	(32.06)	(45.05)
ADF	32.15	24.51	8.12	13.28	12.50	12.90
	(36.09)	(25.94)	(23.18)	(14.93)	(13.03)	(13.78)

PSC – Pumpkin seed cake; PSC10 – 10% replacement of concentrate with pumpkin seed cake; NFE – Nitrogen free extract; NDF – Neutral detergent fiber; ADF – Acid detergent fiber; concentration of nutrients is presented in air dry matter, and values presented in brackets are concentrations of nutrients in avsolutelly dry matter.

Results of our study showed that pumpkin seed cake was very high in crude protein concentration (62.84%). Replacing 10% of concentrate with pumpkin seed cake increased crude protein concentration and decreased NDF and ADF concentrations, due to lower NDF (30.76%) and ADF (12.50%) in pumpkin seed cake compared to NDF and ADF (42.07% and 13.28%, respectively) concentration determined in the sample of concentrate. As expected, concentrate contained higher crude fiber (7.11%) concentration compared with the pumpkin seed cake (5.57%), resulting in a medium range of crude fiber concentrations among diet components. Enishi et al. (2004) reported that pumpkin is a valuable feedstuff for ruminants because of its high content of total digestible nutrients, and it is also an excellent source of

protein. Zdunczyk et al. (1999) pointed out that pumpkin seed cake contains more crude protein than soybean meal (598 g kg-1 versus 474.2 g kg-1, respectively). Budžaki et al. (2018) indicated that pumpkin seed cake has high protein content ranging from 19.4% to 62.3% and is characterized by lower average NDF as well as ADF content compared to other seed cakes.
Milk production of lactating dairy cows fed with PSC10 during 21 days are presented in the Table 2.

Table 2. Milk production of lactating dairy cows fed with PSC10, kg day^{-1}

	Cow	Control	1-7 day	8-14 day	15-21 day
	RSXXXXXX-5416	$23.14^{b}\pm0.8$	$23.28^{b}\pm0.7$	$24.28^{ab}\pm0.4$	$24.57^{a}\pm0.5$
60-90	RSXXXXXX-3698	$23.57^{b}\pm0.5$	$23.57^{b}\pm0.5$	$24.00^{b}\pm0.8$	$24.42^{a}\pm0.5$
day of	RSXXXXXX-9032	$25.57^{b}\pm0.5$	$25.85^{b}\pm0.9$	$27.28^{ab}\pm0.9$	$27.85^{a}\pm0.7$
lactation	RSXXXXXX-4210	$26.85^{b}\pm0.7$	$27.7^{ab}\pm0.5$	$29.28^{a}\pm0.7$	$29.00^{a}\pm0.6$
	RSXXXXXX-4470	$19.57^{b}\pm0.8$	$19.85^{ab}\pm0.9$	$20.42^{a}\pm0.9$	$20.85^{a}\pm0.7$
120-150	RSXXXXXX-0808	$16.71^{b}\pm0.7$	$17.00^{b}\pm0.8$	$17.71^{b}\pm1.1$	$18.14^{a}\pm1.0$
day of	RSXXXXXX-0821	$17.14^{b}\pm1.0$	$17.14^{b}\pm0.7$	$18.14^{a}\pm1.0$	$18.14^{a}\pm1.0$
lactation	RSXXXXXX-3206	$14.57^{c}\pm1.1$	$14.85^{b}\pm1.3$	$15.28^{ab}\pm1.2$	$16.14^{a}\pm0.9$

Different letters denote significantly different means ($P< 0.05$).

Results obtained in this study showed that pumpkin seed cake substituted concentrate component of the diet significantly increased milk production in both cows groups (Table 2). The milk production increasing ranged from 3.6% to 8.9% in the first cows group (60-90 days of lactation) and from 5.8% to 10.7% in the second cows group (120-150 days of lactation). This could be explained by the fact that pumpkin seed cake is characterized by lower structural carbohydrates content and by higher crude protein content. An increase in milk yield was recorded in studies where cow’s diet was supplemented with rapeseed cake (Johansson et al., 2006; Minhejev et al., 2007), hempseed cake (Goiri et al., 2021) and linseed cake (Minhejev et al. 2007), while other studies found no statistically significant changes in milk yield for supplementation with rapessed cake, linseed cake, sunflower seed cake, camelina seed cake or pumpkin seed cake (Lerch et al., 2012; Johansson et al., 2015; Józwik et al., 2016; Li et al., 2023).

Conclusions

There has been an increasing interest in investigating the possibility of incorporating pumpkin seed cake into the diets of dairy ruminants. In both groups of cows in this study, regardless of the inclusion level, the significant effects were observed on milk production. This indicates that pumpkin seed cake has the potential to be used as a feed source for dairy cows, but more investigations shoud be undertaken before valid conclusions about the potential impact of using pumpkin seed cake in ruminant nutrition can be made.

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THE CONTENT OF UREA IN THE MILK OF SIMMENTAL COWS IN DIFFERENT SEASONS AND HOUSING SYSTEMS

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Abstract

Based on the content of urea in milk (MU), the balance of the ration for dairy cows can be determined, in terms of protein and energy content. Also, it is claimed that content of MU is influenced by other factors like season, housing system, breed, lactation stage, and parity. The aim of this study is to examine the influence of season and housing system on the chemical composition of milk, including its urea (MU) content. 7,679 milk samples of Simmental cows from ten farms in Vojvodina were collected and analyzed. The milk samples were divided into four groups based on the season, and two groups based on the housing system, from free and the tied housing systems. The average content of urea in the analyzed milk samples was 16.54 mg/dl. The average content of other milk ingredients: milk fat 4.06%, protein 3.34%, lactose 4.62%, and the average amount of milk in a container was 11.08 kg. Statistically significant differences in urea content were found between seasons, while significance of differences between housing systems varied depending on the statistical model used. The highest average MU content (18.12 mg/dl) was recorded in summer, while the lowest average MU content (14.46 mg/dl) was observed in winter. The average content of MU was lower in Simmental cows in the free housing system (15.08 mg/dl), compared to cows in the tied housing system (17.19 mg/dl), contrary to previous data. Based on the results, it is evident that the average content of MU in the milk of Simmental cows during the winter is at the lower recommended limit for the content of MU (15-30 mg/dl), while effect of housing system is harder to interpret.

Keywords: *Milk, urea, season, Simmental breed, housing system.*

Introduction

The cow's milk is a nutritionally valuable food, containing proteins, fats, carbohydrates, vitamins, minerals and urea, which is part of the non-protein fraction of nitrogen. The content of urea in milk depends on a large number of factors, the most important of which is nutrition (Čuklić and Kalember, 2004; Konjačić et al., 2006; Stipić and Ivanković, 2008; Bendelja et al., 2011; Čobanović et al., 2017). An increase in the content of urea in milk is most often the result of a high protein content in the diet, which indicates an unbalanced diet of dairy cows. For efficient utilization of consumed nitrogen in lactating cows, it is of great importance to provide the appropriate amount and quality of protein in the meal, as well as the optimal energy content (Stojanović et al. 2006). Many researchers have found a high and positive correlation between urea content in blood and milk (Roseler et al., 1993; Prpić et al., 2005; Bendelja et al., 2011). Based on the content of urea in milk, we can estimate the content of urea in the blood, considering that milk is more available and easier to sample (Konjačić et al., 2006).
The urea content of milk varies from herd to herd, as well as between cows in the same herd. The milk urea can be a useful tool to diagnose herd problems and identify opportunities to correct the protein content of the cow's ration. When the protein content in milk ranges from

3.2 to 3.8%, the optimal content of urea in milk should be within the limits of 10 to 15 mg MUN/100 ml of milk (Carlsson and Pehrson, 1994), that is from 15 to 30 mg MU/100 ml of milk (Čuklić and Kalember, 2004; Pintić et al., 2007; Konjačić et al., 2010).
The content of urea in milk is increasingly used as a practical parameter for monitoring the intake of raw proteins and meal energy, that is, checking the utilization of nitrogen substances from food in dairy cows (Jonker et al., 2002; Čuklić et al., 2008). The high content of urea in milk is most often associated with excessive protein intake, which adversely affects the health and fertility of cows, as well as environmental pollution and increased feeding costs (Budimir and Mahmutović, 2013).The season is one of the potentially important factors that affect milk production, its chemical composition, including the content of urea in milk. Thus, total nitrogen and protein (mainly casein) in cow's milk decrease during the summer period, while the content of non-protein nitrogen, which includes urea, increases (Carlsson et al., 1995). Konjacic et al. (2006 and 2010) also determined a significant influence of the season on the urea content in milk, the highest MU content was determined during the summer and autumn periods. They conclude that the observed effect of season on the urea content of cow's milk is a reflection of the overall management of dairy cow farms. Rajala-Schultz and Saville (2003) as well as Bendelja et al. (2011) determined a significantly higher content of urea in milk during spring and summer than during autumn and winter. Hojman et al. (2005) also indicate that the highest content of urea in milk is during spring and early summer.
Cattle production, especially milk production, is characterized by the mechanization of production, which is reflected in the introduction of modern equipment, and therefore in the increase of work productivity, balanced nutrition, and the creation of favorable ambient conditions within stable buildings. Many factors go into deciding which dairy cow housing system to go for. Since the construction of stable space is very expensive, it must be used as economically as possible, that is, that it accommodates as many head as possible and that they are provided with all the necessary conditions for the normal expression of production abilities (Jovanović, 2010). In the construction of farm buildings for dairy cows today, a choice can be made between a tied or free housing system (Lazarević, 1993; Tošić et al., 2002), between these two basic types, there are a number of combinations and transitional forms (Trifunović et al., 2005). Both housing systems have their advantages and disadvantages (Plavšić et al., 2003). Brka et al. (2015) state that when selecting a housing system, special attention should be paid to the health of the animals to prevent injuries such as arthritis, joint injuries, limb damage, and udder injuries. According to Plavša (2021), problems related to health and calving occur in cows in a tied housing system, while in barns with a free housing system, injuries and limb damage are more common. This study aims to examine the average content of urea in the milk of Simmental cows in the area of Vojvodina and the influence of the season and housing system on the content of milk urea and chemical composition of the milk of Simmental cows.

Material and Methods

Milk samples of Simmental cows were collected from ten farms, as part of the regular control of the milk yield of quality breeding cows according to the AT4 method, in the period from 2019 to 2021. All milk samples were analyzed in the Laboratory for Milk Quality Control, at the University of Novi Sad, Faculty of Agriculture, Department of Animal Science. The farms are located in the Province of Vojvodina in Serbia. Of the ten farms included in the research, three farms have a free housing system, while the other seven farms have a tied housing system.
The chemical composition of raw milk was analyzed on the ***FOOS MILKOSCAN^{FT}*** instrument using infrared spectrophotometry and Fourier transform. The content of milk fat,

protein, lactose, dry matter and urea was determined. A total of 7679 milk samples were analyzed.
Data were grouped to better understand the factors influencing milk urea content. The data are grouped based on the housing system, free housing system (three farms - 147 cows) and the tied housing system (seven farms - 353 cows). Also, the data are categorized by season, with Season 1 - Winter (December, January, February), Season 2 - Spring (March, April, May), Season 3 - Summer (June, July, August), and Season 4 - Autumn (September, October, November).
Statistical data processing was performed using analysis of variance (ANOVA) in the *STATISTICA 14.0* software package. The post hoc procedure (Duncan's test) determined the statistical significance of the differences between the means of individual observed groups, with a significance level P<0.01.

Results and Discussion

Data on the average chemical composition of the analyzed milk samples of the Simmental breed are presented in Table 1.

Table 1. Average chemical composition of the analyzed samples

Parameter	N	Average	Minimum	Maximum	SD	CV
Fat (%)	7679	4.06	2.50	8.50	0.87	21.57
Protein (%)	7679	3.34	2.33	4.84	0.34	10.24
The amount of milk in a given milking (kg)	7679	11.08	1.70	22.00	2.60	23.45
Dry matter (%)	7679	12.76	9.09	17.87	1.02	7.97
Lactose (%)	7679	4.62	3.08	5.38	0.25	5.34
MU (mg/dl)	7679	16.54	10.00	53.30	7.61	45.99
MUN (mg/dl)	7679	7.72	4.66	24.87	3.55	45.99

SD-Standard deviation, CV-Coefficient of variation

The analyzed milk samples, from all farms included in the research, on average contained: MU 16.54 mg/dl (MUN 7.72 mg/dl), milk fat 4.06%, protein 3.34%, lactose 4.62%, dry matter 12.76%. The average amount of milk during milking was 11.08 kg. In comparison with the optimal recommendations for MU content of 15 to 30 mg/dl of milk (Pintić et al., 2007; Čuklić et al., 2008; Konjačić et al., 2010), it can be seen that the average MU content in the analyzed milk samples is at the lower limit of the recommendation. A relatively low MU content may indicate an insufficient protein content in the meal. Pintić et al. (2007) reported similar results for urea content in Simmental breed milk, along with other milk quality parameters. Gantner et al. (2006) state that based on the content of protein and urea in milk, the energy supply of cows in the meal can be estimated, i.e. their nutritional status, they also state that during the assessment it is important to take into account the content of milk fat, protein, the lactation phase and the level of production milk.
As part of the research, the influence of the season on the investigated parameters of milk quality and quantity, and MU content was observed (Table 2).

Table 2. Average chemical composition and quantity of milk by season

Season	N	Fat (%)	Protein (%)	The amount of milk in a given milking (kg)	Dry matter (%)	Lactose (%)	MU (mg/dl)
Winter	2147	4.22[a]	3.37[a]	11.26[a]	12.99[a]	4.64[a]	14.46[a]
Spring	2118	4.05[b]	3.30[b]	10.75[b]	12.74[b]	4.67[b]	17.24[b]
Summer	1812	3.79[c]	3.26[c]	11.19[a]	12.36[c]	4.56[c]	18.12[c]
Autumn	1602	4.17[a]	3.44[d]	11.14[a]	12.93[a]	4.59[d]	16.62[b]
F		95.10*	97.00*	16.3*	152.00*	75.00*	88.59*

*Values in the same column with the same lowercase letters do not differ statistically significantly (p>0.01); Between arithmetic means in the same column, with different labels (a,b,c,d), there is a statistically highly significant difference (p<0.01); *p<0.01.*

Based on the results presented in Table 2, it is evident that the lowest average MU content (14.46 mg/dl) was observed during the winter (season 1), while the highest average MU content (18.12 mg/dl) occurred during the summer (season 3). Analysis of variance revealed statistically significant differences in MU content between seasons ($F_{3,7675}$=88.59; p<0.01), indicating a significant seasonal influence on MU content. Duncan's test assessed the significance of differences between individual seasons, revealing statistically significant distinctions in MU content among seasons, except between MU content in spring and autumn. Analysis of variance revealed that the season has a statistically significant effect on the content of milk fat ($F_{3;7675}$=95.10; p<0.01), protein ($F_{3;7675}$=97.00; p<0.01), lactose ($F_{3;7675}$=75.00; p<0.01) and dry matter ($F_{3;7675}$=152.00; p<0.01), as well as on the average milk production ($F_{3;7675}$=16.3; p<0.01). Similar results were obtained by Prpić et al. (2005) who state that in cows with a production of about 7,500 kg of milk per lactation, the content of urea in the milk was higher during the summer period. Likewise, Konjačić et al. (2006) concluded that the season significantly impacts milk urea content, with higher content observed during summer (24.65 mg/dl) and autumn (24.97 mg/dl), while lower content was found during spring (18.90 mg/dl) and winter (18.29 mg/dl). Čobanović et al. (2017) also point out that the season significantly affects the urea content of milk. Nateghi et al. (2014) and Bernabucci et al. (2015), state that among the environmental factors, the season has a very significant effect on the quantitative and qualitative properties of milk, including climate changes that occur in almost all regions of the world. Yang et al. (2013) state that the season has a substantial influence, first of all, on the composition of milk.

On 10 farms included in the research, milk samples from 500 dairy cows were analyzed, the results are shown in Table 3.

Table 3. Average composition of raw milk, observed by housing systems

Housing systems	N	Fat (%)	Protein (%)	The amount of milk in a given milking (kg)	Dry matter (%)	Lactose (%)	MU (mg/dl)
Free	2373	3.94[a]	3.46[a]	11.70[a]	12.74[a]	4.61[a]	15.08[a]
Tied	5306	4.12[b]	3.29[b]	10.80[b]	12.76[a]	4.62[a]	17.19[b]

Values in the same column with the same lowercase letters do not differ statistically significantly (p>0.01); Between arithmetic means in the same column, with different labels (a,b), there is a statistically highly significant difference (p<0.01)

The results presented in Table 3 indicate a lower MU content in the free housing system (15.08 mg/dl) compared to the tied housing system (17.19 mg/dl). Utilizing analysis of variance, it was determined that there exists a statistically highly significant difference in MU content between the observed housing systems ($F_{1;7677}$=128.06; p< 0.01). In the free system,

the content of MU is in the lower recommended value of 15 to 30 mg/dl of milk. Humski et al. (2018) also observed differences in MU content in between farms in different housing systems, as did Ruska et al. (2017). Contrary to our data, they found MU to be higher in free housing system. It is hard to explain these opposite findings, yet it should be pointed out that research by Ruska et al. (2017) was done on just 4 farms, and Humski et al. (2018) on just 2 farms.
Analysis of variance showed that the housing system and the season have a statistically significant effect on the urea content in milk. Further analysis, using a linear mixed model, observing the interaction and farms as a third factor, found that the housing system (fixed factor) ($F_{1;6784.646}$=0.4443; p>0.01) does not have a statistically significant effect on the content of MU, compared to the farm (random factor) ($F_{8;8621,574}$=182.43; p<0,01) and season ($F_{1;6201,892}$=186,89; p<0,01) which have a statistically significant influence. Most probable interpretation is that in this research, as well as in previous two (Ruska et al., 2017, Humski et al., 2018), MU is strongly influenced by a farm and not by a housing system. This seems logical, since feeds, feeding practices and diets are unique to each farm, and are not correlated to a housing system in a single and predictable way.

Conclusions

Based on the presented research results, it can be concluded that the content of urea in cow's milk depends on a large number of paragenetic factors, among which nutrition stands out as the most important. In addition to diet, seasonal variation has been found to affect MU content. Also, differences in the content of urea in the milk of Simmental cows can be observed between farms in different housing systems. In practice, the urea content in milk is increasingly used as a parameter for monitoring the intake of raw proteins and energy, that is, for checking the efficiency of nitrogen utilization from feed in dairy cows. The application of this control method has an important role in the optimization of nutrition, because excessive intake of crude protein can negatively affect reproduction and milk production, in addition, excessive consumption of crude protein increases the energy needs of cows, some protein supplements are very expensive, and excessive excretion of nitrogen has a negative impact on the environment. Ammonia is the main component of harmful gases that cause the so-called greenhouse effect, and dairy cows are considered the biggest emitters. Reducing greenhouse gases, especially ammonia emissions from cattle farms, is one of the main goals in achieving ecologically sustainable milk production.

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CLAW LESIONS IN DAIRY COWS IN SERBIA

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Abstract

Lameness in cows is a major problem, affecting welfare and production results. We aimed in this study to identify claw lesions in lame dairy cows in Serbian farms. A total of 94 Simmental dairy cows (26 primiparous and 68 multiparous) from eight small dairy farms located in the Macva district, Serbia were observed during regular hoof trimming. The clinical examination was focused on all possible alternations on the hooves which were observed. The trimming procedure was performed by the Dutch method. Of the 94 animals presented for a hoof trim, 41 cows (43.6%) had no claw lesions noted. In this study, lesions were present in 53 (56.4%) of all examined cows. In the primiparous group, 13 (50%) cows had affected claws: white line disease 15.9% (4/26), sole ulcers 11.5% (3/26), digital dermatitis 7.6% (2/26), aseptic pododermatitis 7.7% (2/26), overgrown hoof 3.9% (1/26) and double sole 3.9 (1/26). In multiparous cows, 40 (58.8%) had affected claws: white line disease 14.7% (10/68), sole ulcers 16.2% (11/68), digital dermatitis 1.5% (1/68), aseptic pododermatitis 4.5% (3/68), overgrown hoof 11.8% (8/68), and double sole 10.3% (7/68). Our results indicate a wide range of claw lesions. Attention should be paid to treatment and reduction of lameness in dairy cows.

Keywords: *Cows, lameness, claw lesions, Simmental, Serbia.*

Introduction

Lameness is a significant problem on many dairy farms and is ranked as the third most common disorder, behind mastitis and reproductive disorders. There are numerous factors that lead to the appearance of lameness: genetics, diet, laminitis, the type of holding, insufficient wear of the horn, improper processing of the hoof, irregular hoof trimming, soft horn, wet conditions (Barker et al., 2010). Factors such as the way of cleaning of faeces, environmental conditions, and hygiene in stalls are predisposing factors for the appearance of lesions on the hooves (Laven et al., 2006; Bell et al., 2006). Often mistakes in the diet lead to the appearance of subclinical acidosis of the rumen, which the resulting toxic products lead to the appearance of cow laminitis. The main reason is that a concentrated diet fed without enough functional fiber from animal feed leads to less chewing, there is a drop in the pH of the rumen and the production of histamine and toxins originating from the rumen (Bergsten et al., 2003). Laminitis is an aseptic inflammation of the corium of the hoofs, which often leads to non-infectious diseases of the hoofs, such as sole ulcers, white line disease, aseptic pododermatitis (Bojkovski et al., 2023). In Serbia, the frequency of sole ulcers and white line diseases in Simmental cows were 15.7% and 17.5%, respectively (Ninković et al., 2021). Cows with hoof infections such as digital dermatitis suffer from lameness, which leads to reduced milk production and reproductive disorders (Laven et al., 2006). Analyzing the data in the literature, there is not enough data for some claw lesions that are often neglected, such as overgrown hooves, double sole. The aim of this research is to provide information on the frequency claw lesions in cows in Serbian dairy farms.

Material and Methods

A total of 94 Simmental dairy cows, comprising 26 primiparous and 68 multiparous individuals, from eight small dairy farms in the Macva district of Serbia, were observed during routine hoof trimming. The study took place from January to February 2024. All cows had their hooves trimmed in the first 120 days of lactation. All cows enrolled in this study were kept in tie-stall farms, ages from 25 to 92 months with an average yearly milk yield from 5500 to 6800 kg. The average milk production per cow in the first 100 days of lactation was 25.3 L. The nutrition on all farms is identical, based on the use of silage, alfalfa hay and concentrates with 16% protein. All farms had a concrete stall base with deep litter straw beds. Date was collected during regular hoof trimming. The trimming procedure was performed using the Dutch method, with cows fixed in a mobile chute for hoof trimming. Hoof processing was performed by two professional hoof trimmers. All changes to changes on the raisin recorded and treated.
Descriptive statistics were prepared using the office software package (Microsoft® Excel® MSO, 2016). The chi-square test was used to evaluate the significance difference between primiparous and multiparous cows. The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09) and Ordinance on the conditions for registration for experimental animals and the keeping of such a register, training programs on welfare on experimental animals, request forms for approval of conducting experiments on animals, standing, treatment and killing experimental animals and reproduction, circulation, or implementation experiments on animals (Official Gazette of the Republic of Serbia No 39/10).

Results and Discussion

Our results established that 41 (43.6%) cows had no acropodium lesions, while 53 (56.4%) cows showed the following claw lesions. All claw lesion noted author s’ elaboration based on the obtained results and showed in Table 1. A chi-square test showed no significant difference between primiparous and multiparous cows in presence of claw lesions (the p-value is 0.440325) the result is insignificant at $p < 0.05$).

Table 1. Distribution of lesions in examined cows.

	Primiparous n=26	Multiparous n=68	P value
White line disease	4 (15.9%)	10 (14.7%)	
Sole ulcer	3 (11.5%)	11 (16.2%)	
Digital dermatitis	2 (7.7%)	1 (1.5%)	
Aseptic pododermatis	2 (7.7%)	3 (4.5%)	
Overgrown hoof	1 (3.9%)	8 (11.8%)	
Double sole	1 (3.9%)	7 (10.3%)	
Total	13 (50%)	40 (58.8%)	0.440325

Lameness of cows is a major economic problem due to the decrease in milk production. According to the results Onyiro et al., (2008) reported milk losses between 0.78 kg and 5.5 kg per day depending on the level of lameness and the stage of lactation. In our previous research, we found that hoof diseases such as hoof ulcers and white line disease lead to a decrease in the content of milk components such as milk fat (Ninković et al., 2023). This study is a continuation of the study of the epidemiology of hoof disease in cows in Serbia. In the tied holding system, white line disease and sole ulcers stand out as the dominant pathology (Ninković et al., 2022). Previous authors have estimated the frequency of sole ulcers 21.83% cows on seven farms with tie stall housing (Bojkovski et al., 2019). Based on the statements of the owners, they cite the appearance of lameness and reduced production as the reason for hoof trimming. As a consequence, white line disease of the hoofs and overgrown hoofs are the most common lesions in multiparous cows in our study. In numerous studies, risk factors for the appearance of foot disease have been identified, related to genetics, diet, posture, environmental conditions, hoof trimming, footbath routines, age (Barker et al., 2010; Dippel et al., 2010).
Claw overgrown arises due to when horn production occurs at a faster rate than the wear and and often occurs on the hind legs (Bell, 2015). Dembele et al., (2006) reported that that there is no significant difference between farms that practice regular hoof trimming compared to farms that do not implement regular hoof processing in cows with overgrown hooves. The main risk factors may have caused the findings in our study are irregular hoof trimming and presence of laminitis as as the main phenomenon that precedes the onset of lameness and the development of pathological hoof changes. One reason for the wide spectrum of lesions on the hooves may be the fact be due to the fact that some cows develop pathological lesions without noticeable lameness (Solano et al., 2016).
The main reasons for the occurrence of widespread pathology on farms are the economic situation of the owners, lack of education of the owners about the importance of lameness and its consequences for the health of the cows and the profitability of the farm. Based our data, approximately 25 % dairy farms twice a year performed hoof trimming all cows in farms.

Conclusion

In summary, this study it showed a wide range of hoof diseases in cows from Serbian dairy farms. Regular trimming of the hooves twice a year of all cows on farms can be useful in order to monitor the health of the hooves and prevent lameness.

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ANIMAL PRODUCTS AS FUNCTIONAL FOOD AND ALIGNING THEIR USE WITH EU REGULATIONS

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Abstract

Contemporary lifestyle unfortunately entails increasing environmental pollution, compelling us to consider that the food we consume should aid the human body in combating harmful agents it is exposed to daily, thereby preserving health. Functional food refers to food containing natural or artificially added functional ingredients that positively impact human health, used in preventing and treating various diseases. Initially targeting cardiovascular and digestive issues, it has evolved to even preventing or reducing the transmission of sexually transmitted diseases. Omega-3 fatty acids, plant sterols, dietary fibers, bioactive peptides, probiotics, prebiotics, and synbiotics are common functional food additives. These can be incorporated into various foods, with fermented milk products being the easiest. Whey, initially underutilized, gained importance as food scarcity increased. The food industry often uses nutritional and health claims for marketing purposes, as such claims are more visible and understandable to consumers compared to chemical composition and nutritional facts.The aim is to describe the concept of functional food, its historical development, significance, major additives, health effects, and notable products available in our market, within the regulatory framework of the Republic of Serbia, with necessary adjustments for EU legislative compliance.

Keywords: *Animal products, Functional food, EU legislation, Environmental protection.*

Introduction

At the beginning of the 21st century, the industrial world is facing increasing healthcare costs, longer life expectancy, the development of new technologies, and their increasingly harmful effects on human health. To address all these challenges, nutritionists have embraced the concept of "optimal nutrition," which focuses on optimizing the quality of daily diet in terms of nutrient content and other food properties that promote human health preservation. This is where optimal nutrition comes into play, based on the application of functional food, aiming to optimize physiological functions to provide each individual with a healthier, longer, and higher quality life. To achieve this, the optimal selection of nutrients must be based on a better understanding of the interactions between genes, nutritional factors, and diseases, as only such a comprehensive picture can determine an individual's response to beneficial and harmful food ingredients (Ashwell, 2002).

The concept of functional food emerged in Japan in the 1980s as an attempt to reduce healthcare costs required for treating diseases caused by an aging population (Kralik et al., 2010). Functional food refers to foods whose regular consumption can influence certain aspects of human health. Its impact on human health can be preventive or therapeutic. The concept of functional food is based on the fundamental knowledge that a diet rich in certain foods has a direct link to improving the quality of life. Over the years, there has been a growing trend towards enriching food with functional ingredients to prevent diseases, improve health, and thus reduce treatment costs (Jeremić, 2022).

Functional food is food that, in addition to the basic nutrients necessary for growth and development, contains ingredients that improve health or reduce the risk of disease, consumed as an integral part of daily diet (Kralik et al., 2010). Omega-3 fatty acids, plant sterols, dietary fibers, bioactive peptides, probiotics, prebiotics, and synbiotics are common additives to functional food. These ingredients can be incorporated into various types of food, with fermented dairy products being the simplest to apply. Whey, which was initially underutilized, has gained importance with the increase in food shortages.

Numerous scientific studies have shown that consuming food containing functional ingredients improves a person's physical and mental well-being, strengthens the immune system, prevents cardiovascular and malignant diseases, acts as an antioxidant, and maintains the integrity of the digestive organs (Jeremić, 2022). Proper nutrition can help control the body's oxidative state and, therefore, overall health. Generally, the concentration of substances needed for antioxidant synthesis in food can be increased through genetic modifications and animal nutrition or by adding these substances to the finished product (Sretenović et al., 2009).

Miletić et al. (2008) state that functional food possesses functional properties due to the presence of one or more components, biologically active compounds, with favorable physiological effects. After consumption, the biologically active compound is released in the digestive tract and acts at the site of release (fiber and probiotic) or is absorbed and transported to targeted tissues, where it exerts beneficial effects. The amount of biologically active compounds in functional food must be equal to the amount for which a beneficial effect has been proven.

Different markers are used to assess the effectiveness of functional food on health, as it is often impossible to directly measure the impact on health or the reduction of the risk of chronic diseases. Markers of targeted functions and markers of different stages in the onset of health damage are used. The presence of probiotic colonies in the colon indicates successful passage of probiotics through the upper parts of the digestive tract and represents a marker of the targeted phase. Bone density can be used as a marker to assess the effectiveness of functional food that can reduce the risk of osteoporosis (Novaković and Torović, 2014).

The objective of this study is to examine how functional foods, which contain bioactive compounds, can enhance human health and reduce healthcare costs through both preventive and therapeutic effects. Additionally, the study aims to analyse the impact of specific ingredients, such as omega-3 fatty acids and probiotics, on physiological functions and overall quality of life.

Classification of Functional Food

From a simple and practical point of view, functional food can be divided into five groups of products. The five types of functional food are (Čalić et al., 2011):

1. Unmodified and unprocessed food - the simplest functional food category includes fruits, vegetables, and herbs. This is fresh food rich in physiologically active ingredients that positively affect the physiological state of the body and help reduce the risk of disease.
2. Nutrient-enriched products that are naturally found in food - functional food with increased amounts of nutrients lost during processing and food production. Examples of these products include fruit juices with increased amounts of vitamins A, C, and E, as well as milk enriched with vitamin D.
3. Nutrient-enriched products not naturally found in food - a group of functional products with added nutrients not naturally found in food or not present in large quantities.

Examples of these products include fruit juices with added calcium or margarine enriched with plant sterols. This group also includes products with added probiotics and prebiotics.

4. Modified products - food in which potentially harmful and undesirable components are replaced by components that have a positive and beneficial impact. The idea is that this replacement does not affect the quality of the product. An example is products where dietary fibers produced from grains are used to replace fats.
5. Enhanced products - food in which the content of functional ingredients is increased during the production process. Examples include corn with increased lysine content, fruits and vegetables with increased levels of vitamins achieved through special growing conditions. Another example is eggs with increased omega-3 fatty acid content, achieved through altered poultry nutrition. Functional food of animal origin can generally be classified, based on the raw material used, into meat and meat products, and milk and dairy products.

Legal Framework Regulating Functional Food

In 2006, the European Union adopted Regulation 1924/2006 of the European Parliament on food and health claims. This regulation prescribes conditions for labeling, advertising, and presenting food with food and health claims, all with the aim of providing consumers with accurate information about a specific product. The Ministry of European Integration of the Republic of Serbia opened Chapter 12 - Food safety, veterinary, and phytosanitary policy, within which proposals for sublegal acts in the field of food production and food for special groups were developed.

The relevant EU legislation consists of six regulations and three directives that comprehensively regulate this area. The activities of the Ministry of European Integration were based on the transposition and implementation of EU regulations falling under negotiating Chapter 12.

The main goal of nutritional and health claims is the well-being of consumers, indicating the nutritional benefits and potential health advantages provided by the consumption of certain foods. When making food and health claims, they must be accurate and supported by evidence to prevent consumers from receiving incorrect information. A food claim implies, suggests, or concludes that the food has certain nutritional properties determined by its energy value, which can be natural, reduced, increased, or absent, based on the quantity of its natural, added, or subtracted nutrients or other substances. Food claims do not pertain to the reduction of the risk of a specific disease but may only indicate a reduced content of a basic element or that the product contains increased and/or added functional ingredients (Čalić et al., 2011).

The most significant health potential on food labeling is believed to come from messages we call nutritional and health claims, which aim to highlight the nutritional benefits and potential health advantages provided by the use of these foods. The food industry often utilizes these statements for marketing purposes since they are more visible and understandable to consumers than chemical compositions and nutritional facts (Novaković and Torović, 2014).

Health claims establish a connection between the nutrients in food and diseases or various health conditions and must be well formulated. Health claims must not provide false information and should be supported by scientific research (Čalić et al., 2011).

According to the regulation on labeling, advertising, and presenting food, making dietary and health claims must not (Čalić et al., 2011):

- Be incorrect, ambiguous, or misleading,
- Cast doubt on the safety and/or nutritional value of other food,
- Encourage or restrict excessive consumption of a specific food,

- Imply or suggest that a balanced and varied diet cannot provide the necessary amounts of nutrients,
- Indicate, suggest, or imply changes in physiological functions of the body that could cause fear in consumers.

According to the Codex Alimentarius, a nutritional statement is any statement claiming, suggesting, or leading to the opinion that food has special favorable nutritional properties due to the energy it provides in reduced or increased quantity, the nutrients and other components it contains in increased or decreased quantity. The conditions under which the use of nutritional statements is permitted are specified in Article 30 of the Regulation on the Declaration and Labeling of Packaged Foods from 2004 (Novaković and Torović, 2014). Table 1 below shows some nutritional statements and the conditions under which they can be used.

Table 1. Nutritional Statements and Conditions for Use (Novaković and Torović, 2014)

Nutritional Statement	Conditions for the Use of Nutritional Statement
Low energy value	Energy value less than 170 kJ or less than 80 kJ per 100 ml of the product
Reduced energy value	Energy value reduced by at least 30% compared to the energy value of the same or similar food
No energy value	Energy value less than 17 kJ/100 ml
Low fat content	Fat content less than 3 g/100 g or less than 1.5 g/100 ml
Fat-free	Fat content less than 0.5 g/100 g or ml
Low content of saturated fatty acids (SFA)	Saturated fatty acid content less than 1.5g or 0.75 g per 100 g of the product, with SFAs not providing more than 10% of the energy value in both cases
Source of fiber and high fiber content	Fiber content should be at least 3 g (1.5 g) per 100 g of the product as a source of fiber. While high content, fiber content should be at least 6 g (3 g) per 100 g of the product

The European Union supports the development of two types of health claims essential for functional food, namely (Kralik et al., 2010):

- Claims of "improved function" relating to specific physiological, psychological, and biological functions beyond established growth, development, and other normal body functions. This type of claim does not mention diseases or pathological conditions, e.g., "certain indigestible oligosaccharides enhance the growth of specific bacterial flora in the intestines," and
- Claims of "reduced risk of disease development" associated with the consumption of food or ingredients that can help reduce the risk of a particular disease or condition due to specific nutrients or nutrients contained in such food, e.g., "adequate calcium intake can help reduce the risk of osteoporosis in later life."
-

When applying health claims, two types of errors may occur (Bulatović, 2015):

- Type I errors relate to making harmful health claims:
  (a) consumers pay more for food unjustifiably believing they will have health benefits from it, or even worse, expose their bodies to food that poses a danger to their health,
  (b) the credibility of all health claims diminishes, and companies slowly lose their market position.

- Type II errors refer to banning beneficial health claims, which may result from incompetence, irresponsibility, and inadequacy of government management leading to: (a) consumers not being effectively informed and
- (b) companies losing an important source of market positioning and differentiation, while public authorities lose useful means of managing public health.

This is why authorities should collaborate closely with the private sector, consumer groups, academic individuals, and research communities to unleash the full potential in the future development of the functional food market.

Conclusion

Modern society is faced with the fact that the quality of consumed food is often not optimal and lacks necessary nutrients. By adding specific ingredients, it is possible to improve the quality of food and alter its purpose. While food was initially only intended for growth and development, today it represents a measure of life quality.

Functional food not only provides the body with necessary nutrients for normal functioning but also has a positive impact on human health, aiding in the prevention and treatment of various diseases. Therefore, it offers more health benefits compared to conventional/traditional food. The functional ingredients themselves are already present in some foods, while others are added naturally or artificially.

The food industry often uses nutritional and health claims for marketing purposes, as such claims are more noticeable and comprehensible to consumers than chemical compositions and nutritional facts.

Functional food is only at the beginning of its development, and the question is what positive health effects may be achieved through its further advancement. The current challenge is that a significant portion of the population, especially in Serbia, is still unfamiliar with the concept of functional food and its benefits are not fully realized. To overcome this problem, it is necessary to educate people and introduce them to new insights and benefits of using functional food.

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THE INFLUENCE OF POLLUTANTS ON THE CHEMICAL AND MICROBIOLOGICAL QUALITY OF THE RIVER IBAR

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Abstract

The impact of pollutants on the quality of the Ibar River was analyzed in the middle and upper course of the river. Based on the results of the analysis of physico-chemical and microbiological parameters and their comparison with the legal norms of water quality, all samples were found to exceed the maximum permissible concentration (MPC) for one third of the tested parameters, which is why the water of the Ibar River belongs to the third quality class surface water. High concentrations of primarily nutrients and microbiological indicators at these two profiles are a consequence of the inflow of untreated municipal and industrial water, leachate from landfills and polluted water from agricultural land. Increased concentrations of heavy metals of iron and lead in all water samples tested indicate permanent pollution from the active mining plants and flotation tailings whose wastewaters flow into the Ibar. According to the research results, the values of the SWQI index ranged from 72 to 81 points, which corresponds to the descriptive indicator "good" water at all three locations, while according to the Bylaw (Official Gazette of the Republic of Serbia No. 50/2012) the water of the Ibar River at all three locations during both sampling periods corresponds to moderate ecological status.

Keywords: *Ibar river, pollution, water quality*.

Introduction

Rivers in Serbia have a significant role in supplying the population with water, irrigating agricultural land, developing tourism, maintaining sports activities and recreation. However, most of the rivers are the direct recipients of polluted water generated by human activity. Due to the anthropogenic impact, harmful substances (heavy metals, pesticides, fecal microorganisms, etc.) can reach into the surface waters, which in a concentration higher than the MPC negatively affect the physical and chemical quality of water, aquatic organisms and indirectly human health.

The Ibar River is the largest and most important tributary of the West Morava, which springs from six springs below the mountain Hajla (1360 m above sea level) in Montenegro, and flows into the West Morava near the town of Kraljevo. The most important tributaries of the Ibar on the territory of Serbia are the rivers Sitnica, Raška, Studenica, Jošanica and Ribnica (Radojičić, 2005).

The Ibar River is the final recipient for most of the wastewater in its confluence area. The main source of pollution of the Ibar River is untreated communal water from suburbs, garbage dumps, wastewater from mines and mine tailings containing heavy metals, industrial wastewater, as well as agricultural activities in the immediate vicinity of the river. The active

drives of RMHK "Trepča" plant (mines, surface mines, flotation plants), flotation and separation tailings of the plant`s former drive pose a special pollution problem of the Ibar with high concentrations of heavy metals, which are direct polluters of the Ibar River.
The aim of the study was to examine the physico-chemical and microbiological quality of the Ibar River in the period of high (May) and low waters (September) during 2019, as well as the anthropogenic impact on the pollution of the Ibar River. For each sampling location, 24 surface water quality parameters were analyzed. The study included three profiles of the Ibar River, located in the geographical area from the settlement of Rudnica, near the administrative border with the Autonomous Province of Kosovo and Metohija, to the settlement of Baljevac, which is about 17 km from the town of Raška.

Material and Methods

The water of the Ibar River was sampled (large and small waters) at three locations belonging to the middle and upper course of the Ibar River during 2019 in two periods: location L1 settlement Rudnica, location L2 - town of Raška, immediately after the confluence of the Raška River into the Ibar and location L3 - Baljevac.
Sampling was performed in accordance with the standards for surface water sampling SRPS EN ISO 5667-6: 1997 at all sites. Water samples were taken in 5 liters plastic balloons from a 10 cm depth from the surface of the water, and for the analysis of heavy metals in plastic bottles of 500 ml. Water sampling vessels for heavy metal analysis were purified with 5% nitric acid HNO_3 and then washed with distilled deionized water. Before taking the samples, the dishes were washed three times with water from the sampling location. Water samples for microbiological analysis were taken in sterile glass bottles with a volume of 500 ml according to the standard procedure SRPS EN ISO 19458: 1997. Conservation and transport of water samples were performed according to the guidelines SRPS EN ISO 5667-3: 1997.
Physico-chemical analysis of the general water parameters was performed by standard laboratory-analytical procedures defined in the Manual of Standard Methods for Potable Water Testing (1990) and APHA AWWA WEF standards (2012). The total number of coliform bacteria and fecal coliforms in 100 ml of water was determined in accordance with the standard SRPS EN ISO 9308-1: 2010. The metal concentration in the water samples was determined on an AAS Solar Unicam 969 atomic absorption spectrophotometer manufactured by Pye Unicam, England in accordance with the methods of APHA AWWA WEF (2012) and EPA (1983, 2002). Water samples were acidified with 10% HNO_3 and filtered through a 0.45 μm membrane filter (Whatman Merck Millipore Corporation, Darmstadt, Germany), and after the filtration the metals were determined on an AAS apparatus. Prior to heavy metal analysis, the AAS was calibrated according to the manufacturer's instructions.
Water quality assessment was also performed on the basis of the Regulation on the National List of Environmental Indicators (Official Gazette No. 37/2011) by method of calculating the SWQI index - Serbian Water Quality Index (Veljković, N., 2006; Veljković et al., 2012).

Results and Discussion

The results of physico-chemical and microbiological analyzes of the Ibar River for all three profiles are shown in Table 1. The determined values of the examined parameters of the Ibar River were compared with legal regulations, based on which the water classification was performed at each examined site, which is shown in Table 2.

Table 1. Results of physico-chemical and microbiological analyzes of the Ibar River by localities

Parameter	Unit	May			September			MPC II class
		L1	L2	L3	L1	L2	L3	
Water temperature	^0C	15.7	15.2	16	18	18.2	18.1	-
Suspended matter	mg l^{-1}	19	20	12	9	10	7	25
Dissolved Oxygen (DO)	mg l^{-1}	8.7	9	9.2	8.5	8.6	9.2	7
Oxygen saturation of water	%	91.84	94	97.74	94.17	93.44	90.84	70-90
Total hardness ($CaCO_3$)	mg l^{-1}	175	182	178	205	216	207	-
pH	-	8.21	8.15	8.25	8.38	8.2	8.1	6.5-8.5
Electrical conductivity	µS/cm	415	430	395	400	391	407	1000
Ammonium ion (NH_4-N)	mg l^{-1}	0.10	0.22	0.15	0.18	0.35	0.28	0.10
Nitrite (NO_2-N)	mg l^{-1}	0.021	0.038	0.011	0.015	0.052	0.041	0.030
Nitrate (NO_3-N)	mg l^{-1}	1.55	1.75	1.60	1.52	1.96	1.89	3.00
Total Nitrogen oxides	mg l^{-1}	1.321	1.788	1.111	1.415	2.001	1.942	-
Total Nitrogen (TN)	mg l^{-1}	2.15	2.31	1.9	1.95	2.4	2.83	2.00
Orthophosphate (PO_4-P)	mg l^{-1}	0.082	0.102	0.091	0.098	0.125	0.108	0.10
Consumption $KMnO_4$ (COD)	mg l^{-1}	5.3	5.8	5.1	7.2	12.7	11.1	10
B. Oxygen Demand (BOD_5)	mg l^{-1}	2.1	3.5	3.3	3.1	4.2	3.9	4.5
Chlorides (Cl^-)	mg l^{-1}	11.4	9.6	10.3	8.5	14.9	13.5	100
Total Coliform bacteria	no/100 ml	16500	24800	21000	19000	25000	20000	10000
Fecal coliform	no/100 ml	8500	19800	15600	9500	20000	14500	1000
Iron (Fe)	mg l^{-1}	1.396	1.15	1.11	0.863	0.715	0.504	0.50
Manganese (Mn)	mg l^{-1}	0.052	0.038	0.042	0.049	0.035	0.03	0.10
Zinc (Zn)	mg l^{-1}	0.093	0.08	0.052	0.071	0.062	0.038	0.20
Copper (Cu)	mg l^{-1}	0.028	0.012	0.01	0.018	0.01	0.012	0.10
Chromium (Cr)	mg l^{-1}	0.021	0.016	0.01	0.014	0.016	0.015	0.05
Lead (Pb)	mg l^{-1}	0.038	0.033	0.025	0.027	0.021	0.017	0.014
Nickel (Ni)	mg l^{-1}	0.025	0.022	0.015	0.021	0.015	0.018	0.034

Table 2. Classification of the Ibar River by localities based on water parameters examined and SWQI index

Parameter	May			September		
	L1	L2	L3	L1	L2	L3
Suspended matter	II	II	II	II	II	II
Dissolved Oxygen (DO)	I	I	I	I	I	I
Oxygen saturation of water	I	I	I	I	I	I
pH	II	II	II	II	II	II
Electrical conductivity	I	I	I	I	I	I
Ammonium ion (NH_4-N)	II	III[1.2]	III[1.2]	III[1.2]	III[1.2]	III[1.2]
Nitrite (NO_2-N)	II	III[1.2]	II	II	III[1.2]	III[1.2]
Nitrate (NO_3-N)	II	II	II	II	II	II
Total Nitrogen (TN)	III[1]	III[1]	II	II	III[1]	III[1]
Orthophosphate (PO_4-P)	II	III[1.2]	II	II	III[1.2]	III[1.2]
Consumption $KMnO_4$ (COD)	II	II	II	II	III[1]	III[1]
Bioc. Oxygen Demand (BOD_5)	II	II	II	II	II	II

Chlorides (Cl^-)	I	I	I	I	I	I
Total Coliform bacteria	III[1.2]	III[1.2]	III[1.2]	III[1.2]	III[1.2]	III[1.2]
Fecal coliform	III[1.2]	IV[1.2]	IV[1.2]	III[1.2]	IV[1.2]	IV[1.2]
Iron (Fe)	III[1]	III[1]	III[1]	III[1]	III[1]	III[1]
Manganese (Mn)	II	II	II	II	II	II
Zinc (Zn)	II	II	II	II	II	II
Copper (Cu)	II	II	II	II	II	II
Chromium (Cr)	II	II	II	II	II	II
Lead (Pb)	III[3]	III[3]	III[3]	III[3]	III[3]	III[3]
Nickel (Ni)	II	II	II	II	II	II
The final class of water	III	III	III	III	III	III
Number of parameters above MDK	5	8	5	5	9	9
SWQI	81	74	80	80	72	75
SWQI **description water class**	Good	Good	Good	Good	Good	Good
SWQI average value	77 (Good)					

[1]Regulation on emission limit values of polluting substances in surface and groundwaters and and sediment and deadlines for their achievement (Official Gazette of the RS, No. 50/12); [2]Regulation on the parameters of ecological and chemical status of surface water and parameters of the chemical and quantitative status of groundwater (Official gazette of the RS, No. 74/2011);
[3]Regulation on emission limit values of priority and priority hazardous substances which pollute surface waters and deadlines for their achievement (Official Gazette of the RS No. 24/2014).

In the field of water quality, our country`s legislation is harmonized with the EU framework directive (WFD, 2000) whose main goal is the sustainable management of water resources and achieving good hydrological, chemical and ecological status of natural waters. Nutrient concentrations showed the largest oscillations in terms of exceeding the value of the demanding quality of the Ibar water. Based on the measured concentrations of ammonium ions, the water quality did not correspond to quality class II in both sampling periods at none of the profiles. The L2 Raška profile showed a significant deterioration in water quality in terms of nitrite concentrations (May 0.038 mg/l^{-1}, September 0.052 mg/l^{-1}) and orthophosphate (May 0.102 mg/l^{-1} and September 0.125 mg/l^{-1}) which were the largest in relation to the other two profiles in both sampling periods, which is why the water at this profile is the third class quality.
The microbiological parameters of the Ibar River were out of quality for both I and II water quality class during both sampling seasons at all control points. According to the number of total coliforms, the quality of the Ibar water corresponded to the III quality class at all profiles, while according to the content of fecal coliforms, the quality of the Ibar River at locations L2 and L3 corresponded to the IV water quality class.
Based on the increased content of nutrients (ammonia ion, nitrites, total nitrogen and orthophosphates) and microbiological indicators of water quality, it can be concluded that there is a constant influx of organic pollution in the Ibar River, which is expressed in low water level and high water temperatures. Most often, increased concentrations of nutrients and coliforms in surface waters are the result of discharges of untreated municipal water, leachate from landfills and discharges of untreated municipal wastewater and diffuse pollution in river banks (Dalmacija and Ivančev-Tumbas, 2004).

The concentrations of heavy metals manganese, zinc, copper, chromium and nickel in all tested water samples were within the permitted limits of the MPC for the II water quality class. Increased values of iron and lead during both sampling seasons at all locations have conditioned that the Ibar River has the III water quality class. Iron concentrations ranged from 0.504 $mg/l^{-}1$ to 1.396 mg/l^{-1}, and lead concentrations ranged from 0.017 mg/l^{-1} to 0.038 mg/l^{-1}. The increased values of iron and lead are primarily a consequence of the inflow of wastewater from mining plants, flotation tailings and industrial wastewater located directly at the banks of the Ibar River.

It can be concluded that the summary ecological status of the water quality of the Ibar River is of moderate ecological status and the III water quality class during both sampling periods at all three measuring locations. According to the bylaw (Official Gazette of the Republic of Serbia No. 50/2012), the surface waters belonging to this class provide conditions for breeding fish from the *cyprinidae* family and can be used for the following purposes: drinking water supply with prior treatment by coagulation, flocculation, filtration and disinfection, bathing and recreation, irrigation and industrial use.

The calculated average SWQI values for the Ibar River (Table 2) indicate that there were no significant oscillations in terms of index water quality. In both testing periods, numerical values ranged from 72 to 81 points at all three locations, corresponding to the descriptive “good” water indicator.

Based on the data from Table 2, it can be stated that the largest number of exceedances of water parameters in relation to the legal regulations were recorded at the measurement profile of Raška, 8 parameters in May and 9 in September. Then the Baljevac profile follows with 5 parameters in May and 9 in September. At the profile of Rudnica, the value was exceeded by 5 water parameters in both sampling periods. Also, the average values of the SWQI index during both testing periods were the lowest at the measurement profile of Raška where they were 73 points, followed by the profile of Baljevac of 77.5 and the highest average value was at the profile of Rudnica 80.5 points.

In the end, it can be concluded that in its middle and lower course, the Ibar River is a recipient of wastewater from mines, industry, flotation tailings, municipal water and diffuse pollutants (agriculture, illegal landfills) starting from Kosovska Mitrovica and Leposavić to Raška and settlement Baljevac. This is the main reason that many parameters of the water quality of the Ibar River do not meet the limit values of the target I-II class of water quality of the Ibar. The values of the examined water parameters did not show large differences by seasons, on the basis of which it can be concluded that the influence of organic pollutants and heavy metals of iron and lead is constant.

Conclusions

Based on the results of the analysis, it can be concluded that neither of the profiles achieved good chemical status in both sampling periods. The water parameters whose concentrations exceeded the approximate limits for I and II class of water quality are: microbiological indicators, ammonium ion, nitrites, total nitrogen, orthophosphates, chemical consumption of oxygen, iron and nickel. Locations with the worst water quality are Raska and Baljevac, where the largest number of pollutants that contribute to organic water pollution is concentrated. The water quality during both testing periods was of relatively similar quality, indicating that the inflow of pollutants into the Ibar River is constant.

The values of the SWQI index ranged from 72 to 81 points, based on which the water of the Ibar River was classified as "good" water at all three locations. According to the Bylaw (the Official Gazette of the Republic of Serbia No. 50/2012), the water of the Ibar River at all

three measuring locations during both sampling periods corresponds to a moderate ecological status, i. e. quality class III.
For a more precise definition of the water quality of the Ibar River, it is necessary to do a longer research and more detailed chemical tests that would include biological and hydromorphological analyzes. In order for the water quality of the Ibar River to be satisfactory, it is necessary to form a cadastre of concentrated pollutants according to the amount and degree of pollution, take measures for rehabilitation and remediation of mine tailings, build wastewater treatment plants and rehabilitate uncontrolled municipal landfills.

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APPLICATION OF AROMA IN THE NUTRITION OF ANIMALS WITH FUR-CHINCHILLA

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Abstract

In breeding chinchillas, the decisive factor for the success of breeding is the quality and intactness of the fur. In this paper we studied the effect of apple aroma as food additive in concentration of 0,04% on performance and reduction of fur chewing. The obtained results showed that the positive effects of using the tested aroma were reflected in an increase in the body weight of chickens by 1.36%, an increase in daily growth by 21.18%, an increase in feed consumption by 0.48% and an improvement in feed conversion by 17.13%. The additon of apple aroma to the feed also had a positive impact on decreasing fur chewing with chincillas. Only 5% out of the total number of treated chinchillas in this group had chewed fur, in comparison with the control group (without aroma addition) where 30% animals had damaged fur, which is an important statistical improvement ($p<0,01$).

Keywords: *chinchilla, food, aroma, fur*.

Introduction

Achieving successful and profitable production of fur is directly affected by proper environmental criteria and the use of quality food. Chinchilla is a South American rodent, herbivore, originating from South America (Peru, Chile, Bolivia) bread as a pet and for fur. It belongs to the nocturnal animals perform most of their life activities, including feeding, at night. Chinchillas are characterized by a high quality fur which consists of extremely soft and loose hair and which makes it the third most expensive animal in the world fur market. Interesting characteristics of chinchilla fur are softness of fur (50-120 fibres comes out of each hair root) and amazing lightness of the fur which weighs almost as silk. Generally, chinchillas have the thickest fur of all animals where 1 cm^2 has more than 20,000 hairs. It is considered one the softest animals in the world and their fur is 30 times softer than human hair. (Bickel, 1987; Mettler, 1999; Kamler, 2002).

One of the most negative phenomenon when it comes to profitability of breeding these rodents is that for various reasons they chew their fur, which happens mostly at night. Disturbances can occur in all categories of chinchillas during the entire production cycle, but typically it occurs in pregnant females. (Jimenez, 1994; Jaros et al. 2004).

Modern concepts of nutrition in intensive animal farming are based on the use of various additives in order to achieve maximum production results. Having this in mind, we studied the impact apple aroma produced by the company Ireks Aroma from Croatia, as a food additive on production results and the possibility of eliminating fur chewing. The use of aromatic plant extract ingredients in animal feed resulted in many positive results such as increasing growth, improving conversion of nutrients, reducing mortality and improvement

of the general health of the treated animals (Kozelov et al., 2003; Živković et al.,2003). We found that aromatic botanical additives have anti-microbiological, anti-viral, anti-fungal, and anti-helmitical immune stimulating properties (Middleton et al., 1992; Dorman et al., 2000; Wenk et al., 2002; Azaz et al., 2004).
However, there is not much bibliographic data on experimental research in the field of application of aroma as a food additive for chinchillas and its impact on production features.
The aim of this paper is to determine the impact of adding apple aroma on prevention of fur chewing, and as well as on production parameters including growth, consummation, food conversion and mortality

Material and Methods

The study was conducted on eight families of standard chinchillas over a period of 30 days. Each family consisted of four females and one male, totaling 40 adult animals aged over one year. These animals were divided into two equal groups, with 20 individuals in each group. In both experimental groups, there were nine pregnant females. All individuals were housed in cages made of galvanized wire, with dimensions of 60 x 55 x 65 cm.
The control group (A) was fed with pellets without the addition of aromas and the other experimental group (B) was added aroma in the same food mixture in the concentration of 0,04 % by "Ireks Aroma" from Croatia. Besides pellets, both groups were fed with dry and high-quality hay with no mold, and supplied with fresh water during the experiment. Daily potions included 30g of briquettes and 20g of hay was given every other day per animal, while pregnant females received 20g of hay in two days and 35g of briquettes daily. The tested animals had ad libitum access to food and water. During the experiment the conversion of food and nutrients were determined based on data and measurements of food consumption, and consumption of food and degree of chewed fur was monitored daily.
In the cage, dry lime sawdust (chips) was used as a bedding and animals were provided with daily bathing in special volcanic sand for scouring and care of fur which contained in the following proportion: sand - aroma 99: 1. Volcanic sand from the vessel was changed once a week. The used food was sampled and chemically analyzed using standard methods of testing (AOAC,1990). Behavior and lack of fur were monitored by a visual method of observation.
Swabs were taken from the places where fur was lacking in order to determine the presence of the most common causes of fungal infections, such as the following: *trichophyton mentagrophytes, microsporum canis and microsporum gypseum.*

Table 1. Chemical composition of pellet complete mixtures for feeding chinchillas

Components (%)	A-control group	B- experimental group
Oats	21	21
Barley	4,7	4,7
Wheat	11,6	11,56
Soybean meal (44%)	9	9
Sunflower meal (33%)	11,5	11,5
Sunflower meal (42%)	5	5
Wheat bran	6	6
Alfalfa meal	20	20
Yeast	1	1
Soybean groats	2,1	2,1
Sugar	2	2
Peletin	2	2
Chalk	1,7	1,7
Monocalcium phosphate	1,1	1,1

Iodised salt	0,3	0,3
Premix	1	1
Apple aroma	-	0,04
UKUPNO	100%	100%
Chemical mixtures		
Crude protein , %	18,74	18,80
Crude fiber, %	12,35	12,40
Ash , %	8,17	8,13
Ca, %	1,14	1,18
Total fat , %	3,10	3,00
P, %	0,70	0,72
Na, %	0,16	0,17

The composition of the used premix per kg mixture: A (E 672) IU / kg 23000, D3 (E 671) IU / kg 3500, E 90 mg / kg, B1 mg / kg 3, B2 5 mg / kg, B6 2 mg / kg , B12 0.02 mg / kg, K3, 1 mg / kg, C 13 mg / kg, niacin 50 mg / kg, Ca-pantothenate 21mg/kg, Mn (E5) 30 mg / kg, Zn (E6) 50 mg / kg, Fe (E1) 30 mg / kg, Cu (E4) 4 mg / kg, B (E2) 0.7 mg / kg, Se (E8) 0.2 mg / kg, Co (E3) 0.4 mg / kg, biotin 0.1 mg / kg Choline chloride 400 mg / kg, folic acid. 0.2 mg / kg, Lysine 700 mg / kg, methionine 300 mg / kg, Rovabio AP/10 200 mg / kg Phytase 100 mg / kg, Antioxidant BHT (E321) 100 mg / kg.

The results were grouped into appropriate series and statistically processed in a computer using common mathematical and statistical procedures that involve the analysis of variance and evaluating the significance of the results (differences) by using the t-test.

Results and Discussion

The data listed in Table 1 show chemical composition of used food for chinchillas in detail. Based on the shown chemical composition we can conclude that the used pellets contained all the necessary nutrients and chemical composition of complete feeds was approximately the same, which completely satisfied the needs of furry animals.
Based on the obtained results of chewed and damaged fur (Table 2) we can conclude that the presence of apple aroma in the mixture of experimental group B contributed to a significant reduction in fur chewing (5% out of the total number) compared to animals in control group A where the total damage of fur was found in found is 30% of animals. Fur chewing in the control group A was most common on hips and legs with the intensity of 8 points, which is 25% more points compared to the furry animals that were fed with apple aroma, which suggests the existence of highly significant differences ($p<0,01$). In places where fur was lacking, dermatological analysis did not register the presence of *trichophyton mentagrophytes, microsporum canis and microsporum gypseum.*

Table 2. Bitten fur on body regions during the test

Category	Total No.	Number etched	A-control group				
			flanks	neck	back	legs	tail
Males	4	1	+	-	-	-	-
Females	7	2	++	-	-	+	-
Females pregnant	9	3	++	-	-	++	-
TOTAL:	20	6	8 points				

			B- experimental group				
			flanks	neck	back	legs	tail
Males	4		-	-	-	-	-
Females	7		-	-	-	-	-
Females pregnant	9	1	+	-	-	+	-
TOTAL:	20	1	2 points				

Degree of bitten fur on intensity: - no bites (0 points) + small size (1 point), + + pronounced volume (2 points) + + + very strong volume (3 points)

Bibliographic data (Kamler, 2002; Mettler, 1999) suggest different factors that could cause fur chewing. Some of the most significant are inadequate nutrition, inadequate housing and zoohygienic conditions, dirty fur, hormonal and metabolic disorders and genetic factors. Also, fungal diseases can be one of the causes of pathological skin changes that occur due to inflammation of skin, appearance of the cross, peeling, itching and hair fall out. The disorder is easily visible because the areas without hairs are very noticeable. Such animals are anxious, they lose weight, and their feces contains large amounts of hair.

According to Jaros-in et al. (2004) deficiency in vitamin B complex is an important factor for occurrence of the phenomenon of fur chewing, due to the lack of concentration or preventing their absorption in the presence antivitamine. The author also states that overuse of antibiotics and sulfonamides in the treatment of diseases causes disturbances in the form of hair falling. Unfavourable ambient conditions such as high temperature, poor lighting and excessive humidity may cause these adverse effects. Farmers experience shows that parts of chinchilla fur fall off if they are roughly and suddenly caught with hands. Another cause of fur damage can be the fact that animals bite each other.

Adding apple aroma to the mixture in Group B in the quantity of 400 g/t had a positive effect at the end of the experiment and it resulted in weight gain by 1,36% compared to the control group A, but there was no statistical significance (p>0,05). Compared with average daily gain of body weight in the A-group, it can be concluded that the experimental B-group achieved higher daily weight gain of 21,18% which indicates the existence of significant differences (p<0,05). During the test, the added apple aroma in the experimental group improved the average daily intake of food by 0,48% compared to the control group of chinchillas. The addition of aromatic additives in food resulted in an improved feed conversion in trial A group in amounting 17,13% compared to the control B-group of chinchillas, with no additives tested in the mixtures.

Table 3. Production results in the experiment

Days	Categories	GROUP	
		A-control group	B- experimental group
The average body mass of chinchillas (g)			
Home Reflected	Males	555,80	560,30
	Females	580,10	580,50
	Females pregnant	600,90	608,30
	AVERAGE	577,27	583,03
	Index (%)	100	100,1
	Difference (%)		+0,10
p>0,05			
30 day	Males	562,80	570,50
	Females	585,00	590,80
	Females pregnant	615,30	625,70
	AVERAGE	587,70	595,67

	Index (%)	100	101,36
	Difference (%)		+1,36
p>0,05			
Average daily weight gain (g)			
0-30 days	Males	7	10,20
	Females	4,9	10,30
	Females pregnant	14,40	17,40
	AVERAGE	10,43	12,64
	Index (%)	100	121,18
	Difference (%)		+21,18
*p<0,05			
Consumption and conversion of food pellets + hay (g)			
0-30 days	Males (g)	4510	4530
	Females (g)	8190	8220
	Females pregnant (g)	12120	12190
	Total (g)	24820	24940
	Daily (g)	41,37	41,57
	CONVERSION	3,97	3,29
	Index (%)	100	82,87
	Difference (%)		+17,13

The results are in agreement with other authors (Ignatova et al. 2005), who found that adding a mixture of plant extracts to feed for rabbits stimulates the increase of growth and yield in the range from 3.3% to 5.5% and feed conversion improved by 9%. Aromatic plant extracts have a favourable effect on improving digestion and absorption of nutrients, because they encourage the activity of pancreatic and bile secretion (Mellor, 2001; Soliman, 2002). Eiben et al. (2004) concluded that the use of fenugreek seeds and anise in the amount of 6 g / kg, led to improved consumption and feed conversion in rabbits with statistical relevance of ($p<0,01$). Aromatic plants extracts have antibacterial, antifungal and anti-inflammatory properties (Soliman, 2002; Sagdic, 2003), and in addition to the efficiency of digestion, they increase lactation of mammals (Albert-Buel, 1980). The application of yucca aroma in pelleted food for rabbits at a concentration of 250 mg/kg, contributed to the increase in feed consumption by 9,6%, 3,62% for the conversion, and retention of nitrogen by 24,3% compared to rabbits fed without this stimulus (Amber et al., 2004). In general, the obtained results showed that the addition of apple aroma had positive effects both in nutrition and prevention of fur chewing.

Conclusions

The impact of apple flavor as food additive, used at a concentration of 0,04% in the pelleted food for chinchillas in this experiment had a positive effect on all analyzed parameters of production. The positive effects were seen in the following: increase in body weight by 1,36% ($p>0,05$); increased daily gain by 21,18% ($p<0,05$); increased consumption of food is 0,48%; improvement of feed conversion ratio of 17,13%. The addition of the tested flavors contributed to a significant reduction in fur chewing ($p<0,01$). The treated chinchillas had only 5% of chewed fur compared to the control group (without aroma addition) where 30% of animals had damaged fur.

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CHARACTERISTICS OF HERZEGOVINIAN CHEESE IN SACK

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Abstract

Cheese matured in animal skin is produced in several countries around the world and is one of the traditional types of cheese, such as Tulum (Turkey), Bouhezza (Algeria) and Darfiyeh (Lebanon). In the Balkans, this type of cheese is produced in Croatia (sir iz mišine), Bosnia and Herzegovina, and Montenegro (sir iz mijeha). There are two types of this cheese: one is a low-fat cheese made from milk after the production of kajmak (skorup) and is known under various names, such as Torotan, „Vareni" cheese, Tarenik, Tučenik, Mješinski, Diga, Prljo, and the other type is a full-fat cheese made from whole or partially skimmed milk. The technological process of production is not standardized and differs depending on the country in which it is produced and the type of milk used as raw material. The taste and aroma of this cheese depends primarily on the type of milk used for its production, but the specific taste and aroma are also influenced by the sack in which the ripening process takes place. Based on the ripening period (15, 30 and 45 days), three groups of samples were collected from the households in the area of the municipality of Ljubinje, Republic of Srpska. According to moisture on fat-free basis and fat in dry matter all investigated cheeses belong to semi-hard, full fat cheeses. As expected, the highest ripening index was determined in 45 days old cheeses in sack – 19.43% ($p<0.05$).

Keywords: *autochthonous dairy product, cheese, sack.*

Introduction

Several types of cheese are produced in the world that are matured in animal skins. There are certain differences in the production technology and in the type of milk used. Cheeses matured in animal skins are produced in Turkey-Tulum, Lebanon-Darfiyeh and Algeria-Bouhezz (Aissaoui at al., 2011, Medjoudj et. al., 2017, Kamber, 2008, Sert et al., 2014, Yilmaz et al., 2005). In the Balkans, the production of *Sir iz mišine* (Croatia) and *Sir iz mijeha* (Bosnia and Herzegovina, Montenegro) has been recorded (Tudor Kalit et al., 2010; Bijeljac and Sarić, 2005; Brenjo and Sarić, 2020; Samardžić, 2009). The specificity of this type of cheese is ripening in goat/lamb skin. Namely, the animal skin is a medium in which the ripening of the cheese takes place under anaerobic conditions with the presence of naturally occurring lactic acid bacteria, other bacteria of the non-starter microbiota as well as yeasts and moulds, which form the secondary microbiota of the cheese due to the permeability of the animal skin (Tudor Kalit et al., 2020). It is believed that in the past, due to the lack of wooden material for the production of equipment for storing and transporting cheese, nomadic shepherds began to use sheepskin for the preparation of cheese and its transport from the mountains. There are two varieties of Herzegovinian cheese in sack. The first variety is produced from skimmed milk after the production of kajmak, while the second variety uses whole or partially skimmed milk. Due to specific anaerobic ripening conditions in a specially prepared sack, this cheese has specific and recognizable sensory characteristics, and occupies a special place among the traditional dairy products of Herzegovina (Dozet et al., 1996). The aim of this study was to determine the autochthonous technology and overall quality of Herzegovinian cheese in a sack.

Material and method

The recording of the autochthonous technology of cheese from sack was carried out in the village of Ubosko, which is located in the territory of the municipality of Ljubinje, Republika Srpska (Bosnia and Herzegovina). Cheese samples at various stages of ripening (15, 30 and 45 days) were taken from three households between September and October. The samples were transported to the laboratory in an isothermal container and stored at −20°C until analysis.
The following methods were used to analyse the physico-chemical composition of cheese in sack samples: dry matter by the standard drying method at 102±2°C (IDF, 1982); fat content according to the Van Gulik method (IDF, 1986). These parameters were used to calculate the fat content in dry matter (FDM) and the moisture content on a fat-free basis (MFFB). The protein content was determined by the Kjeldahl method (AOAC, 1998), water-soluble nitrogen (WSN) according to Kuchroo and Fox (1982). The NaCl content was determined according to the Volhard method (IDF, 1988). Titratable acidity (°SH) was measured using the Soxhlet-Henkel method (Carić et al. 2000) and pH was measured using a digital pH-meter (Consort, Turnhout, Belgium) (Ardö & Polychroniadou, 1999). All measurements were performed in triplicate.
Obtained results were subjected to a one-way analysis of variance (ANOVA) using Statistica 6.0 software (Stat Soft. Inc., Tulsa, USA). The mean values were compared using a t-test at the 0.05 level of significance.

Results and discussion

Autochthonous technology of Herzegovinian cheese in a sack

Both evening and morning milk is used for the production of Herzegovinian cheese in sack. In most cases, some of the cream is taken from the evening milk and used to make butter at home. The temperature of the milk before rennet is added varies between 30-35°C and the duration of coagulation varies between 40 minutes and 2 hours. Once a firm curd has formed, it is first cut crosswise and then into small pieces to make it easier to separate the whey. The whey is then heated to a temperature of 40-50°C. Curd processing takes 30-90 minutes, during which the whey is gently drained off. After that, the curd is transferred to a cloth and pressed, which usually lasts overnight. A large clean stone is used for pressing. The pressure must be high enough to squeeze out most of the remaining whey from the cheese (Bijeljac and Sarić, 2005). After pressing, the curd is stored in the refrigerator or in the cold for a few days until a sufficient amount has accumulated to fill the sack, which is consistent with some literature data (Kalit, 2016; Tudor Kalit et al., 2010). This also represents the aerobic phase of curd ripening. The curd is then cut into pieces and salted, usually with large grain sea salt, as in the technological production process of *sir iz mišine* (Vrdoljak, 2016). Salting takes place during the night, separating the whey, which is removed before filling the prepared lamb sack. It is very important that the cheese is well packed when filling the bag so that no air is left behind that could spoil the cheese. After the filling is complete, all openings of the sack are tightly closed to ensure anaerobic ripening conditions. The sack is placed on the shelves in the ripening room, where the temperature is 14-17°C. Ripening takes an average of 45 days (1-3) months, which is in agreement with the data provided by Tudor Kalit et al. (2014) for *sir iz mišine*. During the ripening process it is necessary to take care of the sack. In the early stage of ripening, sacks can be turned daily, cleaning the surface, and in the late stage of ripening they are turned every 2-3 days. For the production of 1 kg of mature cheese, 8-10 liters of milk are consumed, which corresponds to the data from the study by Bijeljac and Sarić

(2005). A characteristic feature of the technological process for producing this variety of cheese from the sack is that, unlike the cheese described by Bijeljac and Sarić (2005) and Dozet et al. (1996), there is no manual curd breaking phase, which indicates that production varies from household to household and from region to region.

Physico-chemical properties of Herzegovinian cheese in sack

Considering the non-standardized production, variations in quality were to be expected. The physico-chemical characteristics of Herzegovinian cheese in sack are given in Table 1.

Table 1. Physico-chemical properties of Herzegovinian cheese in sack (*Sir iz mijeha*)

Parameter	Ripening time (days)		
	15	30	45
DM (%)	51.98±1.22^{b}	55.44±1.53^{a}	50.41±1.28^{b}
MF (%)	24.75±1.52^{a}	26.75±2.06^{a}	24.38±2.21^{a}
FDM (%)	47.57±1.83^{a}	48.20±2.42^{a}	48.30±3.22^{a}
MFFB (%)	63.81±0.38^{b}	60.83±0.52^{c}	65.58±0.50^{a}
Proteins (%)	20.51±0.81^{ab}	21.25±0.92^{a}	19.30±0.53^{b}
WSN (%)	0.2872±0.0247^{c}	0.4219±0.0431^{b}	0.5895±0.0968^{a}
NaCl (%)	2.58±0.30^{b}	3.83±0.31^{a}	2.79±0.76^{b}
Acidity (°SH)	65.07±1.18^{a}	66.48±0.87^{a}	73.99±12.73^{a}
pH	5.11±0.03^{a}	5.10±0.04^{a}	5.01±0.16^{a}

DM - dry matter, MF –milk fat, FDM - fat in dry matter, MFFB - moisture on fat-free basis, WSN- water soluble nitrogen; Means within a row marked with the different letter differ significantly at $p<0.05$

According to fat in dry matter (FDM), all cheeses belonged to the group of full-fat cheeses, regardless of the ripening stage (IDF, 2021). In addition, the ripening period had no influence on milk fat content, fat in dry matter, acidity and pH ($p>0.05$). Since examined cheeses were produced from partially skimmed milk, the values for milk fat content were lower (24.38 to 26.75%) than the values reported by Dozet et al. (1996). Significant differences ($p<0.05$) were found between the cheeses in terms of moisture on a fat-free basis (MFFB), but all cheeses belonged to the semi-hard cheeses regardless of ripening time (IDF, 2021). The highest levels of dry matter (DM), proteins and NaCl ($p<0.05$) were observed in cheeses matured for 30 days. However, after 30 days of ripening DM and protein content was lower than in *Sir iz mišine* at the same ripening period (Rako et al., 2018). The porous structure of sack allows the loss of moisture from the cheese during ripening. This leads to an increase in the dry matter content of the cheese (Hayaloglu et al., 2007; Tudor Kalit et al., 2010). Obviously, the highest water loss occurred between the 15th and 30th day of ripening, as the reported DM and protein values were significantly lower ($p<0.05$) in the following ripening period. The lower DM content in 45-day-old cheese is due to a lower fat and protein content resulting from extensive lypolysis and proteolysis, during which many volatile compounds are formed that are responsible for the piquant aroma and flavour of Herzegovinian cheese in sack. In addition, the products of lactose and citrate metabolism contribute to the specific sensory properties of cheese. As can be seen from Table 1, no significant differences ($p>0.05$) were found in the acidity values. However, after 45 days of ripening, large variations in acidity were found between the cheese samples, which could be due to the unequal temperature in the ripening rooms of the different households.

According to Brenjo et al. (2012) and Sarić (2009) salt content in matured Herzegovinian cheese in sack should be 3-5%. However, samples analysed in this study had lower average NaCl content at the end of the ripening period (2.79%).

The ripening of cheese can be characterized through increase of water-soluble nitrogen (WSN) during ripening, and especially by ripening index (RI) which represents WSN in total nitrogen. As expected, ripening time had a significant effect ($p<0.05$) on WSN content (Table 1). At the end of ripening, after 45 days, the samples of Herzegovinian cheese in sack had 0.5895 % WSN, respectively. The increase in WSN led to significantly higher RI values (Figure 1), which indicates a higher content of small and medium-sized polypeptides and free amino acids and thus a more extensive proteolysis.

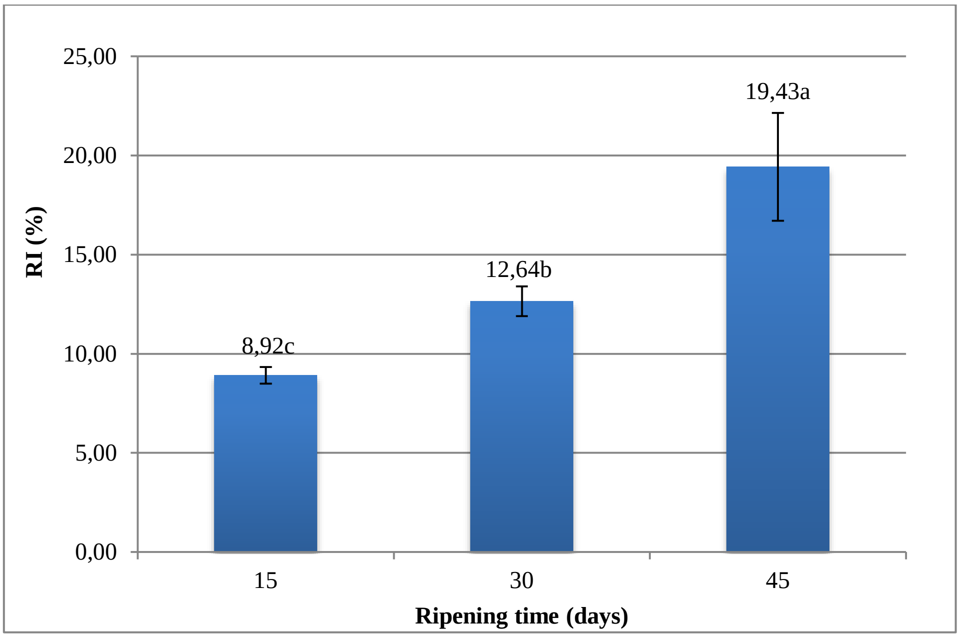

Figure 1. Ripening index of Herzegovinian cheese in sack

As already mentioned, variations in the technological production process led to different cheese quality and thus to different RI values between cheese samples from different households at the same ripening time. These variations become more pronounced as ripening progresses. In comparison to the data for *Sir iz mišine* (Tudor Kalit et al., 2018), Herzegovinian cheese in sack had lower RI on 15th and 30th day of ripening. After 45 days of ripening average RI was 19.45%.

Sensory properties

Herzegovinian cheese in sack has a light yellow colour. When taken out of the sack, the cheese is in lumps of different sizes, with characteristic crumbly texture (Figure 2). The cheese is moderately salty and has a characteristic, spicy flavour and aroma, which it acquires during ripening in the animal skin.

Figure 2. Herzegovinian cheese in sack

Conclusion

Due to its characteristic sensory properties, this cheese is intended for a limited target group of consumers who appreciate this specific and spicy product, which can be classified as a delicacy. Due to the inconsistent technology in the individual households, large differences were found in some quality parameters after 45 days of ripening, particularly in the acidity (73.99°SH) and ripening index (19.43%). It is therefore necessary to standardize the technological process of production and therefore the quality in order to meet the requirements for the protection of the designation of origin. In this way, this autochthonous product would be preserved with all its specific characteristics and would have its place and appropriate price on the market.

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THE EFFECT OF GENOTYPE AND PROTEASE ENZYME ON THE WEIGHT AND PERCENTAGE OF MEAT CLASSES IN CHICKENS

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Abstract

The aim of the present study was to evaluate the effects of adding protease enzymes (Ronozyme ProAct, 0, 200 mg/kg and 300 mg/kg diet) while reducing the crude protein content (control group - standard diet, group E-I - 4 % less crude protein compared to the control group and group E-II, which received 6 % low-protein feed compared to the control group) in fast and medium-growing meat chickens on the weight and percentage of certain classes of meat. In the experiment, fast-growing Cobb 500 and medium-growing Master Gris chickens were used. The genotype had a significant effect on the weight and percentage of the different chicken meat classes ($P<0.05$). The fast-growing Cobb 500 genotype had a higher weight of all meat classes and a higher percentage of meat class I and a lower percentage of meat class II and meat class III compared to the broilers of the Master Gris genotype ($P<0.05$). Significance was found under the influence of the protease enzyme in the hybrid Cobb 500 for meat class III (between C group and E-I group on the one hand and E-II group on the other hand, $P<0.05$) and in the hybrid Master Gris for meat class III (between C group and E-I group, $P<0.05$), while there was no effect on the mass of all three classes of meat ($P>0.05$). Enzyme protease can be used as an additive in a broiler diet for production at a concentration of 200 mg/kg Ronozyme ProAct and with 4% less crude protein compared to the standard diet.

Keywords: *broilers, hybrids, enzyme protease, classes of meat.*

Introduction

One of the biggest challenges in poultry production is the high cost of feed, which can account for up to 70% of total costs. One strategy to overcome this problem is the use of alternative feeds that provide good animal performance at a reasonable cost without harming the environment (da Silva *et al*. 2021). Research studies have shown that exogenous enzymes added to diets improve the digestibility of nutrients and their absorption in the intestines of birds (Borda-Molina *et al*. 2019; Hafeez *et al*. 2020), thereby reducing environmental pollution (Hafeez *et al*. 2021) and production costs (Ding *et al*. 2016). The application of proteases in broiler diets is complex and therefore the properties and dosage of the protease as well as the protein content in the feed should be fully considered when protease is provided in animal feed (Zheng *et al*. 2023). The facts show that the efficacy of exogenous proteases is highly dependent on the amount of protease added. Currently, there is a lack of knowledge on the dose-dependent relationship between commercial proteases and animal growth, organ weight, gut health and nutrient digestibility (Song *et al*. 2023). Therefore, the aim of the present study was to investigate the effect of a commercial protease supplement to corn-soybean meal diet on the weight and percentage of certain meat classes of two commercial broiler chickens with different growth rates.

Materials and methods

Six treatments consisting of a 2 × 3 factorial design of 2 genotypes (fast-growing hybrid Cobb 500 and the medium-growing hybrid Master Gris) and 3 experimental diets (control group - standard diet without protease; group E-I, which received 4 % less crude protein with the addition of 200 mg/kg Ronozyme ProAct in relation to the control group, and Group E-II, which received 6 % low-protein diet with the addition of 300 mg/kg Ronozyme ProAct in relation to the control group). Each treatment comprised 100 chicks. Until 21 day of age, chicks received a starter diet, from 22 to 35 d a grower diet and from 35 to 49 day a finisher diet. Diets for the control groups was formulated to meet the requirements of Cobb 500 (Cobb 500, 2012). The feed was provided in mash form. Feed and water were offered to the chicks ad libitum during the experimental period. At the end of the experiment on day 49, 20 birds (10 males and 10 females) were randomly selected from each experimental unit, weighed, slaughtered and processed to determine the weight and percentage of meat classes (meat class I: breast, drumsticks and thighs; meat class II - wings and meat class III - back and pelvis). The percentages of certain meat classes are calculated in relation to the weight of the ready-to-grill carcass. The data were analysed using analysis of variance (Anova). Significant main effects or interactions were separated using the LSD test. Statistical significance was defined as a P-value of less than 0.05.

Results and discussion

Slaughter yield is an important indicator of the characteristics of meat production in livestock and poultry. The results for the weight of certain meat classes are shown in Table 1.

Table 1. Weight of different classes of chicken meat on the 49th day of fattening, g

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Hybrids	Groups				
Cobb 500	C	X̄	1502.1^{a}	261.9^{a}	529.7^{a}
		Sd	154.9	27.2	59.2
	E-I	X̄	1476.2^{a}	252.1^{a}	519.7^{a}
		Sd	116.7	27.1	77.2
	E-II	X̄	1433.9^{a}	253.8^{a}	532.2^{a}
		Sd	150. 9	30.2	72.5
Master Gris	C	X̄	1105.8^{b}	221.7^{b}	434.1^{b}
		Sd	90.5	19.5	37.7
	E-I	X̄	1067.8^{b}	219.2^{b}	410.0^{b}
		Sd	100.8	20.4	32.2
	E-II	X̄	1077.8^{b}	219.2^{b}	422.1^{b}
		Sd	101.3	23.4	41.9
p-value					
Source of variation					
Sex			0.001	0.001	0.001
Protease			0.204	0.491	0.378
Protease x sex			0.605	0.799	0.806

X̄ - Average, Sd - Standard deviation
Different superscripts (a, b) indicate a significant differences between groups (P<0.05)

Table 1. shows that the investigated carcass traits were predominantly influenced by the genotype (P<0.05). Namely, fast-growing Cobb 500 chickens aged 49 had a higher weight in all meat classes than the medium-growing Master Gris chickens of the same age. However, that crude proteins levels and protease concentrations as well as the interaction of genotype and feeding regime had no effects on the weight of meat classes of broilers in both hybrids (P>0.05). Blagojević *et al*. (2009) determined a higher mass of the carcass parts and thus the meat class in the same fast-growing hybrid compared to the same medium-growing genotype. Dosković *et al*. (2024) also came to similar conclusions about the weight of certain meat classes, that there are differences between hybrids and that there is no influence of the applied nutritional treatments studying the same genotypes at 63 days of age.
Table 2. shows the effects of genotype and diet (with three different protease levels at three different crude protein contents) on the percentage of the different meat classes in the dressed carcass of chicks slaughtered at 49 days of age.

Table 2. The percentage of the different chicken meat classes in the dressed carcass on the 49th day of fattening, %

Treatment			Class I (breast, thighs, drumsticks)	Class II (wings)	Class III (back, pelvis)
Hybrids	Groups				
Cobb 500	C	$\bar{X}$	63.1^{a}	11.0^{c}	22.2^{b}
		Sd	1.3	0.5	0.9
	E-I	$\bar{X}$	63.3^{a}	10.8^{c}	22.1^{b}
		Sd	2.4	0.6	1. 8
	E-II	$\bar{X}$	62.3^{a}	11.0^{c}	23.1^{a}
		Sd	1.7	0.6	1.2
Master Gris	C	$\bar{X}$	60.2^{b}	12.1^{b}	23.6^{a}
		Sd	1.1	0.4	0.7
	E-I	$\bar{X}$	60.5^{b}	12.4^{a}	23.2^{a}
		Sd	0.9	0.4	0.7
	E-II	$\bar{X}$	60.1^{b}	12.2^{ab}	23.5^{a}
		Sd	0.9	0.5	0.8
p-value					
Source of variation					
Sex			0.001	0.001	0.001
Protease			0.122	0.819	0.049
Protease x sex			0.484	0.051	0.178

$\bar{X}$ - Average, Sd - Standard deviation
Different superscripts (a-c) indicate a significant difference between groups (P<0.05)

The genotype has a significant effect on the percentage of the different chicken meat classes (P<0.05). Thus, the proportion of meat class I was higher in the Cobb 500 genotype, while the proportion of meat class II and the proportion of meat class III was lower compared to the Master Gris hybrids (P<0.05).
In our study, significance under the influence of the protease enzyme was found in the Cobb 500 hybrid for meat class III (between C group and E-I group on the one hand and E-II group on the other hand, P<0.05) and in the Master Gris hybrid for meat class III (between C group and E-I group, P<0.05). Differences between strains with different growth intensity in the proportion of individual carcass parts and meat classes (group of carcass parts) were also cited by Aksoy *et al*. (2010), Jaspal *et al*. (2020), Dosković *et al*. (2021) and Tůmová *et al*. (2021).

The different effects of protease application may be due to the type, amount, composition of feed, breed, age and health status of the broilers (Li *et al.* 2023). The results are in some agreement with those of Mahendran *et al*. (2022), Duque-Ramirez *et al*. (2023) and Dosković *et al*. (2024), who reported that supplementation of protease enzyme with different crude protein-reduced diets had no effect on the proportion of meat classes and carcass traits in broilers.
The interaction between genotype and diet was not significant for any meat class, implying that the genotype responses to the diet treatments are the same.

Conclusion

The present study shows that the genotype has a much greater influence on the weight and percentage of certain meat classes of broiler chickens ($P<0.05$) in relation to the applied feeding regime. Namely, the fast-growing Cobb 500 genotype showed a higher weight of all meat classes and a higher percentage of class I meat and a lower percentage of class II and class III meat compared to the broiler chickens of the Master Gris hybride.
Based on the results obtained, it can be concluded that the enzyme protease can be used as an additive in a broiler diet for production at a concentration of 200 mg/kg Ronozyme ProAct and with 4% less crude protein compared to the standard diet, accordingly very small differences in the examined characteristics of the processed chicken carcasses - weights and percentages of certain classes of meat.

Acknowledgement

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EFFECTS OF ROOSTER PRESENCE IN FREE-RANGE SYSTEMS ON EGG QUALITY

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Abstract

The aim of this study was to investigate the effects of having roosters on egg quality in a free-range system. In the study, there were 2 groups (without and with roosters), and each group consisted of 4 replications. The first group was kept with roosters together with hens, and the other group was kept only with hens. Each subgroup in the research contained 15 hens, and each replicate included 1 rooster. Egg weight, eggshell strength (kg), Haugh unit, and shell thickness (mm) were determined as indicators of egg quality. We measured egg quality characteristics at 41 weeks of age. Egg weight was 66.4 g in the group without roosters and 64.2 g in the group with roosters, and the differences between the groups were statistically insignificant ($P > 0.05$). Eggshell strength was 5.412 kg in the group without roosters and 5.183 kg in the group with roosters, and the difference between the groups was statistically insignificant ($P > 0.05$). The Haugh unit was 94.3 in the group without roosters and 90.6 in the group with roosters, and the difference between the groups was statistically insignificant ($P > 0.05$). Shell thickness was 0.399 mm in the group without roosters and 0.405 mm in the group with roosters, and the difference between the groups was statistically insignificant ($P > 0.05$). Therefore, the presence or absence of roosters in a free-range system did not significantly affect egg quality characteristics.

Keywords: *Free-range system, rooster, egg weight, eggshell strength, Haugh unit.*

Introduction

Red jungle fowl, the ancestors of modern egg hybrids, are highly social animals, forming a family in which roosters fertilize females and protect them from predators (McBride *et al.*, 1969; Odén *et al.*, 2005). Chickens, especially in free-range systems, face many dangers, especially from predators such as foxes, martens, eagles and hawks. Chickens have various behaviors to protect themselves from these predators. Depending on the type of predator, they may remain motionless, run to hide in a closed area, or escape by jumping to higher levels. While the chickens are doing their behavioral characteristics (foraging, eating food, dust bathing, etc.) in the outdoor area, the roosters protect them against predators that may come from the environment and warn them of danger (Johnson 1963; Sullivan 1991).

Keeping roosters in a free-range system is not preferred due to increased feed consumption. However, in recent years, as a result of the increase in demand for natural food, it is stated that keeping roosters in flocks has a more improving role in the behavioral characteristics of chickens (Pereira *et al.*, 2017). According to our literature research, there are limited studies on keeping roosters in chicken flocks (Odén *et al.*, 2005; Pereira *et al.*, 2017).

In these studies, mostly behavioral characteristics were examined as there is a lack of literature on the effect of flocks with roosters on egg quality. This study investigated the impact of rooster presence in a free-range flock on egg quality.

Materials and Methods

This study was carried out at Selcuk University, Faculty of Agriculture, Department of Animal Science (Turkey). The study was conducted in June 2022. In the study, the 41-week-old Lohmann Sandy layer genotype was used. In the study, there were 2 groups (without and with roosters), and each group consisted of 4 replications. The first group was kept with roosters together with hens, and the other group was kept only with hens. Each subgroup in the research contained 15 hens, and each replicate included 1 rooster.
The hens were reared in a free-range system. The stocking density in the in-door area is 6 hens /m^2, while the out-door area provides 4 m^2 per hen. Water and feed are given as ad-libitum. The animals were given laying hen feed adlibitum (2720 Kcal kg/ME, 17.60% HP, 3.90% Ca and 0.39% available Phosphorus). The lighting program was applied as 16 L: 8 D. At least eight hours a day are provided for animals for use in the outdoor area. Egg quality analyses were performed on 5 eggs (10/subgroup) randomly taken from eggs produced on 2 consecutive days at the age of 41 weeks. Eggs collected daily were stored at room temperature for one day and then analyzed the next day. Egg weight, eggshell strength, Haugh unit and shell thickness were investigated as egg quality characteristics. Egg weight was measured using a balance and was recorded to the nearest 0.01 g. Eggshell strength (kg) was measured with an ERTEST device (Ankara, Turkey). The height of the albumen was measured using a height gauge. The Haugh unit was calculated using the following formula: Haugh unit = $100 \times \log(H + 7.57 - 1.7W^{0.37})$, where H is the albumen height (mm) and W is the egg weight (g) (Haugh 1937). For eggshell thickness, three parts of the egg (pointed, medium and blunt) were measured with a digital micrometer with a sensitivity of 0.001 mm and the average was taken.

Statistical analysis

One-way analysis of variance (ANOVA) was used in the analysis of data. Tukey test was used in comparisons between groups. All hypothesis tests will be performed at a significance level of 0.05 and the Minitab 16 package program will be used for statistical analysis.

Results and Discussion

Egg weight was 66.4 g in the group without roosters and 64.2 g in the group with roosters, and the differences between the groups were statistically insignificant ($P > 0.05$)(Figure 1). Egg weight is an important criterion for consumers. The most important factors affecting egg weight are genotype, age, body weight and the amount of methionine in the diet (Koelkebeck *et al.*, 1992; Hocking *et al.*, 2003; Wolc *et al.*, 2012). According to the catalog values of the Lohmann Sandy genotype, the egg weight at 41 weeks of age was determined to be approximately 63 g (Anonymous, 2021). The egg weight obtained in our study was higher than these values.

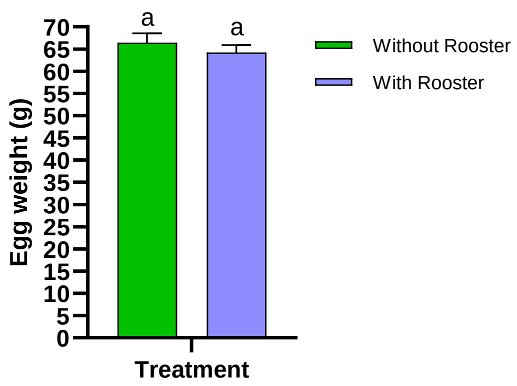

Figure 1. Effect of rearing with and without roosters on egg weight

Eggshell strength was 5.412 kg in the group without roosters and 5.183 kg in the group with roosters, and the difference between the groups was statistically insignificant (P > 0.05; Figure 2).

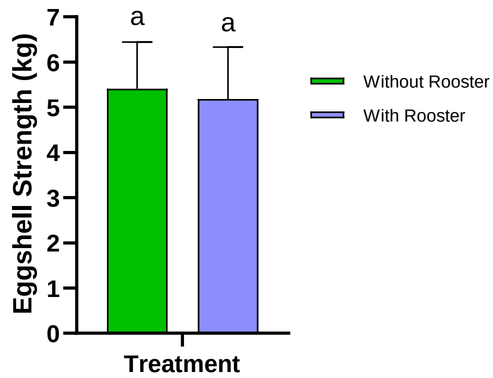

Figure 2. Effect of rearing with and without roosters on eggshell strength

Eggshell strength has an important effect on the collection, transportation and storage processes of eggs. According to the catalog data of the Lohmann Sandy genotype, the eggshell strength was stated to be higher than 4.079 kg, but an average value was not given (Anonymous, 2021). The eggshell strength values obtained from our study (5.355 and 5.400 kg) are better than the catalog data.

The egg Haugh unit was 94.3 in the group without roosters and 90.6 in the group with roosters, and the difference between the groups was statistically insignificant (P > 0.05; Figure 3).

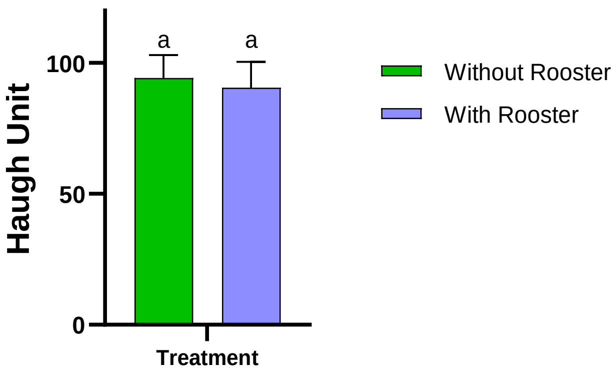

Figure 3. Effect of rearing with and without roosters on Haugh unit

The Haugh unit is an important internal quality trait developed by a scientist named Haugh in 1937 and calculated by egg weight and egg albumen height. The higher the egg Haugh unit, the better the egg quality and the longer the egg can maintain its shelf life during storage. Akyol and Denli (2023) determined the egg Haugh unit as 84.7 in their study with the Lohmann Sandy genotype in the free-range system. Kop-Bozbay (2024) determined the egg Haugh unit as 92.43 in their study with the Lohmann Sandy genotype in the free-range system. Alkan (2023) determined the egg Haugh unit as 92.90 in his study with the Lohmann Sandy genotype in the free-range system.
Shell thickness was 0.399 mm in the group without roosters and 0.405 mm in the group with roosters, and the difference between the groups was statistically insignificant ($P > 0.05$; Figure 4).

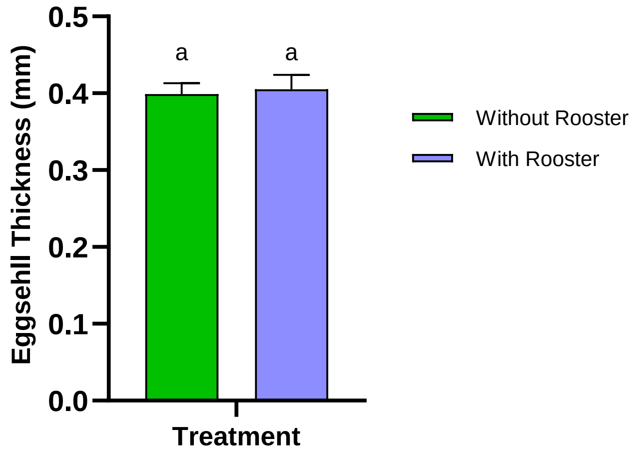

Figure 4. Effect of rearing with and without roosters on eggshell thickness

Alkan (2023) determined the egg shell thickness as 0.400 mm in his study with the Lohmann Sandy genotype in the free-range system. Akyol and Denli (2023) determined the egg shell thickness as 0.360 mm in his study with the Lohmann Sandy genotype in the free-range system. Kop-Bozbay (2024) determined the egg shell thickness as 0.427 mm in his study with the Lohmann Sandy genotype in the free-range system.

Conclusion

Rearing hens with roosters in a free-range system had no effect on egg weight, eggshell strength, Haugh unit and shell thickness. It is believed that having roosters in the flock is a more natural breeding method and since this practice does not have a negative effect on egg quality, roosters can be easily added to the flock. It may also be recommended to investigate the rooster/hen ratio in the flock.

Acknowledgement

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COMPARISON OF THREE DIFFERENT LAYER GENOTYPES RAISED IN A FREE-RANGE SYSTEM FOR EGG PRODUCTION PERFORMANCE

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Abstract

The aim of this study is to compare the egg production performance of three different layer genotypes (Lohmann Sandy; LS, Lohmann Brown; LB; and ATAK-S; A) reared in the free-range system. We determined egg performance characteristics such as egg production (% hen-day), broken-cracked egg ratio (%), egg weight (%), feed intake (g/hen/day), and feed efficiency (g feed/g egg). We measured egg production performance between 28 and 35 weeks of age. Indoor stocking density was set at 6 chickens per m^2, and outdoor stocking density was set at 4 m^2 per chicken. Hen-day egg production (%) was determined in the LS genotype (94.8%), LB genotype (89.56%), and A genotype (86.59%), and the differences between genotypes were statistically insignificant ($P > 0.05$). The A genotype had the highest cracked-broken egg ratio (8%), while the LS genotype had the lowest cracked-broken egg (%1.2; $P < 0.05$). The highest feed intake was detected in the A genotype (115 g), and the difference in feed intake between the LS (100 g) and LB (103 g) genotypes was found to be statistically insignificant ($P > 0.05$). Egg weight was the lowest in genotype A (54.7 g) ($P < 0.05$), and there was no statistical difference between the LS (59.8 g) and LB (59.2 g) genotypes in terms of egg weight. The worst feed conversion ratio was found in genotype A (2.10) ($P < 0.05$), and there was no statistical difference between the LS (1.68) and LB (1.74) genotypes in terms of feed conversion ratio ($P > 0.05$). The results showed that genotype significantly influenced the cracked-broken egg ratio, feed intake, egg weight, and feed conversion ratio, with the exception of hen-day egg production.

Keywords: *Genotype, Free-range system, hen-day egg production, egg weight, feed intake.*

Introduction

Conventional cage egg production has been banned in European Union countries since 2012 and egg production has been suggested to be done in alternative systems (EU, 1999). One of these production systems is the free-range system. It has become increasingly important to identify the layer genotypes that will be used in egg production in the free-range system. White and brown layer genotypes are used in egg production in the free-range system. In our country, the Lohmann Brown genotype is mostly used as a brown layer in the free-range system. In addition, the ATAK-S genotype, one of our local genotypes, has a greater area of use in rural areas due to its feather color (Türker *et al.,* 2017; Tutkun *et al.,* 2018b; Aygun *et al.,* 2024). In recent years, some genotypes that produce cream-colored eggs and are called “tinted” have been developed and used in the free-range system.
There are sufficient number of studies examining the effects of different genotypes on performance traits in free-range system (Şekeroğlu and Sarıca 2005; Türker *et al.,* 2017; Tutkun *et al.,* 2018a; Sözcü *et al.,* 2021). However, there is no literature on the effects of so-called "tinted" genotypes on performance traits in free-range system.

For this purpose, our study aimed to compare the egg production, broken-cracked egg ratio, egg weight, feed intake, and feed efficiency characteristics of brown layer (Lohmann Brown), native genotype (ATAK-S) and tinted (Lohmann Sandy) genotypes in free-range system during 28 and 35 weeks of periods.

Materials and Methods

This study was carried out at Selcuk University, Faculty of Agriculture, Department of Animal Science (Turkey). Three different genotypes, commercial (Lohmann Sandy; (L), Lohmann Brown; B), and domestic (ATAK-S, A), were used in the study. The experiment consisted of 3 genotype groups and each group consisted of four replications. Each replication contained 20 chickens. A total of 240 chickens were used. Performance characteristics of the study animals were examined between 28 and 35 weeks of age. The hens were reared in a free-range system. The stocking density in the in-door area is 6 hens /m^2, while the out-door area provides 4 m^2 per hen. Water and feed are given as ad-libitum. Egg production was recorded daily, and hen-day egg production was calculated. The broken-cracked egg ratio was calculated by dividing the number of broken eggs by the total amount of eggs produced. Egg weight was determined by weighing all eggs produced in the last two weeks of each 4-week period. Feed consumption was determined by determining the remaining feed in 4-week periods. Feed conversion ratio was calculated by dividing feed consumption by the weight of eggs produced.

Statistical analysis

One-way analysis of variance (ANOVA) was used in the analysis of data. The multiple comparison test Tukey test was used in comparisons between groups. All hypothesis tests will be performed at a significance level of 0.05 and the Minitab 16 package program will be used for statistical analysis.

Results and Discussion

Hen-day Egg Production

Hen-day egg production (%) of different genotypes is given in Figure 1. Hen-day egg production (%) was determined in the LS genotype (94.8%), LB genotype (89.56%), and A genotype (86.59%), and the differences between genotypes were statistically insignificant ($P > 0.05$). The results obtained from our study are consistent with the results indicating that genotype does not significantly affect egg production (Türker *et al.,* 2017). The results obtained from our study are inconsistent with the results indicating that genotype significantly affects egg production (Fathel and Elibol 2006; Ketta *et al.,* 2020; Sharma *et al.,* 2022; Sözcü *et al.,* 2023). The reason for these differences may be that many factors affect egg production (Hocking *et al.,* 2003; Englmaierová *et al.,* 2014; Jacob *et al.,* 2014; Yetişir and Sarıca 2018). Indeed, differences in the rearing period, nutrition, lighting, research period, sexual maturity age, etc. may differ in the studies.

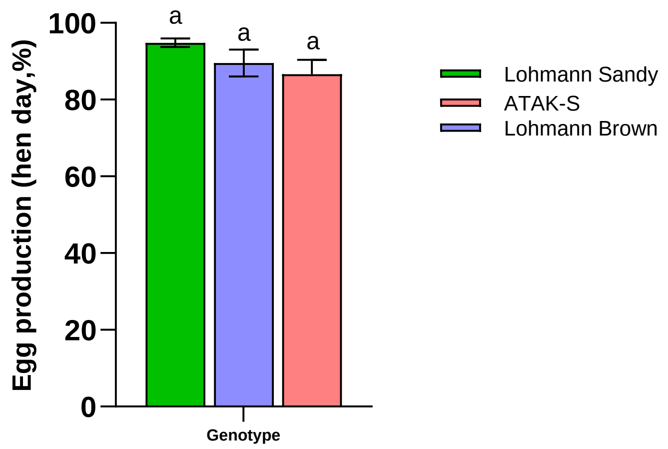

Figure 1. Egg production (hen-day,%) of different genotypes.

Broken-cracked eggs (%)

Broken-cracked eggs (%) of different genotypes is given in Figure 2. The A genotype had the highest cracked-broken egg ratio (8%), while the LS genotype had the lowest cracked-broken egg (%1.2; $P < 0.05$). The difference in the cracked egg rate of the Lb genotype with both the LS genotype and the A genotype was statistically insignificant.

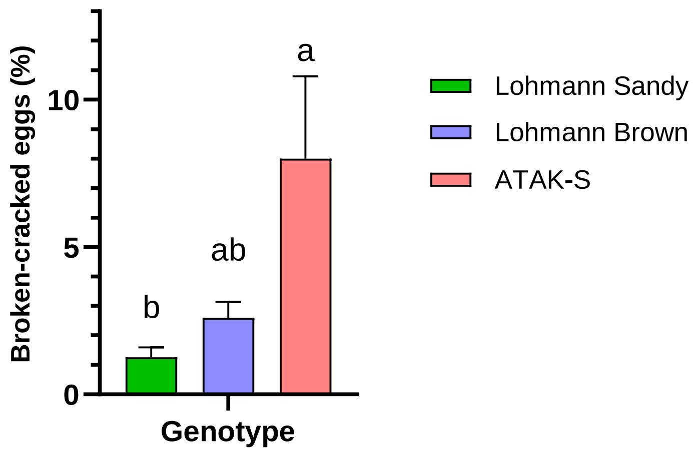

Figure 2. Broken-cracked egg (%) of different genotypes.

[a-b] Differences between groups indicated with different letters are statistically significant ($P<0.05$).

Approximately 8-10% of the eggs produced in egg production enterprises are broken-cracked eggs, which causes economic losses (Ketta and Tůmová 2016). The main factors affecting the broken-cracked egg rate are feeding, temperature, stress and nest management (Koelkebeck *et al.,* 1992; Konca and Yazgan 2002; Daghir 2008; Ketta and Tůmová 2016).

Egg Weight (g)

Egg weight (%) of different genotypes is given in Figure 3. Egg weight was the lowest in genotype A (54.7 g) ($P < 0.05$), and there was no statistical difference between the LS (59.8 g) and LB (59.2 g) genotypes in terms of egg weight. Egg prices are determined by egg weights in the egg market. Egg weight is positively related to body weight but negatively related to egg production (Du Plessis and Erasmus 1972). According to Kraus *et al.*, (2020), the genotype had a significant effect on egg weight and that the Lohmann Brown eggs (65.18 g) were heavier than the Hisex Brown eggs (63.73 g).

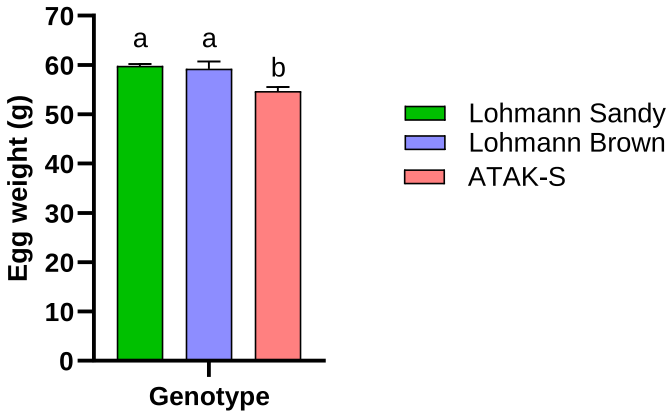

Figure 3. Egg weights of different genotypes.
[a-b] Differences between groups indicated with different letters are statistically significant (P<0.05).

Feed intake (g/hen/day)

Feed intake (g/hen/day) of different genotypes is given in Figure 4. The highest feed intake was detected in the A genotype (115 g), and the difference in feed intake between the LS (100 g) and LB (103 g) genotypes was found to be statistically insignificant (P > 0.05). Feed intake in poultry can be affected by the energy level of the feed, feeding time, feed form, age, genotype, rearing system and environmental conditions (temperature, stress, lighting, stocking density) (McDonald 1978; Küçükyılmaz *et al.*, 2012; Classen 2017; Kahraman *et al.*, 2020; Ketta *et al.*, 2020).

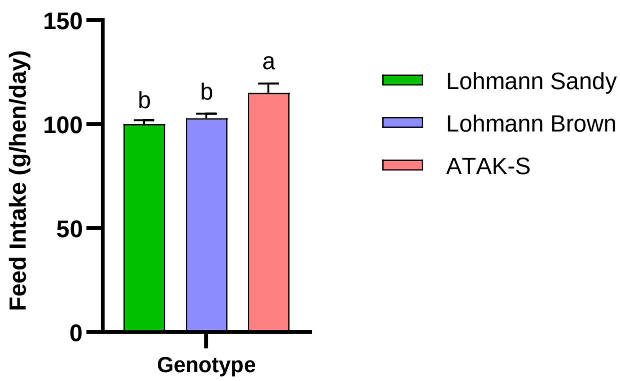

Figure 4. Feed intake of different genotypes.
[a-b] Differences between groups indicated with different letters are statistically significant (P<0.05).

Feed Conversion Ratio (g feed/g egg)

Feed Conversion Ratio (g feed/g egg) of different genotypes is given in Figure 1. The worst feed conversion ratio was found in genotype A (2.10) (P < 0.05), and there was no statistical difference between the LS (1.68) and LB (1.74) genotypes in terms of feed conversion ratio (P

> 0.05). This is consistent with studies showing that the effect of genotype on feed conversion ratio is important (Türker *et al.*, 2017; Sözcü *et al.*, 2023; Aygun *et al.*, 2024).

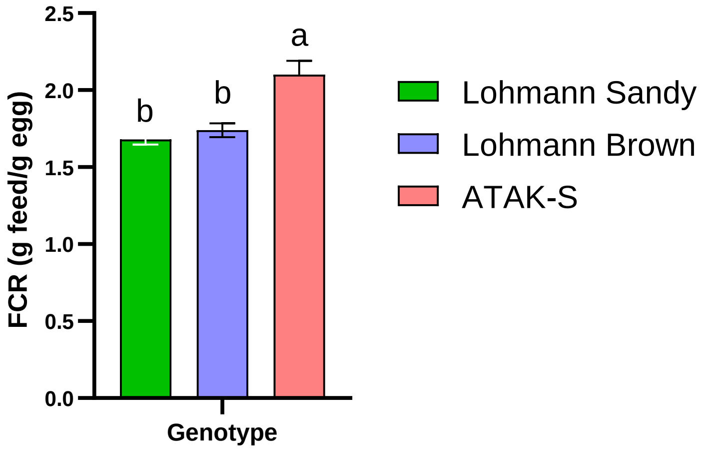

Figure 5. FCR (g feed/g egg) of different genotypes.

[a-b] Differences between groups indicated with different letters are statistically significant (P<0.05).

Conclusion

The results showed that genotype significantly influenced the cracked-broken egg ratio, feed intake, egg weight, and feed conversion ratio, with the exception of hen-day egg production. According to the results of the current study, it would be more appropriate to prefer LS and LB genotypes in the free-range system.

Acknowledgement

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RESPONSE OF BROILERS TO A FERMENTED PROTEIN SUPPLEMENTARY FEED

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Abstract

This study investigated the effect of fermented protein supplementary feed (FPS; 630 g CP/ kg DM, Integro®) as a protein source in broiler diets on performance and relative weight of carcass and non-carcass components. A total of 600 one-day-old broiler chickens (ROSS308) were randomly divided into six groups of five replicates of 20 chicks each in a completely randomized design. During the experiment (40 days), animals in the control group were fed standard broiler starter and grower diets (C), while the diets of the other groups were supplemented with 20 (FPS20), 40 (FPS40), 60 (FPS60), 80 (FPS80) or 100 (FPS100) g/kg FPS. The FPS20 birds had the highest body weight. Both the C and FPS20 groups had higher feed intake than the other groups. The FCR values of the FPS20 and FPS40 groups were better than those of the FPS60 and C group, followed by the FPS80 and FPS100 groups, respectively. The carcass yield and *pectoralis major* were lower in the FPS80 and FPS100 groups compared to the other groups. The neck weight was higher in FPS60 and FPS100 than in FPS80 birds. The tail weight was higher in FPS40 and FPS80 birds than in the group C. The liver weight was higher in the FPS100 group compared to the group C. The whole gut-weight was higher in the FPS40, FPS80, and FPS100 groups compared to the other groups. These results provide clear evidence of the benefits of adding 40 g/kg FPS to broiler diets without any negative impact on growth performance.

Keywords: *Broiler, Fermented feed, Performance, Supplementary feeding.*

Introduction

The search for alternative protein-rich sources aims to address the protein deficit in animal diets, provide sustainable quality feed ingredients and reduce costs on farms. A balanced protein diet is important for maintaining vital activities, strengthening the immune system and reducing the risk of disease.

Oilseeds and oilseed meals are the main sources of protein (İlkdoğan, 2008). However, the fact that imported meal is generally used in our country due to shortages, as in Europe, makes the compound feed sector dependent on imports and causes an increase in prices by being rapidly affected by economic fluctuations. In addition, the lack of a continuous flow of raw materials causes problems in production (Van der Poel *et al*., 2013). Therefore, the search for cheap and reliable protein-rich feed raw materials that can be used in animal nutrition has gained momentum in order to meet the animal product needs of the growing population globally and in Türkiye. For a resource to be used as a raw material for animal feed, it should have adequate nutritional value, be non-toxic, digestible and palatable to animals (Vasey and Powell, 1984). In this context, unicellular proteins such as blue-green algae, yeasts and edible insects such as house flies and mealworms are used in animal nutrition. Since yeasts are beneficial in terms of performance in poultry nutrition (Shareef and Al-Dabbagh; 2009), the present study determined the effect of complex protein raw material obtained by treating beneficial metabolites, nitrogenous compounds and yeast cell proteins formed during yeast fermentation with thermal and chemical reactions on the performance of broiler chickens. The yeast industry by-product vinasse, obtained by fermentation of vinasse with certain cereals and milling industry by-products, has a crude protein content of 63%, is heat-treated and does not contain live microorganisms, and its amino acid profile is considered suitable for poultry nutrition. Therefore, our aim was to determine the possibilities and proportions of using the yeast industry by-product in broiler rations by replacing it with soybean meal in certain proportions.

Materials and methods

This study was conducted at the poultry facilities of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Türkiye. The experimental protocol and implemented procedures were approved by the local Ethics Committee for Experimental Animals (Protocol code HAYDEK-915/2022).

A total of 600 chicks with similar average body weights were divided into 6 treatment groups in 5 replicates of 20 chicks per replicate according to the randomised experimental design. During the experiment (40 days), animals in the control group were fed standard broiler starter and grower diets (C), while the diets of the other groups were supplemented with 20 (FPS20), 40 (FPS40), 60 (FPS60), 80 (FPS80) or 100 (FPS100) g/kg fermented protein supplementary feed (FPS; 630 g CP/ kg DM, Integro®). FPS was added to the diets to replace soybean meal in the FPS groups. The ingredients of the experimental diets are listed in Table 1.

The chicks were housed in an experimental poultry house with environmentally controlled sawdust litter and fluorescent lighting (starting with 23 h of light and 1 h of darkness and gradually changing to 18 h of light and 6 h of darkness at the end of the period) (Kop-Bozbay et al., 2024). Each pen in the poultry house was 1.25 × 2 m and each pen had a nipple drinker with an equal amount of drip and a hanging broiler feeder (tube type).

Body weight (BW) and feed intake (FI) in replicate pens were determined on day 42. These measurements were then used to calculate daily BW gain (BWG, g day^{-1}), FI (g day^{-1}) and feed conversion ratio (FCR, g feed: g gain).

To determine carcass and non-carcass components on day 42, two birds of each pen (10 birds per treatment) were selected and slaughtered. The non-carcass components were collected and weighed. Carcass yield expressed as dressing percentage, relative weights of non-carcass components were calculated as a percentage of BW just before the slaughter of each bird (g per 100 g BW).

Kolmogorov-Smirnov and Levene’s test were employed for normality and homogeneity of variances, respectively. Pen was experimental unit in the present study. IBM SPSS Statistics 25 package programme was used for one way ANOVA. Differences among the means were determined by using Duncan’s multiple range test ($P<0.05$).

Results and discussion

As shown in Table 2, FPS addition had a significant effect on the performance of broilers. The FPS20 the broilers had a higher BW and BWG compared to the other groups ($p < 0.05$). The FPS20 broilers had higher BW and BWG than the other groups ($p < 0.05$). BW and BWG of C and FPS60 birds were higher than FPS60 birds, and in FPS60 birds were higher than FPS80 and FPS100 birds ($p < 0.05$). The FI of C and FPS40 birds was higher than the other groups ($p < 0.05$). The FCR of the FPS20 and FPS40 groups was lower than that of the C and FPS60 groups ($p < 0.05$). The FPS80 groups had a lower FCR than the FPS100 birds ($p < 0.05$).

Table 3 shows that the use of FPS in the broiler diet did not affect the relative weight of the femur, tibia and heart of the birds ($p > 0.05$). The FPS80 and FPS100 treatments decreased carcass yield and *pectoralis muscle* weight of broilers compared to the other treatments ($p < 0.05$). The highest wing weights were found in the FPS100 group and the lowest in the FPS20 group ($p < 0.05$). Neck weights of FPS60 and FPS100 birds were higher than those of FPS80 birds ($p < 0.05$). Tail weight was higher in FPS40 and FPS80 groups than in C group ($p < 0.05$). The liver weight of the C group was lower than that of the FPS100 birds ($p < 0.05$). C, FPS20 and FPS60 birds had a lower whole gut weight than the other groups ($p < 0.05$).

Table 1. The ingredients of experimental diets (as fed)

	1-21 d					
Ingredients (g/kg)	**Control**	**FPS20**	**FPS40**	**FPS60**	**FPS80**	**FPS100**
Corn	545.4	556.5	569.9	580.8	592	602.2
Soybean meal	**376**	**347**	**317**	**287.4**	**257.6**	**229**
Fermented protein supplementary feed (FPS)	**0**	**20**	**40**	**60**	**80**	**100**
DCP	20.2	21.0	20.0	20.0	20.0	20.0
Limestone	12.5	11.5	11	10.5	10	9.3
DL-methionine	1.5	1.8	2.2	2.6	2.9	3.3
L-lysine HCI, 78%	1.4	2.2	2.9	3.7	4.5	5.2
Vegetable oil	43	40	37	35	33	31
Salt	10	10	10	10	10	10
Vit-min premix	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient levels						
Metabolizable energy (kcal/kg)	3048.25	3043.75	3044.35	3046.68	3049.50	3051.93
Crude protein (%)	21.98	22.01	22.01	22.00	22.00	22.03
	22-42 d					
Corn	598.3	611	625	634.2	645.7	658.7
Soybean meal	**319**	**288**	**257.5**	**230**	**200**	**170**
Fermented protein supplementary feed (FPS)	**0**	**20**	**40**	**60**	**80**	**100**
DCP	18.0	18.0	17.5	17.5	17.0	16.5
Limestone	7.8	7.2	6.8	6	5.8	5.2
DL-methionine	1.5	1.9	2.2	2.6	3	3.3
L-lysine HCI, 78%	1.4	2.2	3.0	3.7	4.5	5.3
Vegetable oil	54	51.7	48	46	44	41
Salt	8	8	8	8	8	8
Vit-min premix	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient levels						
Metabolizable energy (kcal/kg)*	3182.70	3184.88	3180.01	3181.80	3185.13	3184.43
Crude protein (%)	19.80	19.75	19.73	19.81	19.79	19.79

*Calculated values

Table 2. Performance of broilers fed on fermented protein supplementary feed (FPS)

Item†	Control	FPS20	FPS40	FPS60	FPS80	FPS100	SEM	P
Body weight, g								
	2259.19^{b}	2565.84^{a}	2180.66^{b}	1905.96^{c}	1407.69^{d}	1241.99^{d}	91.440	<0.001
Daily body weight gain, g								
	55.48^{b}	63.14^{a}	53.52^{b}	46.65^{c}	34.20^{d}	30.05^{d}	2.285	<0.001
Feed intake, g								
	10.49^{a}	108.93^{a}	93.23^{b}	92.07^{b}	91.19^{b}	97.31^{b}	1.858	<0.001
Feed conversion ratio, g FI/g BWG								
	1.99^{c}	1.72^{d}	1.74^{d}	1.97^{c}	2.67^{b}	3.24^{a}	0.104	<0.001

SEM: Standard error of mean. Means with different lowercase letters in the same row are statistically different at P<0.05.
†Values are the means of six pens.

Table 3. Relative weights of carcass and non-carcass components of broilers fed on fermented protein supplementary feed (FPS)

Item†, %	Control	FPS20	FPS40	FPS60	FPS80	FPS100	SEM	P
Carcass yield	70.17^{a}	71.73^{a}	70.05^{a}	69.79^{a}	67.58^{b}	67.77^{b}	0.335	<0.001
Pectoralis muscle	9.96^{a}	10.59^{a}	10.06^{a}	9.76^{a}	8.60^{b}	7.83^{b}	0.190	<0.001
Thigh special	10.05	10.16	10.03	9.81	10.26	10.13	0.097	0.848
Femur								
Boned	5.11	5.62	5.28	5.16	5.42	5.58	0.087	0.430
Deboned	3.85	4.10	3.93	3.85	3.88	3.74	0.049	0.443
Tibia								
Boned	4.80	4.46	4.50	4.47	4.52	4.47	0.065	0.678
Deboned	3.01	2.91	2.93	2.77	2.87	2.84	0.040	0.653
Wing	3.33^{bc}	3.21^{c}	3.41^{bc}	3.50^{b}	3.60^{ab}	3.86^{a}	0.045	<0.001
Neck	2.54^{ab}	2.54^{ab}	2.64^{ab}	2.93^{a}	2.49^{b}	2.93^{a}	0.057	0.034
Tail	0.68^{b}	0.73^{ab}	0.85^{a}	0.81^{ab}	0.87^{a}	0.74^{ab}	0.020	0.048
Heart	0.52	0.46	0.45	0.48	0.49	0.52	0.008	0.140
Liver	1.88^{b}	1.94^{ab}	2.09^{ab}	2.04^{ab}	2.00^{ab}	2.15^{a}	0.029	0.041
Whole gut	7.55^{b}	7.02^{b}	8.65^{a}	7.70^{b}	8.76^{a}	9.18^{a}	0.145	<0.001

SEM: Standard error of mean. Means with different lowercase letters in the same row are statistically different at P<0.05.
† Values are the means of six pens.

The dynamic structure of the broiler sector, which is open to development, allows for the use of a variety of feedstuffs that can be employed to enhance the yield characteristics. Yeasts, which are commonly utilized in the production of bread, cakes, and other food items, can also be utilized in animal nutrition to support growth and development. It has been demonstrated that yeast, when added to feeds as a growth factor, inhibits the ability of pathogenic microorganisms to persist in the intestinal microbial flora. Consequently, yeast enhances the live weight of broiler chickens and can be employed as a growth factor instead of antibiotics. In the present study, it was observed that the addition of 40 g/kg FPS to the diets had no detrimental effect on broiler performance. Even at the 60 g/kg level, FCR, carcass yield and pectoralis major muscle ratio were the same as in the Control animals. The observed outcomes may be attributed to the beneficial effects of metabolites produced during yeast fermentation and the subsequent formation of FPS in poultry diets, which have been shown to influence intestinal morphology by enhancing the humoral immune response while maintaining intestinal microbial balance (He *et al.*, 2021). This resulted in improved performance. It can be concluded that the use of FPS will be economically advantageous, given that 30, 60.25 and 88.8 g/kg less soybean meal was used in the FPS20, FPS40 and FPS60 groups, respectively, compared to the Control group. It is crucial to note that FPS lacks essential amino acids in comparison to soybean. In the experimental groups, essential amino acids were provided in an external supplement. It is therefore recommended that these factors be taken into account when economic analyses are not desired. However, it was unexpected that the live weight and FCR in the FPS20 group were superior to those of the Control. In fact, an additional 13.4% live weight gain and a reduction of 0.27 units in the FCR ratio were observed. This may be attributed to the antimicrobial effect of FPS. The objective was to achieve the same performance by replacing soybean with FPS as that observed in the Contol group. The performance of the 20 kg FPS group, which subtracts 30 kg of soybean meal from 1 tonne of feed from the control group, is promising in that it is higher than the control.

One of the most significant findings of the study was that the incidence of diarrhoea cases decreased as the use of FPS increased, despite the absence of anticoccidial agents in the rations. Consequently, the quality of breast meat will be preserved to a greater extent than in the control group. This may be attributed to the effect of beneficial metabolites formed during yeast fermentation in the present study. A number of studies have demonstrated the potential of probiotics and prebiotics as substitutes for antibiotics in improving immune dysfunction, intestinal morphology impairment, and growth performance in broilers (Ahiwe *et al.*, 2021). Probiotics comprising live yeast contain a plethora of biologically valuable proteins, functional nucleic acids, vitamin B complex, immune enhancers such as mannan oligosaccharide, β-glucan and growth-promoting factors (Berto *et al.*, 2020; Ahiwe *et al.*, 2021). Consequently, live yeast (Saccharomyces cerevisiae) has been demonstrated to confer a number of beneficial effects on poultry, including an improved gut microbial balance, an enhanced humoral immune response and gut morphology, and favourable growth performance outcomes (He *et al.*, 2021). Furthermore, the absence of mortality or health issues during the trial indicates that FPS can be employed in broiler diets at a dosage of 40 g/kg without negatively impacting animal performance.

Conclusion

It is important to meet the animals' essential amino acid requirements, improve digestibility and maintain overall nutrient balance when changing the content of protein ingredients in the ration. These strategies can help maintain chicken health and production performance while reducing costs. Indeed, the study provides clear evidence of the benefits of adding up to 40 g/kg FPS to the broiler diet without negatively affecting performance.

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LAYING PERFORMANCE OF LAYING HENS FED A FERMENTED PROTEIN SUPPLEMENTARY FEED

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Abstract

This study examined the laying performance (body weight change, egg-laying rate, feed intake, egg weight, and FCR) of Lohmann Brown hens (20-week-old) housed with a deep-litter system and fed fermented protein supplementary feed (FPS; 630 g CP/ kg DM, Integro®) as a protein source. The study lasted for 12 weeks. The hens were randomly allocated into four groups, each with four replicates of 25 hens. The control group was fed a conventional diet (C), while the other groups were fed a conventional diet with 20 (FPS20), 30 (FPS30), and 40 (FPS40) g/kg FPS. A corn–soybean meal-based conventional diet in mash form and water were provided ad libitum throughout the experimental period for all treatments. The highest feed intake value was observed in the FPS40 group, while the lowest was observed in the FPS20 and FPS30 groups. The C and FPS20 hens produced eggs with a higher weight than those produced by the FPS40 group. The FPS20 and FPS30 hens exhibited a superior feed conversion ratio (FCR) in comparison to the C and FPS40 hens. The treatments had no effect on the final body weight or egg-laying rate. The study provides compelling evidence that the addition of 30 g/kg FPS to the hen diet is beneficial without negatively impacting laying performance.

Keywords: *Fermented feed, Performance, Supplementary feeding, Layers.*

Introduction

The poultry sector is of great economic and nutritional importance to global food production and continues to be driven by consumer demands and technological innovation. To meet the animal product needs of a growing population, the search for protein-rich feed ingredients that are both cheap and reliable has accelerated. Reducing the level of soybean in laying hen diets can reduce costs, but care must be taken not to compromise hen health and production performance. In this context, fermented protein supplements offer a sustainable and efficient method of animal nutrition, enabling more efficient use of protein resources and providing both economic and environmental benefits.

Yeasts, which are widely used in bread, cakes and other food products, can also be used as growth and development promoters in animal husbandry. Yeasts used as growth factors in feeds are said to make it difficult for pathogenic micro-organisms to attach to the intestinal micro-organism flora. For this reason, yeasts are reported to have a positive effect on egg weight, egg yield, feed conversion ratio and egg quality in laying hens (Gurbuz *et al*., 2011; Cai *et al*., 2016; Li *et al*., 2016; Zhang *et al*., 2020; Park *et al*., 2020; Sjofjan *et al*., 2021). One of the important features of yeast is that industrial organic wastes and other organic waste materials can be used to produce single-celled organisms, and it is believed that such studies will assist in solving the environmental waste problem.

Because of the aforementioned benefits of yeasts in poultry nutrition, the present study will determine the effects of fermented protein supplementary feed (FPS), obtained by thermal and chemical reactions of beneficial metabolites, nitrogenous compounds and yeast cell proteins formed during yeast fermentation, on the performance of laying hens. The by-product of the yeast industry, obtained by fermentation of vinasse with certain cereals and by-products of the milling industry, heat-treated and free of living microorganisms, has a crude protein content of 63%, whose amino acid profile is considered suitable for poultry nutrition. Therefore, our aim is to develop an economically and environmentally sustainable feed formulation by substituting the yeast industry by-product with soybean meal in certain proportions and to determine the possibilities and proportions of its use in laying hen diets.

Materials and methods

This study was carried out from March to May 2023 using the poultry facilities of the Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Türkiye. The experimental protocol and implemented procedures were approved by the local Ethics Committee for Experimental Animals (Protocol code HAYDEK-940/2023).

After a pre-trial period (two weeks) of adaptation to the diet, 400 Lohmann Brown layer hens (22 weeks old) were divided into four groups with four replicates of 25 hens each. The experimental period was 12 weeks. All hens were housed in floor litter pens in a curtain-sided house with mechanical ventilation and both artificial (LED bulbs) and natural lighting through the windows. Each pen (2.5 × 3 m) was equipped with a perch, individual nests (30 × 45 × 60 cm height, 1 nest/5 hens), an automatic drinker and a red circular feeder plate. During the experiment, the temperature was maintained at 20 ± 1 °C. The hens were exposed to artificial plus natural light according to the daily photoperiod to provide 16 h of light (Kop-Bozbay et al., 2021).

The control group was fed a conventional diet (C), while the other groups were fed a conventional diet supplemented with 20 (FPS20), 30 (FPS30) and 40 (FPS40) g/kg fermented protein supplementary feed (FPS; 630 g CP/ kg DM, Integro®). FPS was added to the diets to replace soybean meal in the FPS groups. Throughout the experimental period, all treatments were provided ad libitum with a corn–soybean meal-based conventional diet in mash form and water. The ingredients of the experimental diets are listed in Table 1.

Hens were weighed at the beginning of the experiment and after 12 weeks. During the experiment, feed intake (FI) was recorded at 14-day intervals, while all eggs and their individual weights were recorded on the last 3 days of each 7-day interval per replicate pen. Egg-laying rate (%), FI (total FI/number of days in the experiment) and FCR (g feed: g egg mass) were calculated. For normality and homogeneity Kolmogorov-Smirnov and Levene’s test were used. One way ANOVA was performed by using IBM SPSS 25. Significant differences among the means were determined by using Duncan’s multiple range test at $P<0.05$.

Results and discussion

No differences were found in final body weight, body weight change, and laying rate (Table 2). The FI of the FPS40 hens was higher than that of the C hens, whereas the C hens were higher than that of the FPS20 and FPS30 hens ($p < 0.05$). The egg weight of C and FPS20 hens was higher than that of FPS40 hens ($p < 0.05$). The FCR of the FPS20 and FPS30 groups was lower than that of the C and FPS40 hens ($p < 0.05$).

The C hens had a numerically lower laying rate compared to all FPS groups. In fact, the FPS30 group had the highest laying rate and the egg weight was the same as the control group. Although 63.4% soybean meal was less than the C group, such a result is an indication that FPS will provide an advantage in the use of diets for laying hens. In addition, in the FPS40 group, egg weight decreased despite significantly higher egg production. For this reason, it may only allow a 2% more profitable production in terms of feed costs compared to

the control group. The FPS20 group had the highest egg weight and laying rate was numerically higher than the C group. In fact, the fact that the FCR was lower in the FPS20 and FPS30 groups supports this finding. In addition, the data relating to the final body weight suggests that despite a reduction in the concentrate feed intake, the FPS20 and FPS30 promoted egg weight in the hens kept under the same rearing conditions. The findings demonstrate that the incorporation of FPS into the diet as a substitute for soybean not only improvements FCR in laying hens but also results in eggs of optimal weight, benefiting both producers and consumers. These findings align with those of previous studies, which have demonstrated the necessity of yeast culture supplementation for the detection of positive effects on livestock and poultry performance. (Gao et al., 2008; Zhang et al., 2020).
The results obtained may be due to the positive effect of FPS in poultry diets on intestinal morphology by providing an improved humoral immune response without disturbing the intestinal microbial balance (He et al., 2021). This resulted in improved performance. In addition, the fact that no mortality or health problems were recorded during the trial demonstrates that FPS can be used in laying hen diets without adversely affecting animal performance.

Table 1. The ingredients of experimental diets (as fed)

Ingredients (g/kg)	Control	FPS20	FPS30	FPS40
Wheat	224	224	224	228
Corn	357	366	371	373.5
Sunflower seed meal (36)	149.5	149.5	150	152
Fermented protein supplementary feed (FPS)	**0**	**20**	**30**	**40**
DCP	13	13	13	13
Limestone	79	79	79	79
Salt	2.5	2.5	2.5	2.5
Soybean meal	**115**	**88**	**73**	**55**
Vegetable oil	50	48	47	46
Vit-min premix[1]	2.5	2.5	2.5	2.5
L-lysine HCI, 78%	1.35	1.35	1.5	1.65
DL-methionine	1.45	1.45	1.6	1.75
L-threonine	1.2	1.2	1.4	1.6
Sodium bicarbonate	2	2	2	2
Toxin binder	1.5	1.5	1.5	1.5
Nutrient levels				
Metabolizable energy (kcal/kg)*	2797.48	2797.98	2797.30	2796.45
Crude protein (%)	16.52	16.57	16.54	16.45

*Calculated value [1]Per kilogram of diet: vitamin A (retinyl acetate) 20 000 IU, vitamin E (dl-αtocopheryl acetate) 80 mg, vitamin D3 (cholecalciferol) 6000 IU, vitamin B1 (thiamine monophosphate) 3 mg, vitamin B2 (riboflavin) 12 mg, vitamin B6 (pyridoxine hydrochloride) 8 mg, vitamin B12 (cyanocobalamin) 0.04 mg, vitamin K3 (menadione) 4.8 mg; vitamin H (D-biotin) 0.2 mg, vitamin PP (nicotinic acid) 48 mg, folic acid 2 mg, calcium pantothenate 20 mg, manganous oxide 200 mg, ferrous carbonate 80 mg, cupric sulphate pentahydrate 20 mg, zinc oxide 120 mg, basic carbonate monohydrate 0.4 mg, anhydrous calcium iodate 2 mg, sodium selenite 0.4 mg, choline chloride 800 mg, 4-6-phitase 1800 FYT, DL-methionine 2600 mg, canthaxanthin 8 mg

Table 2. Laying performance of laying hens fed on fermented protein supplementary feed (FPS)

	Control	**FPS20**	**FPS30**	**FPS40**	**SEM**	**P**
Initial body weight (g/hen)	1767.49	1730.41	1740.13	1746.03	9.098	0.570
Final body weight (g/hen)	1862.64	1841.20	1848.98	1837.64	8.119	0.748
Body weight change (g)	95.15	110.79	108.85	91.60	9.201	0.872
Egg-laying rate (%)	90.58	92.59	94.03	93.68	1.232	0.792
Feed intake (g/hen/day)	120.68^{b}	109.58^{c}	108.58^{c}	127.68^{a}	2.098	0.000
Egg weight (g)	61.25^{a}	61.37^{a}	60.75^{ab}	59.72^{b}	0.259	0.045
FCR (g feed: g egg mass)	2.26^{a}	1.98^{b}	1.97^{b}	2.36^{a}	0.536	0.003

SEM: Standart error of mean. Means with different lowercase letters in the same row are statistically different at P<0.05.

Conclusion

It is important to meet the animals' essential amino acid requirements, improve digestibility and maintain overall nutrient balance when changing the content of protein ingredients in the ration. These strategies can help maintain chicken health and production performance while reducing costs. Indeed, the study provides clear evidence of the benefits of adding 30 g/kg FPS to the hen diet without negatively affecting laying performance.

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COMPUTATIONAL FLUID DYNAMICS (CFD) APPLICATIONS IN LİVESTOCK BUILDINGS

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Abstract

Computational Fluid Dynamics (CFD) is a branch of fluid mechanics in which important problems of fluid motion are solved in a virtual environment with numerical data and algorithms. In recent years, CFD has been used for purposes such as increasing production performance in farm buildings, especially in livestock hoseses and greenhouses, determining indoor climate conditions suitable for animal welfare, and predicting problems that may arise in farm buildings and producing solutions. CFD analyses aim to determine the improvement and optical design values by calculating the fluid-structure interaction forces of high and wind-exposed agricultural structures. In analyzing the climate parameters inside the barn in the farm; Using CFD analysis of a three-dimensional model prepared over time will help examine the sensitivity of environmental impacts such as different climate scenarios, management practices and use of different materials on the system before investing. In this way, problem areas that may arise in the barn in terms of structural, climatic and animal welfare can be identified and appropriate solution proposals can be developed without building a farm or a barn. As a result, it will be easier and at lower costs to design new barns that are suitable for animal welfare, which will increase product efficiency in animal production, and to detect any problems that may arise during construction.

Keywords: *Indoor climatic conditions, Computational fluid dynamics, livestock building, farm structures.*

Introduction

CFD (Computational Fluid Dynamics) analysis, a branch of fluid mechanics, analyzes the motion process of fluids and gases by performing mathematical, statistical and algorithmic calculations in order to create 3D models. Computational Fluid Dynamics (CFD) is a branch of fluid mechanics in which important problems of fluid behavior are solved in a virtual environment with numerical data and algorithms (Anonymous, 2024a). While CFD brings computer-based engineering solutions; It offers conveniences such as control of fluids and gases, design optimization related to production and efficient use of resources (Anonymous, 2024b). It is very important to determine the efficiency of a product in any sector at the design stage. The usability of CFD in increasing product efficiency enables the evaluation of the situation and the elimination of problems before they occur (Açıkgöz et al., 2007). Performance models of analyzes during agricultural production are very useful in completing the deficiencies when agricultural test results are missing or when the data is very expensive to obtain. In addition to these analyzes; They allow us to study the distribution of growth effects, economic efficiency, use of sustainable agricultural techniques, climate change resulting from global warming, interactive resource management and use. These models can also be used to determine appropriate management systems that allow us to adapt to targeted economic and climate variability using mixed cooling or distribution techniques (Cros, et al., 2006).

Computational Fluid Dynamics (CFD); provides practical, healthy and precise results for use in today's conditions, and saves cost and time for the employer. CFD analysis and simulations save time in the design process, obtain cheaper and faster results compared to traditional tests, The fact that the tests that need to be done in reality can be done in the desired number using different parameters with CFD analysis can be listed among the reasons for actively preferring the study. CFD has become an engineering tool used to reduce the design, cost and design stages of fluid behavior processes in many areas from aviation to the automotive sector, from biomedical engineering to meteorology, from the food industry to chemistry, and has a much more effective place in solving complex processes (Nicolaï et al., 2006). In recent years, CFD; It is also important in terms of increasing production performance in agricultural structures, determining the indoor climatic conditions in the livestock houses suitable for animal welfare, and predicting the problems that may arise during the implementation phase of projects developed in agricultural structures and producing solution suggestions.

In the analysis of indoor climate parameters, the barn in the farm; using CFD analyses of the simulation model designed in three dimensions depending on time will help to examine the sensitivity of environmental effects such as different climate changes, management practices and different material usage on the system before making an investment. Thus, by determining the problem areas that may arise in terms of structural, climatic and animal welfare in the barn, it will be possible to develop appropriate solution suggestions before the farm structure and livestock buildings are built. This study was carried out to emphasize what CFD applications are in livestock buildings, how they can be used and the importance of these studies.

Importance and Usage Areas of CFD

The representations made to make the system simple and understandable by using all the situations that occur in the universe and what is known about these situations are called "models". Explanation of an idea by analogy is called modeling. Modeling is the oldest and most effective method used to solve the complexity of systems. (CFD) is the branch of fluid mechanics in which important problems of fluid movements are solved in a computer environment with numerical data and algorithms. CFD analysis uses statistical and numerical methods and algorithms for the analysis and solution of problems related to fluid mechanics. In complex structures where analytical methods are inadequate and cannot be calculated; the numerical determination of data such as flow rate, heat transfer, pressure loss provides resource and time savings in terms of design and production. As a result of simulating complex behaviors; fast, safe, economical and comfortable solutions are provided. Parameters such as flow dynamics, flow conditions, flow region can be analyzed by simulating with differential equations. CFD, on the other hand, divides the workspace into finite parts and analyzes each unit separately numerically. In measurements and analyses, more precise and accurate results are achieved while application flexibility and a detailed perspective are achieved (Anonymous, 2024a).

In general, CFD is a method that allows determining the behavior of all kinds of fluids and flows under different conditions. In this method, the numerical solution is carried out with the continuity, momentum and energy equations, which are basically three main equations. While the pressure, velocity and temperature distribution data in the flow are obtained, many data can be easily reached by means of these equations (Ferziger and Peric, 2002).

Complex geometries, viscosity, temperature and heat changes etc. factors complicate the solution point when applied to the basic equations of classical fluid mechanics. While the data that can be solved in classical fluid mechanics cannot be solved when applied to structures (livestock buildings, green houses, etc.) with different geometries, a solution can be obtained by analyzing them with the CFD method. The network structure of the structure to be

simulated is created by differential equations, namely mesh points, and the solution is reached. The usability of simulation models accelerates the analysis process experimentally or by using mathematical optimization techniques. This process increases the speed of agricultural analyses in reducing costs and reaching accurate results. It is a guide for determining the most appropriate management criteria in the development of sustainable and renewable resources (Cros, et al., 2006).
It is a new process to start working on integrated system models in agricultural enterprises. In order to create models, the selection of data to be loaded into simulators, the input and output processes of the data and the evaluation of the results constitute the solution phase of the model. There is a need for more studies with the modeling approach in enterprises where agricultural technologies defined as precision agriculture are used at the highest level. It is thought that using simulations by modeling the software to be developed in agricultural production in such enterprises will eliminate the disruptions in the production process (Cros, et al., 2006).

CFD Applications in Livestock Buildings

In the field of Agricultural Construction, CFD analyses are performed on high and wind-exposed agricultural structures; fluid-structure interaction forces are calculated, and improvement and optical design values are determined. Facade load calculations are performed and wind modeling around the livestock houses is solved with indoor and outdoor CFD analyses. In agricultural production, CFD analyses can be listed as areas where livestock houses, greenhouses, protection and storage structures, tractors and harvesting machines where all planting and maintenance operations are carried out, irrigation systems and equipment, drying equipment needed in the product processing and drying phase in closed agricultural production areas, and in the development of aircraft such as drones, helicopters, spraying jets and airplanes used in agriculture (Saygılı and Çakmak, 2022). Animal welfare and productivity depends on the indoor air quality in the livestock buildings and creating a comfortable environment (Uzal and Uğurlu 2009).
In improving inside air quality, ventilation system analysis can be used to determine the behavior of air flow inside the livestock houses using CFD and therefore the efficiency of the ventilation system can be determined. For example, by examining the air flow behavior in a closed shelter with air openings from the ceiling and doors or windows, the distribution of inside climate conditions in the livestock houses such as humidity, speed and temperature created by the flow movement created by the air entering and exiting the livestock house can be examined (Anonymous, 2024c).
Akdemir et al. (2012), in their study conducted to examine the temperature and humidity distribution movements in cold storages and to increase the economic storage life of agricultural products for longer periods, they examined the relationship between temperature and humidity distribution and cold storage configuration with ANSYS, CFD finite element software. They reported that they produced 3 different scenarios by developing 3 different wing angle simulations for cold storages. They stated that there was less change in the air flow rate measured in the evaporator storage compared to the air flow rate of the storages included in other ventilation systems. In their study conducted to determine the natural ventilation efficiency and effective ventilation height in different greenhouse models suitable for Samsun conditions and at different wind speeds, Atiş (2011); studied the climate parameters of May, June, September and October for 6 different greenhouse models and each greenhouse model with ANSYS computational fluid dynamics (CFD) and the natural ventilation efficiency in different greenhouse models suitable for Samsun conditions and at different wind speeds (0.5, 1 and 2 m s-1). As a result of the study, among the six different greenhouse models determined to be evaluated as a naturally ventilated greenhouse model

suitable for covered plant production in the region, the 3rd Application model was recommended out of 6 different greenhouses due to the superior performance of the effective ventilation height and the appropriate environmental conditions within the greenhouse.
Kim et al. (2007) used the FLUENT program to model the air temperature and relative humidity distribution in greenhouses with a fogging cooling system. In the test conducted on the developed CFD model, data were taken from a greenhouse with a fogging cooling system that was not in production. As a result of the obtained data and the analyses performed on the model, it was reported that a difference was detected between air temperature and relative humidity for greenhouses.
Various researchers (Choi et al., 1988; 1990; Hoff et al., 1992; Harral and Boon, 1997; Mistriotis et al., 1997; Zhang et al., 2000; Tinoco et al., 2001 and van Wagenberg et al., 2004) report that they have conducted many different studies such as ventilation system design with models they created by taking into account relative humidity, indoor air temperature and chemical composition of the air in the shelter using CFD in animal livestock houses designed in different ways.
Demir, V., T. Günhan & H. Bilgen (2022) aimed to reveal the air movements of ceiling type fans with two different rotation circle diameters with the CFD method and to determine the most suitable turbulence model. In the first stage of the study, the air velocity changes in the axial direction at certain distances from the fan center of the ceiling type fans were compared with the measured values, and in the second stage, the values calculated with the CFD analysis method with different turbulence models were compared with the measured values. As a result of the study, they reported that with the appropriate selection of the turbulence model, very close estimates to the experimental data could be obtained with CFD simulation at points at certain heights. In their study, Pakari and Ghani (2021) compared the results they obtained by using CFD simulations with field measurements in the comparison of ventilation systems developed with the help of different machines that cannot be naturally ventilated for dairy cattle barns. As a result of the study, they reported that the solution approaches of the CFD simulation models were very close to each other with field measurements.
Küçüktopcu et al. (2022) conducted a study to evaluate the spatial variability of environmental conditions in a free-stall dairy cattle barn using CFD and compared the temperature and air velocity measurements in the livestock houses with simulated results. As a result of the research, they reported that CFD is an important method to evaluate the variability of environmental conditions and barn characteristics in dairy cattle houses, considering animal welfare, and can be used as an alternative technique air quality analysis in livestock houses. There are turbulence models that allow the use of CFD. These models were evaluated in terms of their ability to accurately simulate the internal turbulent flow of a barn. As a result of the study, the RNG k-3 model was accepted as the best air velocity measurement model among the developed models (Küçüktopçu and Cemek, 2019).
Coradi et al. (2016) tested the simulation of heated air inside the barn with (CFD) in order to increase animal welfare and productivity in their study. As a result of the study, they reported that they obtained data on the temperature distribution, heat flow, heated air pressure and air flow velocity of the cage heating system. They also reported that they observed that poultry did not prefer the sections of the house where the temperature was high.

Conclusion

In recent years, CFD analysis has been widely used in livestock houses to analyze the indoor climate conditions in the livestock buildings, to determine the effect of using different material combinations in the livestock houses on the indoor climate in the shelter, and to identify possible problems and produce alternative solutions. The use of CFD in animal production; It is a very important issue for the country's economy that it enables the development of solution suggestions by determining the possible negativities that may occur because of the construction of new livestock houses designs to be developed in order to increase animal welfare and product efficiency with simulations before the construction of the livestock buildings. In addition, this situation means a new era in terms of labor and time in new livestock houses design. Therefore, increasing the use of CFD in livestock buildings studies is an important issue that needs to be worked on to increase animal welfare and production efficiency.

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THE EFFECT OF DIFFERENT RAISING CONDITIONS ON LIVE WEIGHT AFTER SHEARING AND GREASY WOOL YIELD IN FAT TAILED EWES

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Abstract

This paper was compiled from research carried out to determine wool yield and live weight after shearing of fat tailed ewes in rural farm condition. In these researches, the greasy fleece weight, the live weight after shearing, and the fleece yield value of Karakaş, Morkaraman, Norduz, and Zom sheep raised in different breeder conditions were investigated. One of main objectives of sheep breeding in terms of contemporary sense is to increase the production from sheep. Indigenous sheep breeds raised in rural conditions need to be genetically improved because genetic improvement offers solutions for satisfaction of needs in livestock husbandry. In addition to determining the productivity performance and morphological and physiological characteristics of local sheep breeds raised in cultivation conditions directly, research on determining the breeding infrastructure and breeder tendencies will be useful for more effective animal husbandry. The quantity and quality of fleece obtained from sheep are shaped by the effects of genotype and environmental factors. Environmental factors that affect quantity and quality can be listed as view-feeding, gender, lambing method, live weight, and age. The least squares mean for greasy fleece yield in Karakaş, Morkaraman, Norduz, and Zom ewes were found to be 1.93±0.02 kg, 1.41±0.021 kg, 2.05±0.004 kg, and 1.37±0.02 kg, respectively. When all sheep breeds were generally evaluated, the effect of shearing age and the farm on greasy wool yield was found insignificant ($p>0.05$), but the effect of live weight after shearing was statistically significant ($p<0.01$). The genotype level of the herd and environmental factors can be considered among the reasons for the differences observed between breeds in terms of live weight and wool yield after shearing.

Keywords: *Ewe, Greasy fleece yield, Live weight, Shearing, Wool improvement.*

Introduction

The fleece of sheep has a very important yield; It is the best raw material for the weaving and carpet industry with its properties such as fineness, strength, heat retention, moisture absorption, low wetting, and felting. The fleece of native sheep breeds in Türkiye is generally coarse mixed and suitable for carpet making (Öztürk and Odabaşı, 2011). In recent years, the use of coarse mixed fleece has been increasing in the world textile industry.

Wool is the most important raw material of the textile industry. Wool is a valuable woven material that shows superior clothing physiology such as protection from cold, good heat holding, high humidity take-up, low wetting capability, and felting ability (Kara Uzun 2008). The wool is still able to compete with many artificial fibers due to its unique properties.

In Türkiye, small livestock farming is generally carried out with low-productivity local breeds, pasture-based care, feeding conditions and extensive conditions where production is aimed with limited input, and the production method varies by region (Semerci and Çelik, 2016). The different conditions of the breeding regions cause differences in sheep breeding techniques between regions.

Reasons such as migration from villages to cities, difficulties in finding shepherds and the decrease in meadow and pasture areas can be given as reasons for the increase and decrease in small livestock breeding in numerical terms. As in many regions of Türkiye, in the region where extensive and semi-intensive sheep farming is carried out in a wide area, breeders earn significant income from lamb and yearling fattening and lamb sales.

As in other farm animals, one of the ways to increase the yield per animal in sheep is genotypic improvement of populations. Quantitative characters can be determined more in the later stages of life. This situation affects the selection efficiency negatively by prolonging the time between generations. The probability of genotypic superiority of individuals determined to be phenotypically superior determines the degree of accuracy in selection (Aygün and Karaca, 1999).

Fleece has a special advantage over other fibers in terms of properties such as temperature retention, moisture absorption and felting. For this reason, fleece has been given great importance and fabrics and items made from fleece have an important place in human life. Fleece used in the textile industry must be suitable for the processing techniques used in the weaving industry. Coarse-mixed fleeces are preferred in bed making. Thus, fleeces of different quality are used in various fields. Fleece has an important place in terms of both protecting the sheep against environmental factors and meeting people's needs with better quality. Although many artificial fibers have been introduced to the market, the superior properties of fleece have not been imitated, and artificial fibers, which dominate the market due to their easy and cheap costs, have not been able to capture the superior properties of fleece in terms of quality features. Even though synthetic fabrics such as nylon, orlon, and dacron made with these types of fibers are more numerous, more diverse, and cheaper, it is not possible for them to replace fleece today (Emsen, 1992; Kaymakçı, 2010).

The domestication and breeding of small ruminants are virtually as old as human history. As in many countries of the world, sheep farming activities are attractive for producers due to ease of management, care, efficient utilization of undersized grasslands, resistance to disease and harsh climatic conditions. Sheep (*Ovis aries*) is the most cultivated farm animal in different parts of the world, after goat, among livestock (Galal 2005).

This article was compiled from research carried out to determine wool yield and live weight after shearing of fat tailed ewes in rural farm condition. In these researches, post-shearing live weight, greasy fleece yield and yield characteristics of Karakaş, Morkaraman, Norduz, and Zom sheep raised in different breeder conditions were evaluated. One of the main objectives of sheep breeding in terms of contemporary sense is to increase the production from sheep.

Greasy Wool Yield in Ewes

Least squares means for the greasy fleece weight, the live weight after shearing, and the fleece yield value in Karakaş, Morkaraman, Norduz, and Zom ewes are presented in the Table 1.

Table 1. Least squares means for the greasy fleece weight, the live weight after shearing, and the fleece yield value in Karakaş, Morkaraman, Norduz, and Zom ewes

Genotypes	**n**	**Greasy Fleece Weight (kg)**	**Live Weight After Shearing (kg)**	**Fleece Yield Value (%) (n= 60)**
Karakaş	250	1.93±0.020	54.97±0.30	65.52±1.70
Morkaraman	248	1.41±0.021	53.80±0.40	64.55±5.80
Norduz	260	2.05±0.004	53.76±0.13	65.25±0.20
Zom	300	1.37±0.020	56.14±0.32	64.59±1.76

The least squares mean for greasy fleece yield in Karakaş (Hakan and Aygün, 2015), Morkaraman (İnan and Aygün, 2019), Norduz (Veziroğlu and Aygün, 2017), and Zom (Karakoç and Aygün, 2019) ewes were found to be 1.93±0.020 kg, 1.41±0.021 kg, 2.05±0.004 kg, and 1.37±0.020 kg, respectively (Table 1). According to this data, the highest greasy fleece yield was in Norduz ewes. It can be said that these differences arise from the possible differences in animal genotype levels in the enterprises and the diversity of care, management, feeding and breeder conditions. The most important factor is their different genotypic structures.
In terms of dirty fleece yields, which Tuncer (2008) found to be 2.220±0.175 and 1.700±0.233 kg in Norduz and Karakaş sheep, respectively, the average determined for Norduz sheep is higher than the averages of dirty fleece yields in enterprises.
When it was reviewed the literature studies on the greasy wool production of Norduz sheep, the average values of 2.91 kg reported by Yılmaz and Denk (2004) and 2.22 kg reported by Tuncer (2008) were found higher than average greasy fleece weight of 2.05 kg detected in Norduz sheep in all farms. But, the average greasy fleece weight of 1.96 kg reported by Karakuş et al. (2005) for Norduz ewes was lower than the average greasy fleece weight of 2.05 kg determined in all farms.
When the dirty fleece yield of Karakaş sheep is compared with the local genotypes reported in the literature; Gökdal et al. (2000) found the average dirty fleece yield in Karakaş sheep to be 1.79±0.06 kg, which was found to be lower than the average dirty fleece yield of Karakaş ewes.
Although the wool obtained from sheep in Türkiye loses its importance over time, the scientific studies on wool yield have been increasing in recent years. Wool yield in sheep is under the influence of many factors. As well as the effect of genotype on wool productivity, the effect of environmental factors is at a significant level. There is also a significant relationship between the live weight after shearing and the greasy fleece and husbandry conditions.
It is concluded that the fleece yield varies depending on many external factors such as maintenance, nutrition, business structure, reproductive activity, soil type, climate, disease and parasites. Especially, the differences in the yield value of wool due to the environmental conditions may occur.

Live Weight After Shearing in Ewes

The least squares mean for body weight after shearing in Karakaş (Hakan and Aygün, 2015), Morkaraman (İnan and Aygün, 2019), Norduz (Veziroğlu and Aygün, 2017), and Zom (Karakoç and Aygün, 2019) ewes were found to be 54.97±0.30 kg, 53.80±0.40 kg, 53.76±0.13 kg, and 56.14±0.32 kg, respectively (Table 1). According to this data, the highest live weight after shearing was in Zom ewes. The most important factor is their different genotypic structures.
The general mean of the live weight after shearing of 56.14 kg found in Zom ewes at all the farms was higher than 53.80 kg reported by İnan and Aygün (2019) for Morkaraman ewes, 53.76 kg reported for Norduz ewes (Veziroğlu and Aygün, 2017) and 52.85 kg reported for Awassi ewes (Üstüner, 2007).
In general, compared to native sheep breeds in Türkiye, it has been understood that average live weight after shearing of Zom sheep was similar to some breeds, higher than some breeds, and lower than some ones. In this study, the live weight after shearing of Zom ewes was found to be lower than the means of the live weight after shearing of Norduz, Karakaş, Dağlıç, and Hamdani sheep breeds but to be higher than that of Kıvırcık and Sakız sheep breeds. The live weight after shearing in Zom ewes was found to be lower than the crossbred

genotypes reported in the literature in Türkiye. The reasons for this difference between sheep breeds for live weight after shearing in Türkiye may be the genotypic level observed in the herd, shelter conditions, structure of the farms, climate features, disease and internal-external parasites, region where fleece samples from animal were taken, level of knowledge on management, and planning of research and researcher factor.
In this research, for example, the live weight after shearing of Zom ewes was found to be higher than the live weight after shearing of Kangal genotype and of Akkaraman sheep breed. This difference may be due to the maintenance and the feeding, and the diversity in breeding conditions. When compared to native sheep breeds in Türkiye, it was understood that average the live weight after shearing of Zom ewes was similar to some sheep breeds, higher than some breeds and lower than some ones.

Clean Fleece Percentage in Ewes

The least squares mean for clean fleece yield value in Karakaş (Hakan and Aygün, 2015), Morkaraman (İnan and Aygün, 2019), Norduz (Veziroğlu and Aygün, 2017), and Zom (Karakoç and Aygün, 2019) ewes were found to be 65.52±1.70%, 64.55±5.80%, 65.25±0.20%, and 64.59±1.76%, respectively (Table 1). According to this data, the clean fleece percentage was in Karakaş and Norduz ewes. The reason for the observed difference, albeit small, between breeds may be due to the maintenance, the feeding, and the breeder conditions between the farms. In addition, the clean fleece yield values in Karakaş and Norduz ewes are close to each other.
Fleece yield value was determined 48.18% by Karakuş et al. (2005) in Karakaş sheep grown under semi-intensive conditions. In another study conducted by Tuncer (2008), it was found to be 60.93% and 60.00%, respectively, in Karakaş and Norduz sheep raised under semi-intensive conditions. The most important factor is their different genotypic structures.
Kara Uzun (2008) reported that the averages of fleece yield value in the breeds of Çine Çaparı, İmroz, İvesi, Karayaka, Kıvırcık, Karacabey Merino, Sakız, Menemen, Karakaş, Norduz, and Tahirova were 60.62%, 59.46%, 70.81%, 68.57%, 66.91%, 51.91%, 65.26%, 62.90%, 61.41%, 66.08%, and 66.44%, respectively. Yılmaz and Denk (2004) found that the average of clean fleece percentage in Norduz ewes was 68.25%. This average is higher than the average of Zom ewes. Tuncer (2008) and Karakuş et al. (2005) identified that the average clean fleece percentage in Norduz ewes was 60% and 55.76%, respectively. Greasy fleece yield of Norduz ewes was found to be lower than that of Dağlıç and Hamdani breeds but to be higher than that of Kıvırcık and Sakız breeds. The yield of fleece in Norduz sheep was found to be lower than the crossbred genotypes reported in the literature in Türkiye.

Results and Discussion

Compared to native sheep breeds in Türkiye, it has been seen that averages of the greasy fleece weight, the live weight after shearing, and the fleece yield value in Karakaş, Morkaraman, Norduz, and Zom ewes were similar to some breeds, higher than some breeds and lower than some ones. The reasons for this difference between sheep breeds for the greasy fleece weight, the live weight after shearing, and the fleece yield value in Türkiye may be:
-The genotypic level observed in the herd,
-Shelter conditions,
-Structure of the farms,
-Climate features,
-Disease and internal-external parasites,

-Region where fleece samples from animal were taken
-Level of knowledge on management and
-Planning of research and researcher factor.
It has been thought that wool yield will be obtained much higher than sheep if the factors listed above which reduce the greasy fleece yield and the fleece yield value of sheep are improved.
When compared to native sheep breeds in Türkiye, it was understood that average the live weight after shearing of Norduz sheep was similar to some sheep breeds, higher than some breeds and lower than some ones. In this study, for example, the live weight after shearing of Norduz ewes was found to be lower than that of Kangal genotype but to be higher than that of Akkaraman breed. This difference may be due to the maintenance, the feeding, and the diversity in breeding conditions.
The average of fleece yield value obtained in the study is similar to those reported for other literatures on Norduz sheep. The difference between fleece yield values can be explained by the diversity of conditions in farms. The fleece yield value is also influenced by shelter and pasture conditions as well as breed characteristics. In addition, it can be said that it is also affected from materials such as the grease, the moisture, the fertilizer, the plant materials, the dust, and the soil. The fleece yield values may especially arise depending on the environmental conditions of animals.

Conclusion

In conclusion, sheep breeding activities vary according to various regions of the world and the environmental factors, climatic conditions, natural resources, economy and cultural conditions of the countries.
Indigenous sheep breeds raised in rural conditions need to be genetically improved because genetic improvement offers solutions for satisfaction of needs in sheep husbandry in Türkiye. In addition to determining the productivity performance and characteristics of morphological and physiological in local sheep breeds raised in cultivation conditions directly, research on determining the breeding infrastructure and breeder tendencies will be useful for more effective animal husbandry. The quantity and the quality of fleece obtained from sheep are shaped by the effects of genotype and environmental factors. Environmental factors that affect quantity and quality can be listed as view-feeding, gender, lambing method, live weight, and age. It is hoped that this study will contribute to future studies on the fleece yield and characteristics of sheep breeds. The genotype level of the herd and environmental factors can be considered among the reasons for the differences observed between breeds in terms of live weight and wool yield after shearing.

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OCCUPATIONAL ACCIDENTS, DISEASES AND PREVENTION IN CROP AND ANIMAL PRODUCTION

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Abstract

In this review study, it was aimed to compile information about occupational safety and accidents that can be encountered by workers in plant and animal production. It was also aimed to provide suggestions for occupational health and safety in agriculture. The health and safety problems faced by agricultural workers are very similar to those working in industry. Agricultural workers may encounter physical, chemical, biological, ergonomic and psychosocial factors in their working environments. The agricultural activities in Türkiye have been characterized by the prominence of different applications. The issue of occupational health and safety in crop and animal production is very important as it is in many other areas. In general, possible dangers for workers in the agricultural sector in Türkiye are the ergonomics, the noise, the air conditioning, the chemicals, the pesticides, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases, etc. Taking precautions related to occupational health and safety has been neglected by the workers at agricultural enterprises, factories, field, and laboratories. The health and safety problems that may be encountered in the agricultural sector are diverse. The primary actions to make are identifying hazards (tractors, overhead electrical lines, ladders, confined spaces, processing machinery, and hazardous chemicals), eliminating or controlling hazards (eliminating hazards by using machines, substituting nontoxic for toxic chemicals, and using material handling equipment to lift heavy items), and ensuring that everyone knows how to work safely and applies that knowledge on the job. Some of the causes of fatal work accidents in the agricultural sector are transportation, falling from a height, being hit by moving or falling objects, drowning, deaths related to livestock, deaths caused by machinery and deaths caused by electricity.

Keywords: *Agricultural farms, Crop production, Occupational accident, Occupational illness.*

Introduction

The issue of occupational health and safety in agriculture is very important as it is in many other areas. In general, possible dangers for workers in the agricultural sector in Türkiye are the ergonomics, the noise, the air conditioning, the chemicals, the pesticides, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases, etc. Taking precautions related to occupational health and safety has been neglected for the workers at the agricultural enterprises and factories, the field and the laboratories. Moreover, it is not possible to say that there is enough sensitivity about the occupational health and safety of the workers. However, over the past decade, it has been observed that the relevant ministries have put in legal regulations related to the issue. The primary actions to make are identifying hazards (tractors, overhead electrical lines, ladders, confined spaces, processing machinery, and hazardous chemicals), eliminating or controlling hazards (eliminating hazards by using machines, substituting nontoxic for toxic chemicals,

and using material handling equipment to lift heavy items), and ensuring that everyone knows how to work safely and applies that knowledge on the job (Aygün, 2018).
One of the most important issues encountered in the livestock sector in Türkiye is occupational diseases and zoonotic diseases transmitted from animals to humans. The livestock sector has different health problems compared to other sectors.
Zoonoses and occupational diseases, which are among the most important health problems encountered in every field and stage of the livestock sector in Türkiye, and what should be the preventive health measures related to these diseases are among the most important issues. It is seen that the economic losses of these accidents, which can cause disability or cost to the life of the workers in the livestock business as a result of work accidents and occupational diseases, are at very serious levels (Aygün, 2021).
Workers engaged in the livestock sector take part and play an important role in all phases of animals from breeding to birth and rearing, from feeding to obtaining products. In addition to the high working potential in the animal husbandry sector, it is understood that their efforts cannot be rewarded despite the intense need for workers, and therefore, their share of production is quite inadequate due to the excess of unregistered activities in the sector. Due to its unique characteristics in the livestock sector, problems in terms of security, salary, accommodation, health, transportation, education, social and cultural aspects can be seen quite often, despite intense working hours and high performance (Aygün, 2017; 2022).
One of the most important risk factors in animal husbandry is animal shelters. Regular ventilation is one of the most important issues. It is emphasized that workplace ventilation should be done to control exposure to pollutants in the air (Eğri et al., 2014).
One of them is production losses due to workplace accidents and occupational illnesses. The issue of occupational health and safety in animal production is very important as it is in many other areas. Occupational diseases and accidents that can be encountered by workers at businesses have caused losses of very serious economic and qualified persons in animal production. In addition, the sustainability of production is negatively affected. Especially, the animal hitting and zoonotic diseases are very important in animal husbandry. Therefore, precautions related to occupational health and safety must be taken for the workers at the livestock enterprises, the field, and the factories such as the feed, the skin and the meat (Aygün et al., 2019).
It is true that more occupational health and safety intervention research focusing on preventing illnesses and injuries needs to be conducted. Conducting this type of research is difficult and time-consuming; however, without increasing the number and methodological rigor of these studies, it will be difficult to identify effective intervention methods and confidently encourage their use (Goldenhar and Schulte, 1996).
In this review, information about occupational diseases and zoonotic diseases observed in animal husbandry was compiled. And brief information about the possible risks in livestock production and the measures to be taken against them were presented. In addition, it is aimed to provide sensitivity of breeders about the occupational and the zoonotic diseases in the sector of animal production. It is also aimed to provide suggestions for the occupational and the zoonotic diseases in animal husbandry.

The Place and Importance of Occupational Health Safety in the Sector of Crop and Animal Production

Agriculture, by its nature, is the field of activity that requires people the most and brings people and nature closer. Therefore, the place, function, problems and solutions of the labor element in the agricultural sector should be discussed in terms of development and social welfare and the issue should be given due importance (Ahioğlu, 2008).

Different health and safety problems faced by agricultural workers; they occur in the environment where agricultural work is carried out, when the tools, machines and materials used, and the technology or method used are brought together by the employee in order to produce a job. This from problems; those that occur momentarily during work are defined as accidents, and those that occur in employees within a certain period of time are defined as diseases.
Unfortunately, all legal regulations made to increase employee welfare and safety in the agricultural sector, which is among the sectors with the highest rates of informality and child labor, have not affected the living standards of agricultural sector employees in active working life. Currently, due to long working hours, working tempo, lack of competent personnel, socioeconomic and political factors, low earnings, widespread employment of child workers and most importantly, the integration of agriculture into all life by seeing it as a way of life rather than a profession, therefore the lack of social security. Insecurity against work accidents is among the problems awaiting solution (Çamurcu and Seyhan, 2015).
In fact, zoonotic and occupational diseases, the number of which has reached an undeniable size, have become a very important issue for employees, businesses, the state and increasingly for society. While these studies are being carried out, it is necessary to create an occupational health and safety culture adopted within a system understanding by taking the views of the group, including the employers, as well as being aimed at those dealing with animal husbandry in the country (Aygün et al., 2018).
One of the biggest problems for agricultural sector workers arises at this stage. It is essential to ensure the hygiene of these environments in order to prevent the lack of clean drinking and utility water, unsuitable conditions for personal hygiene, and to prevent diseases caused by pests and deterioration. Agricultural sector is unique in nature. It has serious differences from other business lines in terms of working conditions, living standards, and business environment. It is one of the rare sectors that is generally carried out as a family business and where business and home life cannot be separated from each other by certain lines (Çamurcu and Seyhan, 2015).
Agricultural workers know issues on tractor safety but they can’t transfer their knowledge to behavior. This result revealed that agricultural workers or farmers do not have suitable attitudes about tractor safety, they know what they must do but they don’t know why they must do that. It is believed that training programs for farmers or agricultural workers should involve practices and educational content based on a change of attitude in this subject (Ahioğlu, 2008).

The Most Common Occupational Diseases in the Sector of Crop and Animal Production

In a nationwide research on agricultural equipment-machinery and tractor accidents, it has been shown that a significant portion of accidents are caused by employees' lack of technical knowledge about the tools and machines they use. Therefore, manufacturers and dealers should inform agricultural workers about the technical features of the machines they will use, safe usage methods, especially occupational safety and accidents, and ensure that they learn these in the training they will provide both during the sale and after-sales of agricultural work machines (Gölbaşı, 2002).
Animal production is associated with a variety of occupational illnesses and injuries. Occupational Health and Safety (OHS) has significant economic implications, particularly in terms of medical costs and economic productivity losses. Zoonotic (zoonosis) diseases can be defined as diseases that humans and animals can transmit to each other and that are shaped jointly in individuals belonging to both groups. In addition to zoonotic diseases caused by farm animals such as cattle, sheep, cats, dogs, chickens, birds, poultry, donkeys, mice, etc.;

the zoonotic diseases of many animal species, such as wild mammals and rabbits, are transmitted to humans and cause serious problems.

An “occupational disease” is any disease or disorder contracted primarily as a result of exposure to risk factors arising from work activity. “Work-related diseases” have multiple causes, where factors in the work environment may play a role, together with other risk factors, in the development of such diseases. The World Health Organization emphasizes the following: Carrying out estimates of the global burden of disease from major occupational risks, such as injuries, airborne exposures, carcinogens, ergonomic stressors, noise and other specific risks. Incorporating occupational diseases and their causes in the 11^{th} revision of the International Statistical Classification of Diseases and Related Health Problems; working with the International Labor Organization (ILO) to develop diagnostic and exposure criteria for occupational diseases and to enable primary and secondary health care providers to detect and report such diseases (WHO, 2020).

The World Health Organization (WHO) defines zoonotic diseases as ‘any diseases or infections that are naturally transmitted between vertebrate animals and humans.’ Agents causing zoonotic diseases may be bacteria, fungi, viruses, parasites or any other communicable agents, for example, prions. Currently, there are over 200 recognized zoonoses, some of which have a worldwide distribution and others which are localized to specific regions. Occupational zoonotic diseases are most common where there is close contact between animals and humans at work, for example in animal husbandry and agricultural occupations, although workers in a wide range of other occupations may also be exposed to zoonotic agents, including those employed in the outdoor leisure industry or the wastewater industry and laboratory workers. There are many occupational zoonotic diseases in the world, many of which occur very rarely, although some do pose a significant health risk for workers in certain occupations. While the incidence of specific zoonoses varies from country to country, there are many occupational zoonoses that occur across Europe, although not every disease is present in every country (Cook and Farrant, 2020).

Other health and safety risks include skin problems, hearing loss, stress, and mental well-being issues particular to farming and the rural way of life. Occupational skin disorders are common in livestock workers. The effects of sun exposure are an important cause of morbidity in the shepherds’ group.

Farmers' lung is one of many forms of *hypersensitivity pneumonitis*. This problem is becoming rare, which is likely due to reduction of exposure to organic dust from increasing mechanization of agriculture and the effect of livestock health and safety programs. Another danger for breeders is the waste of animals. Animal wastes are frequently stored underground and are a source of toxic gases. Entering confined spaces used for manure storage can lead to fatalities, which are often caused by hydrogen sulfide exposures (Von Essen and McCurdy, 1998).

Occupational diseases can go undiagnosed and untreated and worst of all, effective preventive measures are not taken because of a lack of awareness of the problem. It is therefore believed that the knowledge of Occupational Health and Safety and the ability to apply this knowledge in recognizing potential accident situations (hazards) would assist in decision-making and taking remedial measures (Demba et al., 2013).

Some Suggestions and Possible Preventions for Breeders in Sector of Crop and Animal Production

The most important change in recent years in terms of the scope of occupational health and safety studies is that these studies are now carried out for all employees in the sector. While almost all previous studies were carried out in the form of "Occupational Health and Safety", the new approach has begun to include all employees in a certain sector. This approach is very important, especially for those working in agriculture. Because a significant part of the employees in this sector consists of self-employed and unpaid family workers.

Occupational health and safety in agriculture needs to be expressed in a well-defined strategy and must be integrated into agricultural development policy covering both commercial (large farms) and small-scale farming. The development of progressive occupational prevention services for workers in agriculture requires the implementation of effective national policies, specific programs and strategic action plans that emphasize prevention. Occupational health-related practices need to be integrated into the primary healthcare structure (Ahioğlu, 2008).

It can be easily said that work accidents and occupational diseases will increase if occupational safety and occupational health are not given importance. Those who will be most affected by this will be the first-degree workers. First of all, the income level of the worker and naturally the worker's family will decrease. This will have some negative effects on the worker and his family. The worker who is disabled or who lost certain limb or limbs may also suffer from some psychological disorders. This will negatively affect both workers and society (Yiğit, 2005; Karacan and Erdoğan, 2011).

The nature of animal husbandry requires an organization that is appropriate in accordance with local conditions for occupational health and safety. These organizations should be units that try to prevent dangers by determining them at source. For this aim, the risks at work should first be determined. Then, solution suggestions should be presented to remove or minimize these risks. Zoonotic diseases are one of the most important problems of farmers in animal husbandry. Workers and animals must be vaccinated against various zoonotic diseases (Aygün, 2022).

With regard to the control of occupational zoonoses, there are some general control measures that reduce the risk of infection for a wide range of zoonoses. These include the following:

- good personal hygiene practices, especially washing with soap and warm water;
- covering cuts and scratches with waterproof dressings;
- wearing of appropriate PPE (Personal Protective Equipment), for example, gloves, overalls, respiratory protection – this must provide relevant protection, while also being suitable for carrying out the required task;
- good hygiene practices for animal husbandry; and,
- use of an appropriate disinfectant to clean potentially contaminated areas (Aygün, 2021).

For certain zoonoses, there are effective vaccines available and it may be appropriate to administer them to individuals in high-risk occupations, for example, laboratory workers handling infected animals. In many cases, there are effective prophylaxis and treatments available. For these to be used to maximum advantage, it is necessary for workers to be aware of any diseases they may be at risk from and to be able to recognize early symptoms of these diseases. For certain occupations, it may be required for workers to inform their employer if they have a weakened immune system (Cook and Farrant, 2020).

Conclusion

Occupational diseases and zoonotic diseases that can be encountered by workers have caused the losses of very serious economic and the qualify persons in the livestock sector. Therefore, the precautions related to occupational health and safety must be taken for workers in the husbandry and the field. Sample risk assessment studies for sub-fields of agriculture should be increased and model practices should be disseminated. Taking precautions for occupational health and safety are very difficult, costly and time-consuming. Among the difficulties is the varied nature of agriculture, the many ethnic groups engaged in the activities, the traditionalist view of farming families, and rapidly changing technology. As a result, occupational health and safety in the agricultural sector is one of the areas in need of development and the continuity of the studies carried out in this field is required.

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CURRENT SITUATION OF HORSE BREEDING AND POSSIBILITIES IN BİNGÖL PROVINCE OF TÜRKİYE

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Abstract

The purpose of this survey is to put forward the current situation and the potential of horse breeding and the husbandry opportunities in Bingöl province of Eastern Anatolia in Türkiye. Horse breeding in Bingöl province has continued their traditional structure because of the causes such as climatic and geographical conditions of the region. However, horse breeding with traditional structure has been performed for their needs rather than profit. Horses are used in shooting, riding, transportation, and load carrying, as well as in horse-drawn sports and races, as they were in the past. Local people in this region also use horse breeding mostly for transportation and transhumance activities. The number of people who breed horses for hobby purposes is low. Horses in the region show different characteristics depending on the geographical conditions of their region. Especially, as a result of crossbreeding with breeds brought from other regions, new genotypes with different morphological characteristics have emerged. Since the climatic conditions of the region are quite difficult, it has been observed that there are generally serious problems in the care and feeding of horses. However, it has been reported that the horses in the region are very resistant to cold, can survive even under poor care and feeding conditions, have high survival rate and fertility rate, are resistant to diseases and are very docile horses. With this study, a situation assessment was made and suggestions were put forward for the development and popularization of horse breeding in the region. An attempt has been made to reveal basic information about traditional horse breeding methods in this region.

Keywords: *Bingöl province, Breeding system, Horse breeding, Traditional horse husbandry.*

Introduction

Horses in Türkiye show different characteristics depending on the geographical conditions of the regions where they are raised. Especially as a result of crossbreeding with warm-blooded and cold-blooded breeds brought from abroad, it has become very mixed, and as a result, it has become difficult to distinguish them according to morphological characteristics. However, some researchers have examined them by dividing them into large groups that overlap each other (Bayram et al., 2005; Ünal et al., 2005).

Horse husbandry in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry (Aygün, 2020; TOB, 2024).

Livestock in Eastern Anatolia province are mostly carried out with traditional methods. Therefore, it will provide contribution to knowledge of the current problems of the industry in the province the current situation of the livestock sector and needs to be determined and the current situation to the steps taken to improve (Şeker and Köseman, 2015). The first practices that should be done to increase the profitability in livestock enterprises should be directed to environmental regulations. As the environmental factors improve, the genotype needs to be improved accordingly (Öztürk, 2009).

As is the case with all livestock, various situations that affect horses' wellbeing have manifested since their domestication. However, the number of studies on horse health and welfare is limited. When assessing horse welfare, environmental parameters such as the size of shelters and boxes, characteristics, litter, care and feeding, exercise status, and zoological parameters such as body condition scores, skin injuries, scars, and abnormal behaviors are used (Minero and Canali, 2009; Minero et al., 2015; Sommerwille et al., 2018; Czychall et al., 2019; Lesimple, 2020; Hausberger et al., 2020; Homes and Brown, 2022; Hacan et al., 2023).
The aim of this survey is to put forward the current situation and the potential of horse husbandry and breeding opportunities in Bingöl province of Eastern Anatolia in Türkiye.

Demographic Structure of Bingöl Province

Bingöl province is located in the Upper Euphrates section of the Eastern Anatolia Region. It is surrounded by Muş in the east, Erzurum and Erzincan in the north, Tunceli and Elazığ in the west, and Diyarbakır in the south. Bingöl Province is located between 41º - 20 and 39º - 56º east longitudes and 39º - 31 and 36º - 28º north latitudes. Its area is 8.125 km^2 (Anonymous, 2024). Economy of Bingöl province is an economy mainly based on agriculture and animal husbandry, and aquaculture. The livestock sector in Bingöl province has been developing and gaining importance day by day.
There are 7 districts of the province, namely Adaklı, Genç, Karlıova, Kiğı, Solhan, Yayladere, and Yedisu. The city center is located at an altitude of 1151 meters from the sea, in the northwest corner of the Capakçur plain, on a plain overlooking a branch of the Göynük stream, which meets the Murat water around Genç District.
Bingöl, which has an ideal structure for forestry in terms of climate and land structure, is one of the provinces with the richest forest area in the Eastern Anatolia Region. However, the use of forests to meet the need for fuel for a long time and to be used in animal husbandry has resulted in it becoming a degraded coppice (Anonymous, 2024). Different plant species, distributed in meadow and pasture areas in Bingöl province, are seen as the main food source for nutrition of cattle, sheep, and goat.
Bingöl city is sixty-second the most crowded city in Türkiye. As of the begin of 2024, its population is 285.655 people. The educational status of the animal breeders and families of Bingöl province is low. This will have a negative impact on the care and feeding of animals and the productivity of the products to be obtained. For this purpose, meetings where business owners can get technical information can be organized and small units can be created and information can be transferred from village to village. Thus, it will be contributed both to the conscious raising of animal husbandry and the economy of the country (Anonymous, 2024).

The Importance of Horse Husbandry in Bingöl Province

Horse breeding in villages of Bingöl province has been continued their traditional structure because of the causes such as climatic and geographical conditions of the region. However, horse breeding with traditional structure was performed for their needs rather than profit.
In the province of Bingöl and its surroundings, the presence of meadow and pasture areas in areas where animal husbandry is carried out especially in the form of pasture livestock is very important in terms of making livestock more economically as it will provide suitable natural environment conditions for livestock. For this reason, the existence of meadow and pasture areas in any place provides the development of animal husbandry and the first source of livelihood as an economic activity (Kılıç, 2018).
In this region, animal husbandry is mostly done with traditional methods. In the animal husbandry activities carried out in the region, animals are taken to the pastures with the

removal of snow from the ground in the spring and stay in the pastures for a total of 6-7 months until the end of autumn. During this period, animals meet their nutritional needs from meadow and pasture areas. In the winter season, a significant part of the nutritional needs of the animals is met by the dried grass piles that are collected and dried from the pastures in the summer period. However, sometimes straw and ready-made fodder are used in cases where the pastures are not sufficient (Kılıç, 2018).
Horse husbandry is not an industrial sector that they transform the natural vegetation cover pasture and the pasture not used in the agriculture into the products such as meat and milk. Bingöl province is suitable for both small ruminant breeding and cattle husbandry in terms of large pasture areas, water resources, and climate characteristics.
The purpose of sheltering animals is to eliminate the negative effects of the environment on animals within economic limits and to provide comfortable living conditions suitable for their behavior. For this reason, when designing animal shelters, they should be dimensioned so as to provide sufficient space and internal detail for the movement, social, feed and water drinking behaviors of animals, and should be kept within economic and optimal limits in care management and hygienic conditions (Mutaf et al., 2001). Meanwhile, it is directly dependent on the involvement of the breeder to be successful in all studies to improve breeding and environmental factors at the breeder level.
Although goat husbandry used to be very common in this province, its population has gradually been declined in the last 20 years. Because, roughage and concentrate feed costs have gradually increased. Actually, this province is suitable for both the small ruminant breeding and the cattle and the water buffalo husbandry in terms of large pasture areas, water resources, and climate characteristics (Esen, 2017). It can be said that the province has an important horse and donkey breeding potential. The enterprise makes a significant contribution to the horse and donkey husbandry and economy of this province.

Number of Horses in Bingöl Province

Characteristics of horse husbandry in Bingöl province are small farms with 1 to 2 horses per farm. There are more hybrid horse genotypes in the region. It is very important to define the condition, potential and problems of breeding sector especially with respect to the horse stock in this city. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. Therefore, important suggestions for the Bingöl region will be presented with this study. Number of horses is presented in Table 1 in Türkiye and Bingöl province.

Table 1. Number of horse in Bingöl province and Türkiye (head) (TÜİK, 2024).

Year	Bingöl province	Türkiye	Percentage (%)
2019	1598	102467	1.56
2020	1428	90007	1.59
2021	1325	83718	1.58
2022	1169	74359	1.57
2023	500	66431	0.75

As seen in Table 1, there is a gradual decrease in the number of horses both in Bingöl province and in Türkiye from 2019 to 2023. The numbers of indigenous horses are low and there is need for conservation and spread of indigenous pure breeds on other suitable areas. In Bingöl province of Eastern Anatolia in Türkiye, the indigenous horses were found low in numbers. There is need to increase the numbers of indigenous horses for farmers.

It is seen that they do not use oats, corn, wheat bran, cottonseed meal and soybean meal, which are intensive feed raw materials, in the care and nutrition of horses. It has been determined that all horse breeders use straw in feeding and horse breeders generally use barley. Based on this data, it can be said that the use of intensive feed is limited to barley only, the mentioned intensive feeds are not cultivated in the region or are in very small quantities, it is due to economic reasons, and breeders generally leave the horses to feed on the pasture (Küçükersan, 2004).

Some Suggestions for Breeders in Horse Husbandry

In horse breeding, one of the most important issues is to shelter horses in suitable conditions. Proper sheltering conditions are extremely important for horses.

It can be said that the morphological data and other findings obtained from horses bred in the region coincide with the information about the Eastern Anatolian horse in the literature. Since the phenotypic characteristics of the Eastern Anatolian horse mentioned in the research are not determined with precise limits, a definitive judgment cannot be made as to whether the horses found in this region belong to the Eastern Anatolian horse.

In a survey study, sheltering standards in the visited horse farms were good with the exception of window and chimneys. The feeding practice is thought to be adequate. Although the living area in individual boxes for the animals was sufficient, no enrichment was observed. Abnormal behaviors resulting from inadequate housing is an indication of high social stress in horses. It has been concluded that the absence of tail docking, frequent grooming, hoof care and good transport conditions reflect high standards of welfare in farms. In contrast, the lack of dental care, high parasite burden and potentially poor human-animal interactions caused by caretakers not trained in animal health and welfare pointed to low welfare standards (Hacan et al., 2023).

It is also important to record pedigree information and some other measurements in horse breeding. There are very important rules to be considered in record keeping.

In 30.2% of the farms, horses were identified with microchips. These farms are engaged in raising horses for flat racing and according to the Regulation regarding the Registration of Arab and Thoroughbred horses into the Studbook, their Export, and Import, Arab and Thoroughbred foals born in Türkiye in 2006 and later must be identified with microchips in order to be included in the studbook (Anonymous, 2021).

Records provide information for decisions on which mares to breed and when a mare is most likely to conceive if bred. Records from a mare’s past breeding season aid in preparing her for breeding and allow the manager to more accurately predict her breeding status. Records are used to evaluate the level of success in producing foals and the need for changes in breeding management activities. Records should provide accurate information that is clearly understood and readily accessible. For clarity and consistency, it is recommended that one person be responsible for recordkeeping during the breeding season. The design of records should allow ease of cross referencing from one to another (i.e., breeding record referenced to palpation record). The design should minimize time spent recording, without jeopardizing accuracy and detail. Larger farms should strongly consider computer programs for recordkeeping (Hiney, 2024).

Since horse and donkey breeding has an important place in the region where the study was conducted, they carry out their breeding business based on the breeding experience they have gained throughout the historical process. However, especially in recent years, with the increase in migration from villages to cities, it has been observed that horse and donkey breeders have decreased over time. Therefore, it is thought that the number of horses and donkeys in the region will gradually decrease. As a result of the observations made, it is seen that the number of horses has gradually decreased in the last five years.

Conclusion

The main problems of horse husbandry in the region are the lack of infrastructure and organization. Bingöl province has ecological conditions suitable for husbandry of equid-hoofed animals. This potential of the province should be turned into an opportunity for breeders. As a result, preservation and development of native horse breeds as a genetic source is very important. The number of horses bred and the number of horse breeders in the region is quite low. However, if the current potential especially in the Eastern Anatolian Region and in Türkiye is evaluated, it can become very important in this region.

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EFFECT OF BUCKWHEAT ON PERFORMANCE, EGG QUALITY AND HATCHING CHARACTERISTICS IN JAPANESE QUAIL

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Abstract

This study was conducted to investigate the effect of using buckwheat (BW) as a substitute for corn in quail diets on egg production, egg quality, hatching results, feed intake, and growth performance. One hundred seventy five quails were divided into seven groups: 0% (0% BW was supplemented in diet; control), 5% (5% BW was supplemented in diet), 10% (10% BW was supplemented in diet), 15% (15% BW was supplemented in diet), 20% (20% BW was supplemented in diet), 25% (25% BW was supplemented in diet), and 30% (30% BW was supplemented in diet) in a complete randomized design with five (5) replications, each of which contained five quails. The study lasted for 8 weeks, between the dates of June 2022 and July 2022. Average egg production, egg mass, total feed intake, feed conversion ratio (FCR), hatching characteristics, livability, and egg quality were determined. The obtained results show that the supplementation of 30% BW in the quail diet increased the Haugh unit and albumen height. The highest hatchability was found when 5% BW was supplemented in the diet, and the lowest was found when 10% BW was supplemented in the diet. However, the supplementation of BW at different proportions in quail diets did not have any significant effects on feed intake, egg production, or livability. According to the obtained results, it can be recommended that buckwheat can be used as a diet at a proportion of 5% and 30% in order to improve egg quality and hatchability, respectively.

Keywords: *Buckwheat, Quail, Egg production, Egg quality, Hatchability.*

Introduction

Buckwheat (Fagopyrum esculentum Moench.) is an annual dicotyledon herb belonging to the Polygonaceae family. Buckwheat has long been used as a livestock and poultry feed (Meyers and Meinke, 1994). It appears to be nutritionally superior to cereal grains due to its amino acid composition and has some potential as a protein supplement compared to cereal grains. It is reported to contain 11 to 13% crude protein and compared to the other grains such as corn, wheat and barley it is high in lysine, an essential amino acid (NRC, 1944; Jacob and Carter, 2008). The starch in Buckwheat is similar to that of cereals, whereas it contains higher concentrations of amino acids such as lysine, methionine and cystine, which are more typical of legumes (Qian et al., 1998; Zheng et al., 1998). Some researchers have reported that buckwheat has reasonable nutritional value, comparable to that of oats (Farrell, 1978; Cheeke, 1999). The use of buckwheat as a feed supplement offers the possibility of improving animal growth performance. The partial supplementation of corn (-20%) and soybean meal (-10%)

with buckwheat bran (+30%) in the diets of ISA Brown hens increases the rate of egg production and feed consumption (Benvenuti et al., 2012). In the broiler dietary supplementation of 10% buckwheat seed with 1.5% black cumin seed improved final bodyweight gain (Islam et al., 2016). However, Sayed et al. (2016) found that Buckwheat alone or in combination with chitosan had no effects on the growth and feed intake of the broilers. Jacob and Carter (2008), indicated that up to 60% buckwheat can be included in broiler diets with no significant effect on BW gain and that feed conversion worsens as the level of buckwheat in the diet increases. Gül et al. (2023) reported that adding buckwheat in the diet at a maximum level of 15% does not have a detrimental effect on egg quality. Although buckwheat has been used as poultry feed for many years, there are very few published data available on its effect on hatching results of quails. The purpose of this research was to evaluate the use of buckwheat as a substitute for corn in quail diets on egg production, egg quality, hatching results and growth performance.

Materials and methods

In this research, 175 quails aged 10 weeks were equally distributed in seven treatment groups. Each treatment group was composed of four replicates with 5 quails each. During the eight-week period, the quails were fed with treatment diets containing 0% (Control), 5%, 10%,15%, 20%, 25% or 30% buckwheat. The study lasted 8 weeks. The diets were prepared according to the nutritional requirements of the layer quails (NRC, 1994; Table 1). Feed and water were provided ad-libitum. During the trial, 16 hours of light per day was provided.
The animals were housed in plastic conventional cages. Stocking density was set as 150 cm2/quail. Egg production was recorded daily. Feed consumption was calculated for 8 weeks. Egg weight was determined in 2-week periods. Death was recorded as it occurred. Egg yield, feed consumption and feed evaluation characteristics were calculated from these data.

Table 1. Treatment diets and calculated nutrient contents.

Ingredients	Buckwheat level, %						
	0	5	10	15	20	25	30
Buckwheat	0.00	5.00	10.00	15.00	20.00	25.00	30.00
Corn	53.00	48.40	44.00	39.40	34.9	30.30	25.80
Soybean Meal	35.40	34.60	33.7	32.90	32.00	31.20	30.30
Vegetable oil	4.04	4.43	4.75	5.15	5.54	5.95	6.35
Limestone	5.60	5.60	5.58	5.58	5.58	5.56	5.56
Dicalcium phosphate	1.14	1.15	1.15	1.15	1.16	1.17	1.17
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Calculated nutrient contents							
Metabolizable energy, kcal/kg	2902	2902	2900	2900	2900	2902	2903
Crude protein, %	20.00	20.01	20.00	20.02	20.00	20.02	20.00
Calcium, %	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Available phosphorus, %	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Lysine, %	1.10	1.10	1.08	1.07	1.05	1.04	1.03
Methionine, %	0.45	0.45	0.45	0.45	0.45	0.45	0.45

Egg quality parameters were determined at room temperature and at Selcuk University, Faculty of Agriculture, Egg Quality Laboratory from all eggs collected in the last three days of trial. The egg quality characteristics, such as egg weight, albumen height, and Haugh unit, were measured daily in eggs produced on two consecutive days in the last week of study. After the egg was broken on a flat surface, the height of the thick albumen was measured with a height gauge (Tresna, China). The Haugh unit (HU) was calculated using albumen height and egg weight values (Haugh, 1937). For recording incubation characteristics, all eggs produced for three consecutive days were placed in the incubator. Standard conditions for incubation were provided.

Results and discussion

Egg production and egg quality

Figure 1 and Figure 2 show the effect of supplementing different buckwheat levels in diet on egg production and egg mass. The application of different levels of buckwheat in the diet did not have any effect on hen-day egg production among treatment groups (P>0.05). Also, in terms of egg mass (g/quail/day), no significant effect was found among treatment groups (P>0.05).

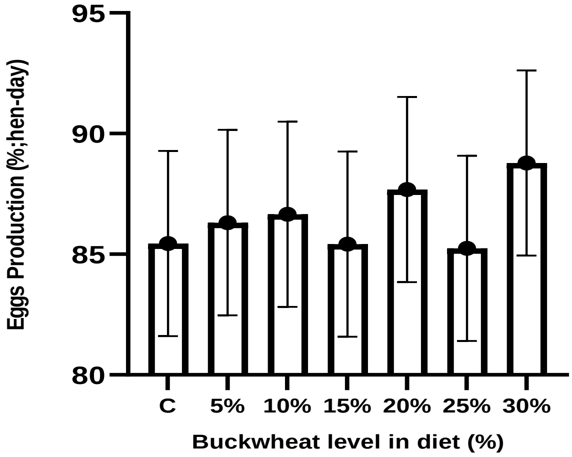

Figure 1. The effect of supplementation of buckwheat on egg production.

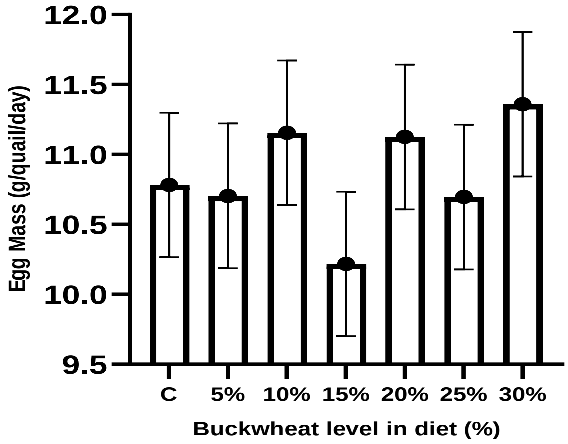

Figure 2. The effect of supplementation of buckwheat on egg mass.

Livability, feed intake and FCR

The supplementation of buckwheat at different levels in the diet did not have any significant effect on feed intake, FCR and livability among treatment groups (P>0.05) (Figure 3, 4, and 5).

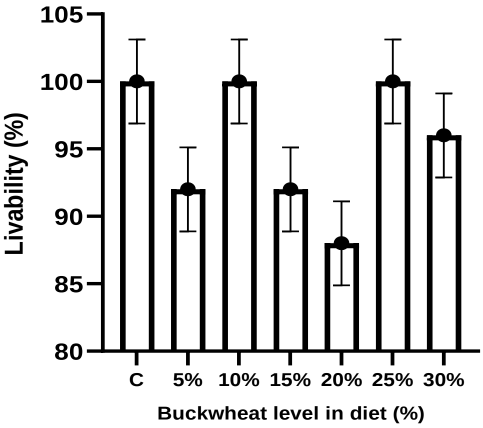

Figure 3. The effect of supplementation of buckwheat on livability.

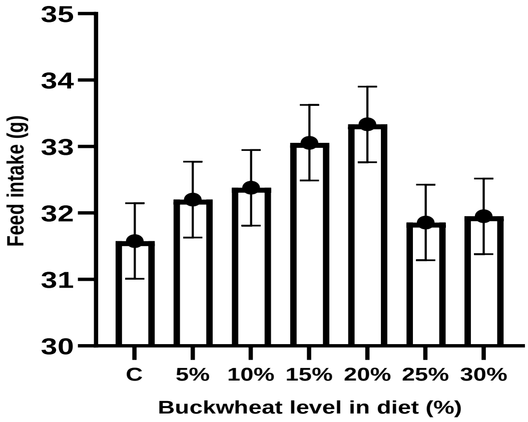

Figure 4. The effect of supplementation of buckwheat on feed intake.

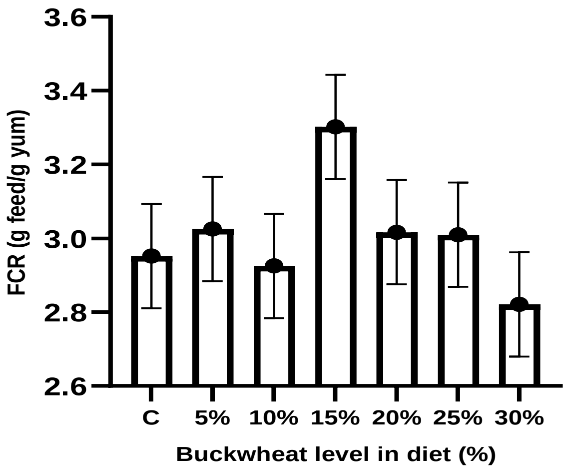

Figure 5. The effect of supplementation of buckwheat on FCR.

Hatching characteristics

The effect of the supplementation of buckwheat at different proportions in the quail diet on hatchability is shown in Figure 6. The highest hatchability was found in the quails which were fed with 5% of buckwheat in the diet and the lowest was found in the quails that were fed 0%

(C) buckwheat (P<0.05). However, quails fed 10%, 15%, 20%, 25%, and 30% buckwheat in diets had similar hatchability to quails fed 0% and 5% buckwheat in diets (P>0.05).

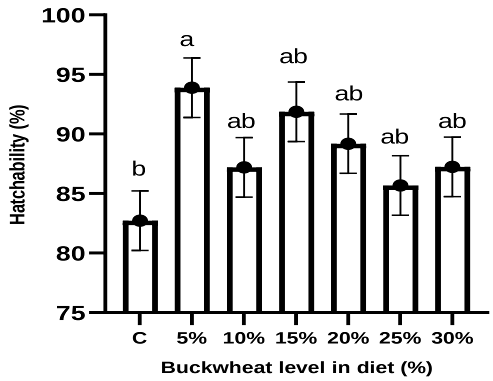

Figure 6. The effect of supplementation of buckwheat on hatchability.

Egg quality characteristics

The effects of the supplementation of buckwheat at different proportions in the quail diet on external and internal egg quality traits are shown in Table 2. The supplementation of a different level in the diet significantly affected almost all internal egg quality parameters. The eggs from quails fed with 10%, 20%, 25%, and 30% of buckwheat were heavier than those from quails fed with 15%. However, the eggs from quails fed with 0% buckwheat and 5% buckwheat in diet exhibited statically similar egg weight to those from quails fed 10%, 15%, 20%, 25%, and 30% of buckwheat in diet (P>0.05). The Haugh unit and albumen height significantly increased with increased buckwheat levels in diet (P<0.05). The eggs obtained from the quail fed with 30% of buckwheat in the diet exhibited the highest HU and albumen height while the lowest was found from the eggs of quails which were fed with 5%, 10%, and 15% of buckwheat in the diet. However, eggs from quail that were fed with 0% (C) of buckwheat exhibited statistically similar HU and albumen height than those fed with 5%, 10%, and 15% (P>0.05). In addition, the eggs from quail fed with 25% of buckwheat in the diet had higher HU and albumen height than those fed with 5%, 10%, and 15% (P<0.05). The supplementation of different buckwheat proportions in the quail diets did not show any significant effect on eggshell strength (P>0.05).

Table 2. The effect of supplementation of buckwheat on egg quality characteristics.

Groups	Egg weight (g)	Eggshell strength (kg)	Albumen height (mm)	Haugh unit
C	12.57^{ab}	1.56	2.42^{cd}	74.47^{cd}
5%	12.44^{ab}	1.49	2.14^{d}	72.31^{d}
10%	13.19^{a}	1.50	1.81^{d}	68.91^{d}
15%	12.03^{b}	1.41	2.03^{d}	72.24^{d}
20%	13.22^{a}	1.39	3.26^{c}	79.88^{c}
25%	12.89^{a}	1.41	4.63^{b}	87.13^{b}
30%	12.90^{a}	1.48	6.71^{a}	98.02^{a}
SEM	0.19	0.05	0.20	1.43
P-value	0.000	0.110	0.000	0.000

[a-d] Differences between groups marked with different letters in the same column are statistically significant (P<0.05).

In the current study, the supplementation of different levels of buckwheat in the quail diet did not have any effect on hen-day egg production nor feed intake among treatment groups. Our findings were in contrast to the observations by Benvenuti et al. (2012) that show that egg production rate and feed intake increased with the partial introduction of buckwheat bran in laying hens' diet. Jacob and Carter (2008) also reported that the inclusion of buckwheat in the broiler's diets resulted in increased feed consumption. Furthermore, buckwheat supplementation in the quail diet did not have any effect on egg mass and the FCR. Islam et al. [9] reported that the supplementation of 20% and 30% buckwheat in broiler chickens' diet increased total feed intake and FCR. Similar results were found by Benvenuti et al. (2012) who reported that the partial introduction of buckwheat did no significantly improved egg mass production and FCR and Sayed et al. (2015) reported that total feed consumption was not significantly different between treatments. In the present study, the supplementation of 20%, 25%, and 30% of buckwheat in quail's diet significantly improved egg weight, albumen height, and Haugh unit. These are in contrast with the observations reported by Benvenuti et al. (2012) who reported that no egg trait quality was significantly affected by the introduction of buckwheat in the hen diet, whereas, our findings show that the supplementation of different proportions in the quail's diets did not have any significant effect on eggshell strength. This was in contrast with Leiber et al. (2009) who reported that feeding whole buckwheat grains can improve the shell strength of the eggs. Leiber et al. (2009) also reported that buckwheat diets may have distinct effects on egg quality. The current study also found that the quail diet supplemented with 30% buckwheat had the best egg quality traits among the treatment groups. These findings are similar to the results of research conducted on hens by Benvenuti et al. (2012), and on broilers by Gupta et al. (2002). Our findings suggest that the eggs from quails fed with control exhibited statically similar egg weight compared to those from quails fed 10%, 15%, 20%, 25%, and 30% of buckwheat in diet, which is in contrast with the observations reported by Leiber et al. (2009) who reported that including whole grain buckwheat or shelled buckwheat at a dietary level of 40% dry matter in layers' diet resulted in significantly heavier eggs compared to a wheat-based control diet. Our study revealed that buckwheat supplementation in the quail diet had a significant positive effect on hatchability. Furthermore, among diet groups supplemented with buckwheat, the highest hatchability was found in the group fed with 5% in the diet. On the other hand, the obtained results of the current study show that the supplementation of buckwheat at different levels in the diet did not have any significant effect on livability among treatment groups.

Conclusion

In the present study, quails fed buckwheat performed equally or better than those fed corn. The finding of the study indicated that dietary supplementation of buckwheat at a proportion of 5% and 30% can be recommended as a diet in order to improve egg quality and hatchability, respectively. Furthermore, dietary inclusion at those levels has no deleterious effect and does not affect livability.

Conflict of interest statement
The authors declare no conflict of interest.

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A STUDY CONCERNING THE EVALUATION OF THE BALANCE OF ENERGY DURING EARLY LACTATION

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Abstract

Following the changes observed in milk parameters throughout the lactation period will offer an easy and practical method for the evaluation of the feeding programs of animals as well as presenting opportunities to improve the quality of milk. For this purpose, the milk samples collected from 1390 Holstein cows in the town of Bursa in Turkey on the test day of the month (at an interval of 30 days) were examined with Bently FTS/FCM COMBİ 400 (Bentley Nexgen 400) and their fat, protein, dry matter and lactose contents were measured. The cows with boundary values for the fat/ protein accepted for the metabolic states of the cows in the study are assumed to have an acidosis risk if they are lower than 1,2, to be healthy if they are between 1,2 and 1,4 and to have a ketosis risk if the values are over 1,4. The results of the study have shown that the risk for acidosis among the animals are 39.5%, 32.4% and 33.9% respectively during the lactation periods 1, 2 and 3. The risk for ketosis is 30.2%, 37.9% and 36.6% respectively. The percentages of the animals at the energy level are 30.3, 29.7 and 29.6 respectively. The estimates conducted for early, mid- and late lactation periods have indicated that 31% of the cows have acidosis, 37% have ketosis, and 32 % have an energy balance during early lactation; during mid-lactation, 31% have acidosis, 28% have ketosis and 32% have an energy balance, whereas 34% have acidosis, 34% have ketosis, and 32% have an energy balance.

Keywords: *Feeding, Acidosis, Ketosis, Milk protein, Milk fat.*

Introduction

In a nutritionally balanced dairy cattle ration, approximately seventy percent of the animal's protein needs will be met by microbial protein produced as a result of the fermentation of nutrients in the feed in the rumen. Achieving this important task largely depends on the supply and adequate consumption of energy and protein in appropriate amounts and proportions in the ration. Reaching the optimum level of milk yield and composition in dairy cattle can only be achieved by ensuring good rumen fermentation. For this, the amount and degradability of energy and protein sources in the diet must be balanced to ensure the optimization of rumen functions (Beever, 1993). Metabolic disorders caused by nutrient deficiency or imbalance in the diet also cause changes in the chemical composition of milk. Therefore, changes in the protein and fat content of individual milk samples during the lactation period of cows are important parameters that directly affect the health, reproduction and productivity of the animals.

In the late lactation period, as the productivity of cows decreases, roughage can meet the energy and other nutritional needs of cows. In such cases, milk composition reflects forage quality. High-quality forages are rich in both energy and protein content and can provide milk yields of around 30 kg or more per day with little or no added nutrients. Negative energy balance, which occurs due to inadequate feeding of cows despite high milk yield in the early lactation period, is an important problem that reduces milk yield and threatens milk quality in

the postpartum period. Monitoring the symptoms of subclinical ketosis is important in terms of milk quality, herd health and reproductive performance, and preventing economic losses in the enterprise.
Both the fat and protein percentage of milk on the control day are significantly associated with the risk of subclinical ketosis, and it has been reported that high milk fat percentage and low milk protein percentage significantly increase the risk of subclinical ketosis. While an increase of 1% in milk fat increased the risk of subclinical ketosis by at least twofold, an increase of 1% in milk protein reduced the risk of subclinical ketosis by more than 50% (Duffield et al., 1997). In ketosis, which is a metabolic disorder frequently seen in early lactation, milk protein content generally decreases while milk fat content increases. However, in subclinical acidosis, milk protein content increases and milk fat content decreases (Pavlata et al., 2008). It has been reported that milk protein and especially casein levels depend on the increase in the level of starch (cereal) consumed in the diet, but this increase raises the risk of rumen acidosis (Beever, 2006; Yang and Beauchemin, 2007). Mackle et al., (2000) reported that increasing energy level in the diet affects milk protein, and this may be related to the increase in microbial protein synthesis in the rumen.
The amount of fat and protein in milk is an effective parameter for monitoring the efficiency of the ration. Changes in the concentrations or mutual ratios of these two milk components in milk can give important clues about changes or problems in health as well as nutritional deficiency or balance in the ration (Nelson and Redlus, 1989; Rathwell, 1990; Lean and Golder, 2024). This study aimed to evaluate the milk fat/milk protein ratio of cows at various stages of lactation as an indicator of metabolic disorders and to use this ratio in estimating energy balance.

Materials and methods

The research findings were obtained from individual data collected from 1390 Holstein dairy cattle in the commercial dairy cattle farm registered to the central association of dairy cattle breeding in Bursa province in Turkey. On the farm where the research was conducted, animals housed in free stall barns are grouped according to their milk yield level, and the number of milking is 3 for those with high milk yield and 2 for animals with low milk yield. 9,920 data consisting of fertility and milk yield records of individual animals were evaluated. The research was conducted to determine milk fat, milk protein, milk solids and milk lactose values of milk samples collected on monthly test days during a 10-month period between August 2022 and May 2023 (30 days apart) with a Bently FTS/FCM COMBI 400 (Bentley Nexgen 400) model device. On the farm where the research was conducted, animals are fed with full rations suitable for milk yield. KM, HP, HY, NDS, ADS, HK analyses of the ration samples taken during the morning feeding on the first control day and monthly (on test days) throughout the duration of the research were carried out in the Feed Analysis Laboratory of the Department of Animal Science, Faculty of Agriculture, Selçuk University. NDS and ADS contents of feed materials and rations were determined with Van Soest, (1994), and other nutrients were determined through methods specified by Akyıldız, (1984). The average nutrient compositions of the rations used on the farm are shown in Table 1. Statistical analyses in the research were carried out using the SPSS 21 package program. Differences between groups were determined by repeated one-way ANOVA.

Table 1. Feed analysis information on the farm.

	Feed analysis information on the farm	
Farm Groups	MY*<35	MY≥35
KM (%)	35.22	40.85
HY (%)	4.68	6.31
HK (%)	6.70	6.39
HP (%)	13.54	14.50
ADF (%)	20.62	18.31
NDF (%)	34.92	32.19
ME(Mcal/kg KM**)	1.73	2.63
NFC***	34.41	34.24
Roughage/Concentrate Feed	56/44	45/55
FOK/HP	2.54	2.36
ME/HP	0.19	0.11
Ration ID	1	2

*MY: milk yield, kg/day
**ME (Mcal/kg KM):(3227+62.86%HY-31.79%HK-32.50%ADF)/1000
***NFC (non-fiber carbohydrate):%KM-(HP+HY+NDF+HK)

Results and discussion

Changes in the biochemical composition of milk can be used as a reflector of the physiological state of the cow (Hamman and Krömker, 1997). Especially in large-scale commercial herds, applying a feeding program appropriate to the lactation periods throughout lactation is very important in terms of monitoring the health status of the animals and milk yield and quality (Stoop et al., 2009). The optimum milk fat/milk protein ratio is between 1.2-1.4, indicating that cows are in positive energy balance, while values higher than 1.4 (or values where the milk protein/milk fat ratio is equal to or lower than 0.75) are a signal of energy deficiency and indicate a high risk of subclinical ketosis. A milk fat/milk protein ratio lower than 1.2 is most probably an indicator of subclinical acidosis, and it has been reported that low values may negatively affect the reproductive performance of cows and increase the possibility of disorders in mineral metabolism (Cejna and Chladek, 2005). Insufficient dietary fiber levels in herds can cause health problems, such as a decrease in milk fat level, acidosis and laminitis (Heuer et al., 1999). It has been reported that a milk fat/milk protein ratio below 1.2 indicates ration cellulose deficiency and energy excess, values between 1.2 and 1.4 are the optimum value, and above 1.4 is considered an indicator of ration energy deficiency and fiber excess (Cejna and Chiladek 2005; Alphonsus et al., 2013).
In the study, the percentage of animals at risk of acidosis in the 1st, 2nd and 3rd lactation was 39.5, 32.4 and 38.9%, respectively, and 33.9% for the whole herd. While the percentage of animals in energy balance was highest in the 1st lactation (30.3%), it was 29.7% in the 2nd lactation and 22.2% in the 3rd lactation, and was found to be 29.6% in the herd overall (Table 2). The percentage of animals at risk of ketosis in the 1st, 2nd and 3rd lactation was 30.2, 37.9 and 38.9%, respectively, and 36.5% for the herd overall. According to the results of the research, the problems seen in energy balance were found to be higher in young animals in the first lactation than in adult animals, especially since young animals in the first lactation,

which constitute the majority of the herd, are more sensitive to pregnancy, hormonal changes and diseases.
It is so important to take into account the indicators used to estimate metabolic energy balances, to detect possible errors in feeding early enough and to manage the herd as well as to take the necessary precautions, especially for animals in the first lactation. If the negative effects observed in energy balance cannot be prevented in the 1st lactation, it is highly likely that more significant health and productivity losses will be experienced in the 2nd and 3rd lactation periods.

Table 2. Energy balance estimation according to lactation order

Limit values for milk fat/milk protein ratio		1. lactation		2. lactation		3. lactation		TOTAL	
		N	%	N	%	N	%	N	%
<1.2	Acidosis	68	39.5	227	32.4	7	38.9	302	33.9
>1.4	Ketosis	52	30.2	266	37.9	7	38.9	325	36.5
=1.2-1.4	Energy balance	57	30.3	208	29.7	4	22.2	264	29.6
TOTAL		172	100	701	100	18	100	891	100

According to the research results, the percentage of ketosis was lowest (28%) in the mid-lactation period, and the highest percentage value (37%) was detected in the early lactation period (Table 3). Hanus et al. (2013) reported that ketosis is a lack of energy and means an insufficient level of glucose in the blood. In ketosis, milk fat content increases due to the breakdown of body fats, while on the contrary, protein content decreases. Negative energy balance, which occurs due to inadequate feeding of cows despite high milk yield in the early lactation period, may be the possible cause of reproductive disorders such as abomasum displacement, mastitis and retention of end, which reduce milk yield and threaten milk quality in the postpartum period (Hanus et al., 2013). It has been reported that ketosis cases, which are mostly seen in the first 50 days of lactation, can be diagnosed accurately within the first 10 days of lactation and that the use of milk analysis records in the early lactation period is common in practice, but the use of daily milk records for the first 10 days of lactation, which is a risky period for ketosis, will give more accurate results. (Manzenreiter et al., 2013).

Table 3. Estimation of energy balance according to lactation stage

Limit values for milk fat/milk protein ratio		Early lactation		Mid- lactation		Late lactation		TOTAL	
		N	%	N	%	N	%	N	%
<1.2	Acidosis	54	31	44	30	228	35	326	34
>1.4	Ketosis	65	37	41	28	226	35	332	34
=1.2-1.4	Energy balance	56	32	63	42	191	30	310	32
TOTAL		175	100	148	100	645	100	968	100

In a study conducted by Cejna and Chladek (2005), the milk fat/milk protein ratio of individual milk samples taken from Holstein cows on days 25, 45, 73, 101, 133, 166, 199, 224, 253 and 280 of lactation was found to be 1.91, 1.45, 1.38. 1.28, 1.22, 1.14, 1.26, 1.21, 1.09 and 1.18 respectively. The high milk fat/milk protein ratio seen in the first phase of lactation has been attributed to energy deficiency. The sediment quality of milk obtained from these animals was also found to be low. Researchers have reported that the milk fat/milk protein ratio changes throughout the lactation period, and that a high ratio at the beginning of lactation indicates that the cows are undernourished in terms of energy and have a negative energy balance.

In the current study, the percentage of animals at risk of acidosis showed the highest value (35%) in the late lactation period, while the lowest value (30%) was observed in the mid-lactation period (Table, 3). The rate of animals at risk of acidosis in the herd overall is 34%, and the rate of animals at risk of ketosis is 34%. While the percentage of animals in energy balance was highest in the mid-lactation period (42%), it was lower in the early and late lactation periods (32% and 30%, respectively).

Conclusion

The variations observed in the components of milk may reflect metabolic disorders and changes in energy balance. Preliminary symptoms of low milk fat percentage problems, as well as important yield and health problems in the 2nd and 3rd lactate periods, which are commonly encountered on large farms in Turkey, can be obtained by using individual milk analyses. Thus, they will be able to prevent economic losses by protecting herd health by means of making changes in their feeding programs. In the research, the suitability and effectiveness of the feeding program applied in the evaluation of metabolic disorders were evaluated by using individual milk analyses on the farm. Although the nutrient and forage ratio of the rations used in the enterprise seems appropriate, there is a need to evaluate the ration roughage quality and piece size. It may be useful to group cows according to lactation stage or increase the number of groups if it is to be done according to productivity level. In addition, feeding rations with higher roughage levels may be a useful practice for mid- and late lactation periods. It is necessary for milk producers to watch out for the indicators of factors affecting milk quality and take precautions in this regard for a sustainable and profitable production.

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THE EFFECT OF EGG WEIGHT ON CHICK QUALITY AND HATCHING RESULTS IN BROILER BREEDERS

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Abstract

The aim of the study was to evaluate chicks obtained from broiler breeder eggs of different weights (low and high). Various parameters were assessed including hatchery criteria (hatchability, hatch rate, fertility rate, embryo mortalities), chick quality parameters, performance indicators (egg weight, body weight, feed consumption), and internal organ weights (liver, thymus, bursa of Fabricius, spleen, pancreas, heart, ileum, duodenum, jejunum, proventriculus, gizzard) at 8th, 21st, and 42nd days of age. Additionally, tibia and femur lengths (mm) and fracture resistance (N) were measured. The chicks used in the study were obtained from a breeder flock of 35-week-old Ross-308 genotype, with a total of 210 incubated eggs categorized into low (45 – 55 g) and high (56 - 60 g) egg weights. The study revealed a positive relationship between egg weight and chick quality during both incubation and rearing periods. Early, mid, and late stage embryonic mortalities and sub-shell mortalities were lower in the low-weight eggs compared to the heavy-weight eggs. It was observed that extreme egg sizes (either too large or too small) resulted in lower hatchery performance, chick quality, and hatchability. Generally accepted views suggest that moderate egg weights lead to better hatchability. There were no significant differences ($p<0.005$) found in the internal organ weights of chicks obtained from high and low egg weights at 8th, 21st, and 42nd days. However, chicks from higher egg weights exhibited greater tibia fracture resistance (N) at the 42nd day.

Key words: *hatching, egg weight, chick quality, breeders.*

Introductıon

In broiler chicken production, there are many factors that affect profitability, and one of these is chick quality. High-quality chicks are defined as those that have shown optimal development throughout the incubation period, resulting in high growth rates, a high proportion of breast meat, and high survival rates. It is well known that chick quality significantly influences performance in broiler chickens.

In a production that starts with low quality chicks, it may be inevitable that yield losses may reach an unrecoverable level in the future. For this reason, since producers want chickens with high growth potential and high carcass yields at the end of the production process, hatchery operators are obliged to produce not only high hatching yields but also high quality chicks.

For achieving high hatchability and ensuring that the resulting chicks are of high quality, it is crucial not only to maintain optimal incubation conditions but also to use eggs of appropriate quality. It is essential that the eggs placed in the incubator are as fresh as possible. As eggs age, their internal quality characteristics deteriorate, which can negatively impact hatchability (Doğan, 2008). Therefore, hatchery operators should prioritize using fresh eggs to maximize hatchability and produce high-quality chicks. Since egg weight is quite easy to control, many researchers have tried to establish a relationship between egg weight and hatchability. The accepted view is that hatchability is better in medium weight eggs. (Wilson, 1991; Narushin and Ramonov, 2002; Şeker et al., 2004). There is a linear relationship between egg weight and

hatchability. Excessively large or small hatching eggs do not give good results and hatchability and chick quality are low in eggs that are larger or smaller than normal.
Depending on the factors affecting chick quality, chick quality increases depending on the necessary sensitivity. Genetic factors affecting the quality are factors such as shell thickness, white height and breaking resistance of the eggshell. Lines that have good egg quality characteristics and can maintain these characteristics for a long time also have good chick quality. In addition, it was emphasised that the increase in egg weight.

Material And Methods

Experiment Design And Egg Incubation

The study was carried out in the incubators and rearing poultry houses in Aydın Adnan Menderes University in Turkey, Faculty of Agriculture, Department of Animal Husbandry. The chicks used in the study were obtained from fertilized eggs (n=210) from 35-week-old breeder flock of Ross -308 genotypes.
At the incubation stage, the breeder eggs were divided into 2 experimental groups. The eggs were incubated under standard incubation conditions (Zhang, 2018). The experimental groups and their characteristics are as follows; **Group 1**: Low weight eggs (45 - 55 g) were collected from Ross-308 breeders. **Group 2:** High egg weights (56 - 60 g) were collected from Ross-308 breeders. 210 eggs were assigned to each of 2 preassigned treatment groups (trays) on each of 8 incubator tray levels (replicate blocks) in Nest Box Incubator, Turkey
Incubator air temperature and relative humidity were recorded every 7 min using (TFA Dostmann GmbH & Co. KG, Germany) wireless data loggers during the 21 d of incubation (doi) period. At hatch, all chicks belonging to a replicate basket in each treatment group were counted and weighed together to determine mean hatchling body weight (BW).

Growth Performance

During the growing period, 24 h light for the first 3 days, 18 h light: 6 s dark lighting program and standard growing temperatures were applied (first 0-3. Day 34 C, days 4-7 32 C, week 2 28 C, week 3 24 C, and 22 C in the following weeks). Feed and water were provided ad libitum. Feed containing 23.0% crude protein and 3,100 kcal/kg ME on days 0-10, 22.0% crude protein and 3,150 kcal/kg ME on 11-21 days, and 20.0% crude protein and 3,200 kcal/kg ME on 22-42 days. The body weight BW (g), feed intake (FI) of the birds were determined . Percentage mortality and feed conversion ratio (FCR; g feed/g gain) adjusted for bird mortality, were calculated for the same time periods.

Internal Organ Weights: Chicks hatched from incubation were sampled on days 8, 21, and 42 of the rearing period. Eight samples were taken from each group. The weights of the liver, thymus, Bursa Fabricius, spleen, pancreas, heart, ileum, duodenum, jejunum, proventriculus, and gizzard were measured.

Tibia and Femur On days 8, 21, and 42 of the rearing period, after slaughtering, the meat and fat tissues on the left side of chicken carcasses' tibia and femur bones were cleaned. Subsequently, the bones were dried by incubation at 60°C for 24 hours, and their lengths were measured using a digital caliper (Mitutoyo 500-181-30). The breaking resistances (Newton, N) of the same bones were determined using the Warner-Bratzler method.
This translation provides a clear understanding of the procedures involved in measuring internal organ weights and tibia/femur bone criteria during different stages of chicken rearing.
Data were analysed using SPSS (PASW statistics 20). A linear mixed model was used to analyse the effect of egg weight (low and high) on hatchability, mortality chick quality and

chick weight. All data were analyzed by one-way ANOVA using the procedure for general linear mixed models. Differences were considered significant at P < 0.05.

Results and Discussion

Tona et al. (2004) conducted a study in which 42 daily live weight and live weight of 7-10 days old chicks weight was positively correlated with chick quality. The second method used for evaluating chick quality The quantitative method is chick length. Wolanski et al. (2003), Meijerhof (2006) and Molenaar et al. (2007) in their studies, chick length at the age of 42 days
positively correlated with live weight they reported. Chick weight and chick length the most widely used quantitative method for assessing quality method, but according to recent studies used to assess chick quality in broilers the most reliable quantitative method is chick length (Joseph et al., 2006; Meijerhof, 2009).

Chick length at six weeks of age in broilers the effect of day-old chick weight on performance (Molenaar et al., 2008). In different studies, hatching output was correlated with chick length.
positive correlation between seventh day live weight also found to have a longer hatching time.
the chick may have better developed organs. is estimated. Hatching chicks lengths and visceral weights were compared. study, heart, liver and spleen in long chicks weights were higher than short chicks (Anonymous, 2006). Also digestive system length increases in parallel with the chick length and this the intestinal tract is better developed in longer chicks in the hatching process. According to the researches, at hatching 1 cm difference in chick length; 264 at 38 days of age grams more live weight and 45 grams more breast muscle formation (Molenaar et al, 2007). Quantitative determination of chick quality the consideration of the parameters alone is not sufficient but also the qualitative characteristics of the chicks. There are studies indicating that it should be evaluated (Tona et al., 2004). Qualitative evaluation is performed by the principle of estimation from their general appearance based on Qualitative characteristics of chicks Tona score and pasgar score are commonly used in scoring. is being used
The effects of different egg weights on, total (0 to 21 days) embryo mortality rate (%) (p=0.025) and fertility rate (p=0.030), 21d BW, Total Feed Intake (p=0.03), chicks (BW), Lenght (cm) at 0 day, tibia strenght (N) at 42d, were found to be significant.
However; the effects of egg weight on the 18th day, egg weight loss in grams (p<0.001), egg shell thickness (p=0,042), number of deformed chicks (p=0.001), number of healthy chicks (p=0.003), hatchability (p=0.003) and hatchability yield (p=0.002) weren’t found to be significant.

Table 1. The effect of egg weight on hatching results

Treatment	Hatchability (%)	Fertility (%)	Mortality (%)
LEW	91.0	95.23	8.57
HEW	89.42	99.04	10.47
P	0.045	0.030	0.025

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 2. Chickens were Body Weight (BW) on 8d, 21d, 42 daily and Total Feed Intake (g) from Different Breeder Eggs Weight

Treatment	8 d	21 d	42 d	Feed Intake
LEW	220	910	2940	4900
HEW	230	950	3030	5200
SEM	0.03	0.10	0.40	1.20
P	0.285	0.028	0.207	0.03

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 3. Post-Hatching Chick Weight (g), Length (cm) and Tona Chick Scoring Score of Breeder Eggs of Different Weights

Treatment	BW (0 d)	Lenght (cm)	Total Score
LEW	41.10	11.80	7.29
HEW	45.83	12.38	7.16
SEM	2.03	0.65	1.36
P	0.00	0.00	0.523

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 4. Internal Organ Weights (g) of 8 days Chick

Treatment	liver	thymus	Bursa Fab.	Spleen	Panc.	Heart	ileum	jejenum	Duode.	Pro-vent.	gizzard
LEW	6.08	0.29	0.29	0.13	0.85	1.27	4.56	4.09	2.67	1.63	5.90
HEW	6.58	0.31	0.38	0.15	0.94	1.25	4,45	4.60	2.66	1.52	6.37
SEM	0.86	0.08	0.12	0.03	0.21	0.12	0.97	1.32	0.22	0.24	0.84
P	**0.263**	**0.636**	**0.166**	**0.266**	**0.421**	**0.758**	**0.180**	**0.461**	**0.691**	**0.402**	**0.275**

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 5. Internal Organ Weights (g) of 21 days Chick

Treatment	liver	thymus	Bursa Fab.	Spleen	Panc.	Heart	ileum	jejenum	Duode.	Pro-vent.	gizzard
LEW	23.39	1.81	1.82	1.01	3.31	5.11	5.36	17.20	19.97	4.53	15.97
HEW	27.25	1.50	1.87	1.03	3.46	5.25	5.30	17.24	19.99	5.15	17.63
SEM	4.25	0.37	0.48	0.29	0.63	0.52	1.17	3.13	2.19	0.77	2.03
P	**0.068**	**0.101**	**0.856**	**0.905**	**0.651**	**0.612**	**0.914**	**0.985**	**0.351**	**0.112**	**0.106**

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 6. Internal Organ Weights (g) of 42 days Chick

Treatment	liver	thymus	Bursa Fab.	Spleen	Panc.	Heart	ileum	jejenum	Duode.	Pro-vent.	gizzard
LEW	63.75	4.28	2.08	3.52	5.41	15.99	20.36	23.88	15.36	9.78	37.12
HEW	61.74	4.35	2.02	3.17	5.59	15.44	16.41	23.28	14.82	8.57	30.83
SEM	6.39	1.43	0.85	0.66	1.14	2.40	5.24	3.56	2.20	1.39	5.20
P	**0.549**	**0.933**	**0.894**	**0.442**	**0.770**	**0.661**	**0.137**	**0.749**	**0.633**	**0.080**	**0.010**

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 7. Tibia and Femur Bone Lengths (mm) and Strength (N) at 8 days of chicks

Treatment	Tibia Lenght (mm)	Tibia Strenght (N)	Femur Lenght (mm)	Femur Strenght (N)
LEW	36.94	29.58	27.44	46.05
HEW	37.40	34.57	28.15	47.04
SEM	1.77	12.18	1.38	13.03
P	0.625	0.431	0.320	0.886

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 8. Tibia and Femur Bone Lengths (mm) and Strength (N) at 21 days of chicks

Treatment	Tibia Lenght (mm)	Tibia Strenght (N)	Femur Lenght (mm)	Femur Strenght (N)
LEW	68.50	144.62	50.01	142.53
HEW	69.22	136.25	51.02	154.74
SEM	3.82	34.66	2.50	46.63
P	0.719	0.645	0.441	0.618

LEW : Low Egg Weight, **HEW:** High Egg Weight

Table 9. Tibia and Femur Bone Lengths (mm) and Strength (N) at 42 days of chicks

Treatment	Tibia Lenght (mm)	Tibia Strenght (N)	Femur Lenght (mm)	Femur Strenght (N)
LEW	103.50	302.62	72.62	240.75
HEW	100.71	237.5	71.71	230
SEM	4.78	8.64	2.59	8.64
P	0.250	0.073	0.501	0.732

LEW : Low Egg Weight, **HEW:** High Egg Weight

Conclusions

Şahan et al., 2016 reported that early, mid, and late-stage embryonic mortalities and sub-shell mortalities were lower in eggs with lower weights compared to those obtained from heavier eggs. In many studies, it has been determined that the effect of egg weight on the mortality of meat-type chicks after hatching is insignificant (Proudfoot and Hulan, 1981; Narushin and Ramanov, 2002). Tona et al. (2004) reported that despite the decrease in egg weight in young pullets, the eggs obtained had better albumen quality and higher hatchability. Therefore, they obtained a greater number of quality chicks.

The relationship between egg weight and hatching has been widely studied due to the ease of controlling egg weight. The accepted view is that hatchability is better with eggs of medium weight (Wilson, 1991; Narushin and Ramanov, 2002; Şeker et al., 2004).

To increase hatchery efficiency, eggs with high internal and external quality characteristics should be obtained. Additionally, considering the age of the breeding flock, along with flock management, nutrition, and especially measures related to egg quality, can result in obtaining a greater number of chicks and thereby increasing productivity.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

CHALLENGES OF SUSTAINABLE INNOVATION IN THE ALGERIAN FOOD INDUSTRY

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Abstract

Innovation is a competitive development process that is an integral part of firms’ strate- gies. The food industry in Algeria is the leading sector outside of hydrocarbons in terms of value added and employment. It is dominated by small and medium-sized enterprises. This study aims to ascertain the impact of the innovation process and identify the obstacles encountered by food sector enterprises in Algeria using a sample of 162 companies. Descriptive statistics reveal that innovation is an important strategy for food industry companies, as all companies report having innovated over the past five years. Our study shows that the main innovations adopted by food industry are, in order of importance, product innovation, followed by process innovation and organizational innova- tion. This innovation process has facilitated modifying the company model to accommodate a dynamic environment, provide new products or services, establish novel markets, respond to market disruptions, and meet existing yet unfulfilled market demands. However, the innovation process encounters several obstacles, such as insufficient internal and external funding, exorbitant invention expenses, partner acquisition difficulties, and unpredictability in demand.

Keywords : *Food industry, innovation, challenge, Alegria.*

Introduction

Agri-food companies bear the responsibility of ensuring that food is safe, secure, and produced sustainably. In numerous countries, the implementation of traceability systems is mandated to bolster food safety and foster consumer trust in the food supply chain (Costa *et al.,* 2013 ; Kafetzopoulos and Skalkos, 2019). The significance of disruptive occurrences, such as climate change and the global pandemic, has emphasised the crucial requirement for novel capabilities that improve the food supply chain's resilience, visibility, redundancy, speed, and flexibility (Stone & Rahimifard, 2018 ; Belhadi, *et al.*, 2024). Food sustainability has significant obstacles due to waste occurring at several points in the agricultural supply chain, such as production, processing, distribution, retail, and consumption (Lemaire and Limbourg, 2019 ; Wani *et al.,* 2024). Producers must integrate advanced technologies to manage their responsibilities more effectively and efficiently to address this. Potential avenues for process innovation have been highlighted, such as the implementation of automated data gathering and improved visibility across the entire value chain (Oltra-Mestre *et al.,* 2021 ; Lezoche *et al.,* 2020). To fully capitalize on these opportunities, it is essential to embrace their potential comprehensively. The magnitude of this potential is defined by the variety of supporting technologies utilized and the level of integration across various stages of the value chain. The advantages include increased adaptability, expedited processes, augmented manufacturing capability, diminished faults, lowered expenses, superior product excellence, and improved client satisfaction (Zhao *et al.,* 2021; Oliveira et al., 2019). The importance of this field cannot be overstated. One of the critical challenges we face is guaranteeing the long-term viability of agricultural and food production. Projections indicate that a 70% increase in food production by 2050 is needed to satisfy the requirements of an expanding population (Hubert *et al.,* 2010; Odegard & van der Voet, 2014). The European Union's research and innovation programs, in conjunction with the United Nations' sustainable development goals, prioritise advancing food security and promoting sustainable agriculture (Adenle et al., 2019 ; Viana et al., 2022). To meet global demands, agriculture and food systems must transform to enhance efficiency, sustainability, adaptability, and waste reduction. Moreover, the adoption of sustainable agriculture methods can improve food's nutritional profile, thereby promoting public health (Welch & Graham, 1999; Mrabet, 2023). Given the increasing worldwide rivalry and significant technological improvements in the agri-food industry, these challenges hold equal significance at the corporate level. The current trend in the agricultural sector towards the consolidation of fewer, larger farms raises worries about the possibility of reaching global sustainable agricultural development targets (Notarnicola *et al.,* 2012; Borsellino *et al.,* 2020). To meet the growing demand for increased efficiency and financial prosperity in the agri-food sector, agri-food entrepreneurs should adopt a strategic and innovative strategy by incorporating sustainability-focused Integrated Marketing Communication (IMC) strategies (Baipai *et al.*, 2023; Krasnokutska *et al.*, 2024). SMEs in the agri-food sector that have effectively overcome these hurdles frequently achieve this by distinguishing their commercial strategy by creating innovative business models. These models often utilise a diversified approach, a strategy based on networks, or a framework based on value networks (McAdam *et al.* 2016 ; Camanzi & Giua, 2020). These company models' proactive involvement with society is vital, demonstrated by increased community engagement and a strong focus on environmental issues. Many researchers have identified the deficiencies of corporate models that prioritise profit as the ultimate objective. This study is designed to assess the impact of the innovation process and the challenges faced by food sector companies in Algeria, using a sample of 162 operations. The descriptive statistics reveal a key finding-all surveyed organisations have actively participated in innovation activities over the past five years. This underscores the importance of innovation as a crucial strategy for these companies and provides practical implications for industry professionals, equipping them with actionable insights.

Materials and methods

The data was collected through a structured questionnaire developed based on information gathered from 162 Algerian agri-food companies in the wilayas of Blida and Algiers in northern Algeria. After receiving confirmation to participate in the survey, the questionnaires were distributed as requested by the managers. Subsequently, interviews and multiple sessions were conducted to ensure the accuracy of the responses. The face-to-face interviews allowed participants to openly discuss their expertise and direct experiences in the agri-food industry and sustainable innovation within their companies. To successfully carry out this study, we first collaborated with expert consultants with expertise in the agri-food sector, and then a preliminary survey was conducted with a specific group of agri-food companies. The goal was to assess the relevance of the questionnaire and to thoroughly analyze the production processes in these companies, focusing on issues related to innovation. The survey approach was used to collect insights and statistics on sustainable innovation and to gather information from managers. The statistical analyses for this research were conducted using SPSS (Statistical Package for Social Sciences, version 25). Initially, the program was used to categorize and statistically examine the variables using descriptive statistics to characterize the studied agri-food companies, followed by other statistical analyses, such as statistical tests, to explore relationships between variables and analyze trends.

Results and discussion

Main Characteristics of Agri-Food Businesses with an Innovation System

This report outlines the key characteristics of agri-food businesses and the profiles of their managers in the Algiers and Blida regions. The surveyed enterprises are exclusively part of the private sector, representing a complete 100% majority. These agri-food businesses have been in operation for over a decade, with 95% registered under legal forms such as SPA (27%), SARL (63%), and multinational corporations (10%). Regarding group affiliation, 67% of these enterprises are part of a larger group, while 23% are independent. The managers reported that a significant 83% of these businesses have an annual turnover exceeding 20 million Algerian dinars (MDA), indicating their financial success. Additionally, 60% of the enterprises have sought assistance from well-known institutions like IANOR, with ALGERAC, ONML, and INAPI representing 20% of the support received.

When questioned about involvement in cooperation programs, responses revealed that the companies had received support from SME I, SME II, and UNDP programs, collectively accounting for 7.1% of participation. Furthermore, these businesses were also engaged in UNIDO and DIVECO cooperation programs, with a participation rate of 14.3%. Regarding state aid, 66.66% of the managers reported that their companies had benefited from it, while 33.33% indicated that they had not.

Table 1. Main characteristics of the companies.

Characteristics		Frequencies
Contact : Position held in the company	Managing Director	100%
Company sector of activity	Public sector	0%
	Private sector	100%
Year the company was set up	Under five years	5%
	Between 5 and 10 years	15%
	Between 10 and 20 years	80%
Legal status of the company	SPA	27%
	SARL	63%
	Multinational	10%
Belonging to a group	Yes	67%
	No	23%
Workforce	1-250 employees	45%
	250-500 employees	35%
	≥500 employees	15%
Turnover	≤ 20 M DA	17%
	Between 20 MDA and 200 MDA	33%
	Between 200 MDA and 2 Milliard DA	33%
	Between DA 2 Milliard DA and 5 Milliard DA	17%
Has the company already called on the services of quality institutions ?	IANOR	60%
	ALGERAC	20%
	ONML	20%
	INAPI	20%
Has the company already received support from coopera tion programmes such as ?	PME I et PME II	7,1%
	PNUD	7,1%
	ONUDI	14.3%
	DIVECO	14,3%
Companies that have benefited from state aid	Yes	66.6%
	No	33.3 %

Source : Compiled by the authors from survey data

The Innovations Adopted by the Algerian Food Industry

The successful commercialization of new ideas characterizes innovation. Businesses realize innovation when they can bring these ideas to market and profit from them. The extent to which a business can capture the financial returns from its innovations is a crucial determinant of the profitability of those innovations. The 'appropriability regime' concept refers to businesses' ability to retain the profits generated by their innovations. According to this concept, businesses operating under a strict appropriability regime can better protect and retain the profits from their unique resources. Conversely, in a loose regime, these profits are more susceptible to unintended losses or spillovers to competitors (Karantininis et al., 2010 ; Tell et al., 2016). As shown in the table below (table 2), all agri-food companies included in this study have developed an innovative product (100%). When asked about innovation in sustainable business models within the agri-food sector, 91.97% of business leaders confirmed that their company had adopted an innovation-focused business plan. The inquiry investigated practical strategies for attaining sustainability and the degree of advancement and complexity of these techniques. We will provide a theoretical framework to propel sustainable business model innovation advancement by adopting these innovative business concepts. This framework aims to improve understanding of previous studies examining the relationship between business models and sustainability in the agri-food industry (Barth et al., 2017).

Table 2. The Innovations Adopted by the Algerian Food Industry.

Type of Innovation	Agri-food business	Frequency (%)
Product Innovation	162	100
Commercial Innovation	149	91.97
Process Innovation	123	75.92
Organizational Innovation	87	53.70

Source : Compiled by the authors from survey data.

Agri-food producers are responsible for guaranteeing the safety, reliability, and sustainability of food in a world experiencing more disruptions and an increasing unwillingness to tolerate waste in supply chains. To efficiently handle this obligation, they must embrace novel technologies that improve productivity and efficacy. These initiatives can stimulate process innovation within the agri-food sector. When surveyed about innovation practices in sustainable processes, 75.92% of companies reported implementing a process-oriented innovation model (Jack *et al.,* 2014 ; Oltra-Mestre et *al.,* 2021). The surveyed managers regard innovation within their organisations as encompassing the creation of new goods, implementation of innovative production techniques, acquisition of new sources of supplies, investigation of unexplored markets, and implementation of different forms of organisational restructuring. In addition, 53.70% of organisations reported implementing an organisational innovation model when asked about sustainable organisational innovation (Batterink *et al.,* 2006; Castillo-Valero & García-Cortijo, 2021).

Challenges to Sustainable Innovation in the Algerian Food Industry

The sustainability of agriculture and food production is closely linked to numerous socioeconomic difficulties that society is anticipated to face, making innovation within agri-food enterprises a matter of great worldwide significance. Projections suggest that food production must be boosted by 70% by 2050 to satisfy the increasing demands (Barcaccia *et al.,* 2020; Zhao *et al.,* 2021). The United Nations Research and Innovation Programme, in partnership with the Sustainable Development Goals, has emphasised the significance of guaranteeing food security and advancing sustainable agriculture (Conceição et *al.,* 2016; Montagnini and Metzel, 2024). This highlights the need for global food and agricultural systems to enhance productivity, resilience, resource efficiency, and waste reduction. Moreover, there is a widespread belief that implementing sustainable agriculture practices can enhance the nutritional value of food, resulting in improved health. The study conducted a poll of agri-food business managers to identify the primary obstacles to implementing innovation. The most significant obstacles reported were access to finance and investment and the pressures of international competition (100%). When studying agri-food business leaders, access to financing and investment and international competition emerged as the most significant impediments to implementing innovation. The challenges related to obtaining adequate funding and global competition are crucial since they directly influence a company's ability to spend on research and development, adopt new technologies, and maintain a competitive advantage. Literature highlights that competitive pressure often compels companies to innovate or risk losing market share, yet with sufficient financial resources, these companies may be able to invest in necessary technologies. This dynamic highlights the intricate balance between resource availability and external pressures from a competitive market, ultimately determining the success of innovative strategies in the agri-food sector (Vives, 2008). Cultural factors, environmental and resource constraints were also significant challenges, cited by 96.61% of respondents. Cultural factors, environmental constraints, and limited resources pose significant challenges to innovation within agri-food businesses, as they directly affect a company's ability to adapt and respond to external pressures. Cultural factors can impact organisational behaviour and determine the level of openness of a company to change and innovation. Environmental constraints, such as regulations or resource limitations, restrict the ability to implement new practices or technology, impeding innovation. The limited financial and human resources exacerbate these challenges by reducing the capacity to invest in innovative activities and support them. These interconnected challenges highlight the intricate ecosystem within which agri-food businesses operate, where it is crucial to overcome these constraints for innovation to be successful (Coghlan, et *al.*, 2020 ; Collado, *et al.*, 2024). Managers universally agreed that national market demand exceeded 95%. Additionally, 93.82% of participants identified inadequate infrastructure and technology as critical issues. This underscores a significant disparity between market prospects and the capability to take advantage of them. Inadequate infrastructure and obsolete technologies impede organisations' ability to expand their operations and fully leverage the existing market potential. This scenario highlights the necessity of making strategic investments in infrastructure and technical improvements to narrow this gap and improve the competitiveness of agri-food firms (Miranda *et al.*, 2011). Over 86% of managers also raised concerns about a clear regulatory and political framework. Finally, awareness and education were highlighted as essential factors, with more than 75% of respondents emphasizing their importance. An unambiguous legislative and political framework is crucial for effectively executing innovative programs in the agri-food sector. Most managers, namely 86%, have raised worries regarding the lack of frameworks. This absence might result in uncertainty and impede the ability to plan long-term investments and innovations successfully. Furthermore, a significant majority of respondents, exceeding 75%, emphasised the need for awareness and education. They emphasised that organisations may struggle to navigate barriers and seize opportunities if they need more information and understanding of the regulatory landscape and innovation processes. This highlights the necessity for consistent policies and educational endeavours to bolster innovation and sustainability in the business (Solarte-Montufar *et al.*, 2021; Miranda *et al.,* 2021).

Table 3. Challenges to Sustainable Innovation in the Algerian Food Industry.

Responses	Totally agree (%)	Agreed (%)	Little Agree (%)	Strongly disagree (%)
Lack of a clear regulatory and policy framework	86,41	8,59	5	0
Access to finance and investment	100	0	0	0
Insufficient infrastructure and technology	93,82	0	6,18	0
Awareness-raising and education	75,30	12,34	12,26	0
Supply chain issues	95	5	0	0
Market demand	95,67	4,23	0	0
Qualified workforce	81,48	18,52	0	0
Cultural factors Environmental and resource constraints	96,91	3,09	0	0
International competition	100	0	0	0

Source : Compiled by the authors from survey data

Conclusion

Although almost half of the published articles discuss topics linked to sustainability, adopting a sustainable strategy, whether in theory or practice, is still relatively rare in the literature. This is significant, particularly given the growing imperative to integrate sustainability into business strategies in the future. Developing business models without integrating sustainability features may prove challenging, if not nearly impossible. Previous evaluations of business models have provided valuable insights into their formation, but they frequently need to pay more attention to the significance of sustainability and sustainable business models. Although prioritising profitability may be viewed as a short-term imperative, agri-food companies must also exhibit ingenuity by incorporating sustainability into their marketing messages from a strategic standpoint. Our data shows that all agri-food companies assessed have achieved a 100% success rate in creating unique products. When questioned regarding innovation in sustainable business models within the agri-food industry, 91.97% of company leaders confirmed that their organisation had effectively adopted a business plan focused on innovation. The poll of agri-food business managers revealed that the primary obstacles in adopting innovation are access to money, investment, and worldwide competition, cited as the most significant issues by 100% of respondents. Additionally, cultural factors, environmental constraints, and limited resources were reported as significant obstacles by 96.61% of respondents. Managers unanimously acknowledged that national market demand was over 95%. Insufficient infrastructure and technology were also highlighted by 93.82% of respondents as critical issues. Moreover, most managers, precisely over 86%, have raised apprehensions regarding the absence of a well-defined regulatory and political structure. Finally, the significance of awareness and education was recognised, with more than 75% of participants highlighting their crucial role.

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ENHANCING ORGANIZATIONAL MANAGEMENT AND DECISION-MAKING THROUGH DASHBOARD IMPLEMENTATION : A CASE STUDY OF THE ALGERIAN RED MEAT COMPANY, ALVIAR

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Abstract

This study examines the critical role of dashboards in enhancing organizational management and decision-making processes, with a focus on the Algerian Red Meat Company (ALVIAR). In the context of an increasingly data-driven business environment, dashboards serve as essential tools that provide real-time access to key performance indicators, enabling managers to make informed decisions quickly. The research addresses the problem of how effectively dashboards can be integrated into an organization's management practices to improve overall efficiency and strategic alignment. The study employs a descriptive-analytical approach, analyzing how dashboards are utilized across various departments within ALVIAR compagnies, including operations, finance, and human resources. The results reveal that the implementation of dashboards has led to significant improvements, particularly in self-financing capacity, which increased by 18.73%. However, the company also faced challenges, such as a 5% decline in the cash liquidity ratio, indicating areas that require further strategic focus. Additionally, revenue trends show a substantial increase in sales of fresh local meat, doubling from 2020 to 2022, reflecting successful market strategies. Despite these positive outcomes, there were challenges in managing the financial balance indicators, highlighting the need for continuous monitoring and adjustment. The study concludes that dashboards are indispensable in modern organizational management, offering practical insights into how they can be leveraged to enhance decision-making processes, improve financial performance, and drive strategic initiatives within the meat industry in Algiers.

Keywords: *Dashboards, organizational management, decision-making, financial performance, meat industry.*

Introduction

In the rapidly evolving landscape of organizational management, the ability to make timely and informed decisions is paramount for achieving strategic objectives and maintaining a competitive edge. Dashboards, as advanced data visualization tools, have emerged as critical components in the arsenal of management control systems, providing real-time access to key performance indicators (KPIs) and other vital metrics that guide decision-making processes (Bradač Hojnik & Huđek, 2023; Balkrishna *et* al., 2024). These tools are particularly indispensable in complex organizational environments, where the ability to synthesize vast amounts of data into actionable insights can significantly influence the outcomes of managerial decisions (Eckerson, 2010; Edgeman, 2013). This study delves into the pivotal role of dashboards within the context of organizational management and decision-making, with a specific focus on the Algerian Red Meat Company, ALVIAR. ALVIAR operates within the highly competitive and regulated meat production industry, where efficiency, quality control, and responsiveness are critical to both operational success and regulatory compliance. In such a setting, dashboards are not merely optional tools but essential instruments that enable managers to monitor performance, predict trends, and respond swiftly to emerging challenges. The integration of dashboards into ALVIAR's management practices provides a unique opportunity to examine how these tools contribute to enhanced decision-making processes and overall organizational efficiency (Pauwels *et al*.,2009 ; Kumar & Belwal, 2017). The motivation for this research stems from the increasing demand for sophisticated management tools that can address the complexities of modern business environments, particularly in industries that are integral to national economies, such as agriculture and food production (Kourtit & Nijkamp, 2018; Talukder et *al*., 2021). In Algeria, the red meat sector is crucial for both economic stability and food security, making it imperative for companies like ALVIAR to adopt best practices in management control. This aims to analyze the specific ways in which dashboards are utilized within ALVIAR to support decision-making across various functional areas, including operations, finance, and human resources (Talukder, 2021; Poponi, 2022). The study will explore the extent to which dashboards have been integrated into the company's management culture and the impact this integration has had on operational performance and strategic alignment (Few, 2006; Branco *et al*.,2015; Wong & Ngai,2023). The research adopts a case study approach, allowing for a comprehensive examination of dashboard implementation at ALVIAR. This methodology is particularly well-suited for exploring complex phenomena within their real-life contexts, providing rich insights that can inform both theory and practice. By focusing on a single organization, the study aims to uncover the nuances of dashboard usage and its implications for decision-making processes at different managerial levels. The findings of this research are expected to contribute to the broader literature on management control systems, offering practical recommendations for organizations seeking to enhance their decision-making capabilities through the effective use of dashboards (Yin, 2017). In conclusion, this paper highlights the growing importance of dashboards as a cornerstone of modern management practices, particularly in industries where the stakes of decision-making are high. The case of ALVIAR serves as a compelling example of how dashboards can be leveraged to improve organizational outcomes, providing valuable lessons for other companies in similar sectors. As businesses continue to navigate an increasingly data-driven world, the insights gained from this study will be crucial for guiding future implementations of dashboard technology in organizational settings (Chaudhuri *et al*.,2011).

The literature surrounding dashboards in organizational management underscores their transformation from rudimentary monitoring tools to sophisticated components of comprehensive management control systems. Initially, dashboards were primarily used for tracking key performance indicators (KPIs) on a basic level, often limited to financial metrics (Bryceson & Slaughter, 2010; Alonso-Martínez *et al*.,2024). However, as businesses have become more data-driven, the role of dashboards has expanded significantly. Modern dashboards are now multifaceted tools that integrate data from various departments, providing a holistic view of organizational performance (Few, 2006; Manikas *et al*., 2022). Three main types of dashboards are widely discussed in the literature strategic, operational, and analytical (Vlachopoulou *et al*., 2021 ; Poponi *et al*., 2022). Strategic dashboards are designed for high-level executives, offering insights that align with long-term organizational goals. These dashboards typically include metrics related to market trends, financial performance, and strategic initiatives, enabling executives to monitor progress and make informed decisions that affect the organization's trajectory (Eckerson, 2010; Trubetskaya *et al*., 2024; Belhadi *et al*., 2024). Operational dashboards, on the other hand, are tailored for mid-level managers and focus on the day-to-day operations of the company. These dashboards provide real-time data on production processes, supply chain management, and other operational metrics, allowing managers to address issues promptly and maintain operational efficiency (Pauwels *et al*., 2009; Carson *et al.*, 2020). Analytical dashboards are more specialized, providing detailed data analysis capabilities that support decision-making processes at all levels of the organization. These dashboards allow users to drill down into data, uncover patterns, and perform predictive analyses that can inform future strategies (Chaudhuri *et al.*, 2011). The literature also highlights the critical importance of dashboards in enabling real-time data access, which is essential for dynamic decision-making. By presenting complex data in an accessible, visual format, dashboards empower managers to make swift, evidence-based decisions that can adapt to changing business conditions (Serfilippi *et al*., 2022; Bersani *et al.*, 2022). This capability is particularly valuable in today’s fast-paced business environment, where the ability to respond quickly to new information can be a significant competitive advantage (Yigitbasioglu & Velcu, 2012). Furthermore, the integration of dashboards into management control systems enhances transparency and accountability, ensuring that all levels of the organization are aligned with its strategic goals (Benos *et al.*, 2018; Biagi *et al*., 2021). Overall, the literature establishes dashboards as indispensable tools in modern management, not only for monitoring performance but also for driving strategic and operational decisions. The evolving capabilities of dashboards, supported by advancements in data analytics and visualization technologies, continue to shape how organizations manage their resources, evaluate their strategies, and ultimately, achieve their objectives.

Materials and methods

This study adopts a descriptive-analytical approach to investigate the complex dynamics of dashboard implementation in organizational management and decision-making, focusing on a case study of the Algerian Red Meat Company, ALVIAR. The Algerian Red Meat Company operates thirteen subsidiaries nationwide. It plays a significant role in regulating the red meat market and is equipped with modern facilities for slaughtering, processing, packaging, and storage of red meat products. This infrastructure enables the company to maintain quality control and contribute to the stability of the red meat supply chain across Algeria. The study begins with a thorough review of theoretical frameworks related to dashboards and decision-making processes, establishing a solid foundation for the empirical analysis. Data collection was primarily conducted through interviews with key personnel at ALVIAR compagnies and the examination of internal documents, allowing for a comprehensive understanding of how dashboards are utilized within the company. The analysis focuses on identifying patterns and correlations between dashboard use and management outcomes, with a particular emphasis on decision-making efficiency and operational performance. The methodology also includes a comparative analysis with previous studies to contextualize the findings within broader industry trends, ensuring that the conclusions drawn are both relevant and applicable to similar organizational environments. This structured approach ensures that the research findings are robust, providing valuable insights into the role of dashboards in enhancing management practices at ALVIAR.

Results and Discussion

Design and Implementation of a Model Dashboard for Human Resources

Table 1 comprehensively examines employment trends and employee mobility from 2020 to 2022. The data indicates a steady rise in the overall workforce, primarily due to an increase in fixed-term contracts with a 2% growth rate. The categorization of personnel based on their professional positions demonstrates a substantial increase in important undertakings (45%), underscoring a transition towards more prominent and more influential endeavours.

Nevertheless, there was a decrease in project completions, indicating possible difficulties in carrying out the tasks. The gender study reveals a consistent rise in male and female workers, with a particularly notable growth rate among female employees (14%). The analysis of employee mobility reveals a significant rise in the number of employees joining the organization (62%) compared to those leaving (49%), resulting in a favourable net inflow and a robust organizational expansion. These developments are consistent with research highlighting the significance of dynamic personnel management in improving organizational performance and adaptation to evolving economic circumstances. The comprehensive examination of employment trends and employee mobility between 2020 and 2022, as shown in Table 1, aligns with the general literature on implementing HRM, which emphasizes the significance of dynamic processes in HRM practices. The steady increase in the workforce, particularly the 2% growth in fixed-term contracts, indicates a strategic adaptability to evolving organizational needs. The significant increase of 45% in critical commitments indicates a shift towards more significant and influential roles within the company, reflecting a successful implementation of HR policies that promote organizational growth and employee development. This trend emphasizes the significance of ongoing monitoring and iterative improvements to HR practices to ensure alignment with organizational objectives and employee engagement (Trullen *et al.,* 2020 ; McCartney and Fu, 2022). The significant increase in employee mobility, with a 62% rise in employees joining the organization compared to a departure rate of 49%, indicates a favourable net flow, which is a strong indicator of a vigorous expansion of the organization. This trend reflects the successful implementation by the organization of strategies that attract and retain skills, contributing to its growth and stability. The positive net flow suggests that the company effectively manages its workforce, ensuring it can meet increasing demands and support its long-term goals. Attracting more employees than it loses is essential for maintaining a competitive advantage and operational continuity (Campbell *et al.,* 2012 ; Nocker & Sena 2019).

Table 1. Comprehensive Workforce and Employee Movement Analysis (2020-2022)

Leadership Board Indicators	year			Deviation	Deviation Rate
	2020	2021	2022		
Total Workforce	613	627	674	47	7%
By Contract Type					
Fixed-term	331	348	356	8	2%
According to the professional classification					
Small Initiatives	20	23	26	3	13%
Major Initiatives	23	31	45	14	45%
Projects	69	96	89	-7	-7%
Two Worker Teams	72	74	95	21	28%
Executive Workers	429	403	419	16	4%
By gender					
Number of Male Workers	553	562	600	38	7%
Number of Female Workers	60	65	74	9	14%
Development of Employee Movement					
Inflows	78	87	141	54	62%
Outflows	41	63	94	31	49%
Net Inflow	375,717	473,000	478,211	52	12%

*Source: Compiled by the authors from survey data

Analysis of Financial Performance and Strategic Insights for the Meat Industry in Algiers (2020-2022)

The financial indicators presented in Table 2 for a meat company from 2020 to 2022 demonstrate notable changes in the company's financial well-being, consistent with the conclusions drawn from contemporary literature on financial management. Fixed Assets (FA) experienced a significant fall of 68% from 2021 to 2022, indicating a substantial reduction in asset value due to liquidation or depreciation. This sharp decline is consistent with strategic restructuring initiatives, as reported by Smith & Jones (2023). The magnitude of this decrease strongly suggests deliberate asset management decisions rather than routine depreciation alone. However, the company's internal cash-generating capacity (CAF) increased by 18.73%, suggesting greater operational efficiency. This aligns with the known tendency of enterprises to optimise cash flows during economic difficulties (Laghari, *et al*., 2023). However, the declining cash liquidity ratio raises concerns about the firm's ability to maintain enough liquid assets for operational needs, a situation that enterprises frequently face during assertive asset management (Williams *et al*., 2022). In addition, although there has been a consistent increase in financial self-sufficiency, the significant decline of 71% in return on equity indicates a decrease in profitability, which could threaten long-term financial stability. This finding aligns with similar conclusions from studies on financial sustainability in unpredictable markets (Salehi & Arianpoor, 2022). The meat business in Algiers, similar to other industries, has seen substantial economic constraints, mainly due to shifting consumer demand and operational difficulties arising from the epidemic. Financial indicators, such as the ones displayed here, are essential for comprehending the resilience and adaptability of these firms. Research has shown that companies in this industry must prioritize enhancing their internal funding methods and profitability to maintain their operations in a highly competitive market (refer to Table 2). The patterns shown in this table highlight the significance of strategic financial management in guaranteeing the long-term stability and expansion of the meat sector in Algeria.

Table 2. Dashboard for Financial Indicators for the Three Years 20/21/22 Unit : K/DA

Dashboard Indicators	2020	2021	2022	Deviation 2021/2022	Deviation Rate 2021/2022	Deviation 2020/2021	Deviation Rate 2020/2021
Financial Balance Indicators							
FA	-937,640.88	-1,459,358.15	-2,456,410.12	-997,051.97	-68%	-521,717.27	-156%
BFR	359,626.67	-1,459,358.15	-1,818,984.82	1,356,287.24	93%	-103,070.91	-406%
TR	-1,297,267.55	-1,723,577.17	-426,309.61	426,309.61	25%	-426,309.61	-133%
Self-Financing Indicators							
Cash Liquidity Ratio	104%	103%	98%	-0.01	-5%	-0.05	-1%
Private Financing Rate	4%	4%	-2%	-0.06	-150%	0.00	0%
Self-Financing Capacity (CAF)	942,581.00	208,095.00	4,105,885.00	3,897,790.00	1873%	-734,486.00	-78%
Financial Independence Indicators							
	-2%	3%	4%	0.05	33%	0.01	250%
Profitability Indicators							

Return on Equity	1%	-59%	-17%	-0.60	71%	0.42	-6000%
Overall Profitability Rate	-0.2%	-10%	-3%	-0.10	70%	0.07	-4900%

Source: Compiled by the authors from survey data

Revenue Trends and Product Category Analysis (2020-2022)

The income breakdown in Table 3 demonstrates notable changes in several product categories, which mirror larger patterns in the meat sector. The steady growth in income derived from fresh local meat, which has more than doubled from 2020 to 2022, corresponds with customer preferences for locally sourced and fresh goods. These preferences have gained popularity due to perceived high quality and the desire to support local economies (Caputo *et al.,* 2024). The increase in fresh meat imports in 2022, following a decrease in 2021, is likely driven by a combination of changes in global trade regulations and improved supply chain strategies. These factors have collectively created more favorable market conditions for importers (Paul, 1999). Nevertheless, the decrease in income from frozen meat products during the same timeframe is likely attributed to two key factors : changing customer preferences that prioritize fresh choices over frozen ones, and supply chain interruptions. The shift in consumer tastes towards fresher options has significantly impacted the frozen meat market, while logistical challenges have further contributed to the decline in sales (Meat Institute, 2023). In 2022, the company's decision to introduce processed meat products and sausages reflects its commitment to expanding its range of goods. This is a typical approach meat producers employ to target various market groups and adapt to evolving customer preferences (Caputo *et al*., 2024). In summary, the upward trajectory of total revenue, especially in 2022, suggests a prosperous corporate expansion and an effective response to market fluctuations.

Table 3. Annual Revenue Breakdown by Product Category for the Years 2020, 2021, and 2022

Years	2020	2021	2022
Fresh Meat (Local)	60,285.00	119,274.00	159,617.28
Fresh Meat (Imported)	326,526.00	85,341.00	147,107.32
Fish and Seafood	-	8,741.00	6,829.23
Processed Meat Products	1,791.00	-	97,669.23
Poultry	1,238,888.00	208.00	888.03
Dairy Products	102,592.00	11,360.00	14,104.32
White Meat	216,747.00	75,330.00	214,288.96
Offal	16,747.00	20,705.00	9,814.60
Eggs	2,977.00	5,266.00	1,888.68
Frozen Meat	424,921.00	1,138,778.00	1,022,206.11
Sausages and Processed Meat	-	-	1,146.91
Services	70,549.88	64,749.35	19,883.31
Total Revenue	1,024,296.88	1,529,752.35	1,695,443.02
Years	2020	2021	2022

Source : Compiled by the authors from survey data

Conclusion

Dashboards have become increasingly important in performance management, serving as powerful diagnostic tools that provide managers with a quick and comprehensive overview of their organization's key performance indicators. These platforms allow users to monitor real-time data, make informed decisions, and align their actions with the company's strategic goals. Over time, dashboards have evolved to include advanced features like drill-down capabilities, scenario analysis, and customizable presentation formats. This evolution reflects the increasing complexity and demands of modern business environments, where quick access to accurate and relevant information is vital for maintaining a competitive advantage. The study has conducted a comprehensive analysis of ALVIAR's financial and operational performance from 2020 to 2022, with a particular focus on using dashboards to enhance decision-making and overall management efficiency. The results showed significant improvements in several important areas, including an impressive increase of 18.73% in the company's financial capacity. This unequivocally illustrates the company's enhanced internal financial management. However, the investigation uncovered several concerns, such as a 5% decline in the cash liquidity ratio and a substantial 68% drop in financial assets. Moreover, the analysis of revenue trends revealed a consistent rise in the sales of fresh meat supplied locally, with a 100% increase from 2020 to 2022.

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RISK ANALYSIS OF INVESTING IN GREENHOUSE VEGETABLE PRODUCTION

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Abstract

This research aimed to investigate the risk analysis of investing in greenhouse vegetable production, for the three most commonly cultivated species (tomato, cucumber, and pepper) in the region of Bijeljina (Bosnia and Herzegovina). Data were collected from 35 family farms using a face-to-face questionnaire (structure of production, type and area of protected environment, and method of production with inputs and outputs). Results showed positive financial results highlighting the tomato production to be more adaptable to changes in the yield, variable and total cost considering the threshold of the economy obtained by the break-even analysis. By using the Monte Carlo method, we analyzed the risk of the investment in the covered systems with an equal share of tomato and cucumber in production under the following assumptions: an investment in a protected area without additional heating of 4,000 m^2, financed with credit from a bank with an interest rate of 4% per year, while incomes and expenses arise at the end of the year. During the 10-year exploitation period, net cash flow was positive and the net present value of the investment was 73,436.51 EUR, which indicates that the investment is economically justified if the projected values of the net cash flow are achieved. According to the Monte Carlo method, we found that the expected net present value of investments was 57,649.89 and with 90% certainty, it can be concluded that the net present value will be in the interval from 30,438.62 to 81,860.70 EUR.

Keywords: *Vegetables , Break-even analysis, Monte Carlo method.*

Introduction

Greenhouse vegetable production is perceived as an intensive production due to obtained yields, usage of production inputs (seeds, fertilizers, pesticides, irrigation systems), energy consumption, initial investments, and costs (Heidari and Omid, 2010). Likewise, covered systems provide an opportunity to expand growing cycles over the seasons with unfavorable climatic conditions, without using additional heating and lightening, especially in the continental moderate climates. Results indicate that greenhouse systems contribute to a higher productivity and transpiration efficiency compared to open-field production as well as better water usage and economic sustainability justifies the funds invested in infrastructure (Stanghellini, 2013). Results of the life cycle assessment showed that greenhouse structure had the biggest influence in the global warming category, but with the possibility to lower the consumption of water in production (Muñoz et al., 2008).
During the 2011-2020 vegetable production in Bosnia and Herzegovina, had a share of 3.65% of European areas and did not significantly change in the observed period, as well as average yields were below the European averages (FAOSTAT, 2022). Apart from the current situation in production, climate change began to affect Bosnia and Herzegovina's agriculture production in a way of changes in the amount of precipitation and their distribution, increase

in temperature and occurrence of droughts, floods, and other extreme weather events (Bodiroga, 2023). FAO data predicts by 2050 world population will rise to 9.1 billion people and ongoing climate changes will be a major obstacle to global food production (FAO, 2009; Touili et al., 2024). Certain adaptations should provide farmers the opportunity to combat the adverse effects of climate change, and at the same time sustain yields, with extended growing periods, and have a higher antioxidant capacity under relatively stressed conditions (Bisbis et al., 2018).
As the form of a protected environment becomes more advanced, the initial investment requirements also increase. Therefore, it is necessary to carefully analyze and assess the justification of such investments before implementing the production process. Economic evaluation is often used to assess the overall performance of production systems (Mohamad et al., 2018). Risk is commonly defined as a measurable probability of an undesirable event occurring. Considering the significant risks associated with agricultural production, there is a need to analyze the economic feasibility of investments under risky conditions using widely accepted methods (Bodiroga et al., 2018). This is particularly relevant today, as climate change leads to the occurrence of extreme weather events that increase the risk of investment activities in agricultural production. According to Vasiljević et al. (2019), there are five types of risks in the agricultural sector: production risk, financial risk, market risk, institutional risk, and human factor risk. On the other hand, Brzaković et al. (2015) stated that there are three fundamental components of risk in any project: individual risk, market risk, and project risk for the company. Different methods used for risk analysis have their advantages and disadvantages. By utilizing these methods, it is possible to examine risks from various perspectives and take into account different parameters, leading to a greater amount of data regarding their application. A method that can be used to analyze risks in various agricultural investments is the break-even analysis (Gogić, 2010). Apart from the break-even method, the determination of the risk of an individual project is carried out by estimating future cash flows using several different methods, among which the most prominent are: sensitivity analysis, scenario analysis, risk analysis using the security equivalent method, simulation analysis and others. In this paper, from the group of the mentioned methods, the break-even analysis was applied, as well as a simulation analysis during the risk analysis in the production of vegetables in a protected area in conditions without heating in the northeastern part of the Republic of Srpska in Bosnia and Herzegovina.

Materials and methods

The data collected for this research was obtained through a survey conducted on a sample of 35 family farms (method of random selection) from the Bijeljina city area during 2016-2018. The family farm’s production structure consisted of vegetable cultivation in the protected area without additional heating (tomato, cucumber, and pepper were the most commonly cultivated species). During the survey, different data were collected (structure of production, the type and area of protected environment, and the method of production with inputs and outputs). The collected data were processed using descriptive statistical analysis methods. Analytical calculations for the most important vegetable species as well as investment calculations were created based on the obtained data. The volume and market value of production, where the total costs of the production process are equalized with the income from the sale of finished products, that is, the break-even analysis, was determined using what-if analysis. Simulation analysis, the Monte Carlo method, which shows the probability distribution of all possible outcomes of the project, taking into account an unlimited number of combinations of the observed variables, was carried out using the XLstat add-on for Microsoft Office Excel 2019 software (Microsoft Corp., USA).

Results and discussion

The analysis of the individual risk of the project is particularly important for agricultural farms that do not have a diversified portfolio because the allocation of significant funds to long-term high-risk investments can completely jeopardize their business. Therefore, before the realization of the investment itself, it is necessary to carry out the mentioned procedure by analyzing the results on an annual level or during the entire economic life of the project.
Break-even analysis is often used in making financing decisions for different investment projects by commercial banks (Ambalkar et al., 2013). This method analyzes a representative year of the investment project to determine the volume and/or market value of production at which the total costs of the production process equal the revenues from selling the finished products. One of the ways to determine the cost coverage point, in addition to the standard forms that are used for this purpose (Sredojević, 2011), involves the application of "What if" analysis in Excel software. Using data on the amount of variable and total costs, yield per unit area and the market price of products from analytical calculations, it is possible to determine the impact of their change on the financial result. The elements of the analytical calculation entered in the worksheet in Excel must be bound with formulas before using what-if analysis. For example, show the value of production as the product of the realized volume of production per unit of area and the selling price per unit of the obtained product, total costs as the sum of fixed and variable costs, financial result as the difference between the value of production, and total costs. Analytical calculations for the most important vegetable species grown in the protected area (tomato, pepper, cucumber) are given in Table 1.

Table 1. Average financial results of the most important cultivated species in the protected area

Elements of calculation	**Tomato**	**Pepper**	**Cucumber**
Yield kg/m^2	13.25	12.61	12.56
Selling price EUR/kg	0.59	0.40	0.37
Production value EUR	7.82	5.04	4.65
Fixed costs EUR/m^2	0.78	0.78	0.78
Variable costs EUR/m^2	4.02	3.08	2.59
Total costs	4.80	3.86	3.37
Financial result	3.02	1.18	1.28

Source: Bodiroga, 2020

All three vegetable species achieved a positive financial result on average. Tomato production obtained the highest financial result, compared to cucumber and pepper (Table 1). In addition, the break-even analysis, as stated, shows how much the volume of production, value of production, total costs or variable costs can be increased or decreased without the financial result being equal to 0. The results of break-even analysis are given in Table 2.

Table 2. Limit values of calculation elements at break-even analysis

Elements of calculation	**Tomato**	**Pepper**	**Cucumber**
Yield kg/m^2	8.14	9.65	9.11
Selling price EUR/kg	0.36	0.31	0.27
Variable costs (EUR/m^2)	7.04	4.26	3.87
Total costs	7.82	5.04	4.65

Considering the results from Table 1 and Table 2, it can be concluded that the financial result in the production of tomatoes will be equal to zero when the selling price is reduced by 0.23 EUR/kg in relation to the average realized price. The same applies to peppers and cucumbers when the sales price decreases by 0.31 EUR/kg and 0.10 EUR/kg. The threshold of the

economy of production occurs when the yield of tomatoes is reduced by 5.11 kg/m^2, peppers by 2.96 kg/m^2, and cucumbers by 3.45 kg/m^2. Also, production will be at the threshold of the economy if variable costs increase their amount by 3.02 EUR/m^2 for tomatoes, for peppers by 1.18 EUR/m^2’ and 1.28 EUR/m^2 for cucumbers. The same applies when it comes to changes in total costs. Tomato production is more plastic to changes in the yield, variable and total cost compared to the other two species considering the results of the break-even point analysis. In addition to determining the break-even point, the analysis of the individual risk of the project is also carried out through the evaluation of cash flows using simulation analysis methods that provide the most complete information because it shows the distribution of the probability of all possible outcomes of the project taking into account an unlimited number of combinations of observed variables. No risk analysis can guarantee the complete success of a project, but one can analyze what the risks of decision-making are. While deterministic methods provide essential information about the project, but do not take into account possible changes in parameters that may affect the implementation of the project, probabilistic methods, which include simulation analysis, i.e., the Monte Carlo method (Milanović et al., 2010; Crnjac Milić and Masle, 2013), can consider simultaneous variations in several relevant investments projects and can enable an easy and fast complete analysis related to project sustainability (Odavić et al., 2017). The Monte Carlo method includes a large number of mathematical models and algorithms specific to the stochastic approach, like the use of random numbers to solve specific problems (Zekić et al., 2014).
To analyze the risk of investing in a greenhouse-protected area for the production of vegetables, a production economic model was created that includes the following assumptions: investment in a protected area (high plastic tunnels) with a total area of 4.000 m^2 without heating with natural ventilation, financed with credit from a commercial bank with an interest rate of 4% per year. Incomes and expenses arise at the end of the year, and incomes do not include support funds. Annually, in the same area in the protected area, only one type of vegetable is produced, cucumber in 50% of the area, and tomato in the other 50% (For more details on production and economic model, see Bodiroga, 2023).
For this model, the total investments consisting of investments in protected space and irrigation equipment amount to 35.078,94 EUR. Inflows and outflows were projected during the 10-year exploitation period, and based on them, the net cash flow given in Table 3 was determined. Inflows represent the sum of externally realized values of the specified products, while outflows include the sum of total production costs by specified types less the amount of depreciation of fixed assets and interest on fixed assets and permanent working capital.

Table 3. Projected inflows, outflows and net cash flow during the 10-year exploitation period

Year	Inflows (EUR)	Outflows	Net cash flow
1.	24.957,83	12.820,70	12.137,13
2.	23.895,79	12.275,14	11.620,66
3.	25.735,95	13.220,42	12.515,54
4.	26.550,88	19.052,36	7.498,52
5.	27.970,43	14.368,26	13.602,18
6.	28.128,16	14.449,28	13.678,88
7.	24.947,31	12.815,30	12.132,02
8.	27.865,28	19.727,56	8.137,72
9.	27.076,64	13.909,12	13.167,52
10.	46.248,80	13.449,98	32.798,82

Source: Bodiroga, 2020

Results show that the net cash flow is positive during the entire life of the project (Table 3). The net present value (NPV) of the investment is 73.436,51, which indicates that the investment is economically justified if the projected values of the net cash flow are achieved. The Monte Carlo method is cited as a powerful tool that allows managers to visualize risk and uncertainty when analyzing net cash flows (Clark et al., 2010). It is precisely for this reason that it was applied in the analysis of the obtained net cash flow of the investment in the protected area.
In the first stage of the simulation analysis, the key variables for the investment model were selected, as well as the probability distribution for each of them. The key variables affecting the economic justification of the investment were used: the amount of investment, cash inflows during the use of the investment, and cash outflows during the planned economic life of the investment.
A triangular distribution was chosen for the variables used, and the minimum, maximum, and expected value was determined for each of them. The expected values for all the selected variables are the values used when determining the net cash flows and calculating the economic indicators of the investment's profitability. The minimum value of total investments was determined as a value 5% lower than expected, while the maximum value of total investments was determined as a value 25% higher than expected. The minimum cash inflows from the investment were determined as a value 20% lower than expected, while the maximum cash inflows were predicted to be 10% higher than expected. When it comes to the minimum cash outflows, they were determined as an amount lower than expected by 5%, while the maximum cash outflows were assumed to be an amount higher than expected by 20%. The results of the simulation analysis for the investment in the specified vegetable production model in the protected area are given in Table 4.

Table 4. Descriptive statistical indicators for the net present value of the investment in the observed model

Statistical indicator	NPV
Expected value	57.649,89
Minimum	4.824,75
Maximum	98.305,83
Standard deviation	15.592,08
Variance	193.958.785,45
Coefficient of variation	0.27
Coefficient of symmetry	-0.24
Flatness coefficient	-0.43
Median	58.737,17
First quartile	46.605,09
Third quartile	69.110,54
Variation interval	92.113,03

Source: Bodiroga, 2023

The determination of the displayed values was carried out based on 10.000 simulations, i.e., 10.000 scenarios when it comes to different values of the selected variables and the realized net present values of them. Results indicated that the NPV did not have a negative value in any of the scenarios, as the lowest achieved was 3.802,20 EUR. Although the expected value is positive, it is lower than the one determined with the investment calculation for 15.786,20 EUR. Based on the NPV value calculated by quantiles, it is possible to define the probability that the NPV will be within the limits of different intervals. The values are given in Table 5.

Table 5. Quantiles obtained by the Monte Carlo simulation for NPV of observed investment

Quantiles (%)	NPV
5	30.438,62
10	36.198,96
20	43.801,51
25	46.603,33
30	49.489,14
40	54.500,30
50	58.730,54
60	62.711,81
70	66.927,95
75	69.110,54
80	71.530,42
90	77.329,91
95	81.860,70

Source: Bodiroga, 2023

With a degree of certainty of 90%, it can be concluded that the NPV of the observed investment will be in the interval from 30.438,62 to 81.860,70 EUR. With the narrowing of the interval in which the NPV of the observed project is determined, the degree of certainty of the conclusions reached also decreases.

Conclusion

Investing in greenhouses for the production of vegetables in unheated conditions has its economic justification, even when producers grow only one type of vegetable in the same area during the year. Considering several factors that can have an impact on the results obtained by investing in the mentioned production, it is necessary to perform a risk analysis and, based on the obtained results, make decisions about their implementation. Break-even analysis provides significant information on the values of the elements of the calculation where the value of production is equated with costs. Our survey with 35 family farms showed that all three vegetable species gained a positive financial result highlighting tomatoes being more plastic to changing the yield, variable, and total cost considering the threshold of the economy.
The results obtained by the Monte Carlo analysis allow the potential investor to see in more detail the possible future outcomes of the mentioned investments. However, the method used does not decide on the final solution but provides the investor with important statistical data that enables him to gain a clearer picture of possible variations in economic results. The decision to accept the investment will depend on the results of the simulation and the investor's willingness to bear the risk. For the concrete model of the investment in greenhouse production, the created risk analysis showed during the 10-year exploitation period, net cash flow was positive and the net present value of the investment was 73.436,51 EUR, which indicates that the investment is economically justified if the projected values of the net cash flow are achieved. Simulation analysis showed the net present value did not have a negative value and with 90% certainty, it can be concluded that the net present value of the investment will be in the interval from 30.438,62 to 81.860,70 EUR.

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BIODIVERSITY FINANCE – CURRENT FINANCIAL FLOWS AND FUNDING GAPS

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Abstract

As part of natural capital, biodiversity provides ecosystem services on which economic activities depend. According to assessments, ecosystem services delivered by biodiversity (pollination, nutrient-cycling and carbon sequestration) are worth USD 125-140 billion per year at the global level. On the other hand, research by the World Economic Forum indicates that about half of global GDP is highly or moderately dependent on nature. Regardless of these data and their essential importance, modern society faces a dramatic and accelerated biodiversity loss due to human activities. The financial and economic implications of biodiversity loss are often uncertain and potentially quite severe. A recent global analysis implies that an annual investment of between USD 722-967 billion is required to halt the biodiversity decline by 2030 and that there is an international funding gap in biodiversity funding averaging USD 711 billion a year. To tackle the funding gap and the significant risks of biodiversity loss, we urgently need to identify and implement innovative funding mechanisms and policies that can quickly mobilize considerable capital for nature preservation. This paper identifies unrealized opportunities to increase investment in projects and activities that conserve, support and extend global biological heritage. The redirection of finances and financing through financial instruments for biodiversity can be valuable in overcoming funding gaps and can accelerate the shift toward a more resilient, low-emissions future. Preserving biodiversity and closing the current financing gap is a significant challenge for sustainable financing but also a clever investment.

Keywords: *biodiversity finance, biodiversity financing gap, smarter investments, financial instruments for biodiversity, sustainable finance.*

Introduction

For a while now, scientists estimate the world is now losing species at up to 1,000 times the background extinction rate of one to five species per year, with the number of birds, fish, amphibians, mammals and reptiles decreasing by an average of 60% by less than half century (Karolyi, & Tobin-de la Puente, 2023). Humans are destroying nature and biodiversity far quicker than they can regenerate. On the other hand, the risks associated with biodiversity loss, such as the reduction of productivity and resilience of ecosystems along supply chains, have significant macroeconomic and financial implications (Dasgupta, 2021).
Biodiversity financing can be seen as a subset of sustainable financing, generally defined as financial flows supporting activities and projects aligned with the UN Sustainable Development Goals – SDGs (Hall, & Lindsay, 2021; United Nations [UN], 2015). Currently, it is insufficient and inadequately used. Hundreds of billions of USD are utilised annually, along with the mobilisation of private investments to close the financial gap between what is being spent and what has to be spent in the next decade to maintain the integrity of ecosystems and biodiversity and the services they provide. In addition, what is needed is far

more extensive global support for initiatives aimed at improving understanding and raising awareness among institutional and individual investors about the value of biodiversity and the economic and financial risks associated with its loss. Investors are the ones who, by intensifying capital investments, can help reduce and stop the loss of biodiversity, provided that data that is both credible and useful for making investment decisions backs the effects of the loss. Institutional and individual investors can bridge the financial gap by combining appropriate valuation, market incentives, and sensible investments, thus protecting natural wealth and expanding biological heritage for future generations.

One of the most striking examples of the benefits of biodiversity conservation involves whales. Namely, research has indicated that only the restoration of the whale population around the world (a recovery to the population of between 4 and 5 million as there were before whaling with slightly more than 1.3 million today) would remove carbon dioxide from the atmosphere which is equal to two billion full-grown trees (Chami, Cosimano, Fullenkamp, & Oztosun, 2019). In addition to the sheer beauty of preserving these magnificent creatures, scientists have estimated that the economic value of the average whale, based on its various activities, is about 2 million USD per whale.

There is ample evidence that private investors today consider sustainability and nature in their investment decisions. The subject of this research is biodiversity financing. More precisely, the paper identified the amount of financial resources currently spent and or needed to be spent in the next decade at the global level to maintain the integrity of the ecosystem and biodiversity. The research rests upon a review of relevant international scientific literature that dealt with biodiversity financing issues, and reviews and analysis of reports on global biodiversity finance flows of relevant international institutions and organizations, including the analysis of practical examples of the application of biodiversity financial instruments. The research aims to point out the importance of overcoming the gap in financing biodiversity and to propose financial instruments that can be helpful in this large and globally significant undertaking. As a result of this research, a proposal for a group of four innovative financial instruments emerged, whose characteristics can draw the private capital necessary to bridge the financial gap in financing the preservation of biodiversity. The paper is structured as follows: the first part thoroughly discusses the key insights that further shed light on the importance of biodiversity and its placement as the highest investment priority. The second part of the paper focuses on a critical element related to biodiversity protection, i.e. the gap in biodiversity financing between the current total global annual capital flows directed towards biodiversity conservation and the total funds needed for sustainable biodiversity management. After considering this lack of funding for biodiversity and stating insufficient participation of the private sector, in the third part of the paper, we proposed and considered four financial instruments for biodiversity which, due to their characteristics (stable cash flow and rated issuer), are suitable for attracting investment from individual investors and which can contribute to bridging the financial gap. The paper ends with concluding remarks.

Biodiversity as an investment priority

Nature and its services are essential to the global economy, as more than half of the global GDP (about 40 trillion USD) heavily depends on them (Karolyi, & Tobin-de la Puente, 2023). Despite this, our planet is experiencing an accelerated loss of biodiversity due to human activities such as deforestation, pollution, climate change and resource overexploitation. The fundamental problem is that modern economic thought does not recognize that the human economy is embedded in nature; instead, it treats humanity as a customer who relies on nature (Dasgupta, & Levin, 2023).

Biodiversity, as a crucial aspect of our planet, supports life as we know it and encompasses the diversity of all living organisms on Earth, including plants, animals, microorganisms and

the ecosystems in which they live (Faster Capital, 2024). Although the importance of biodiversity is often neglected, it undeniably provides numerous benefits to humans (providing clean air, water, food and medicine). Hence, the preservation of biodiversity is essential for the health of the planet, our own health and economic well-being.
To better understand the significance of biodiversity conservation, it is necessary to delve deeper into the concept itself and consider in more detail the critical insights (Figure 1) that further illustrate the importance of biodiversity (Faster Capital, 2024):

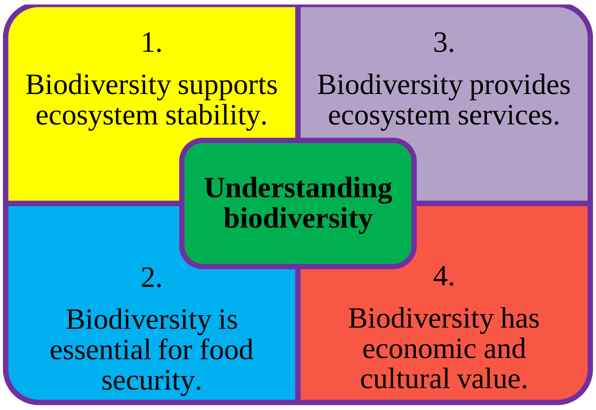

Figure 1. Understanding biodiversity (adapted from: Faster Capital, 2024)

1. Biodiversity supports ecosystem stability: ecosystems consist of various living plant and animal organisms that interact with each other within the environment and thus build a network of complex relationships. Biodiversity appears to be a vital factor in maintaining the stability and resilience of these ecosystems (e.g. a diverse ecosystem is more resilient to disturbances such as disease epidemics or natural disasters than a less diverse one).
2. Biodiversity is of indispensable importance for food security: agricultural production is the primary food source for the world's population and is most dependent on biodiversity. The genetic diversity of crops and livestock in the face of diseases, pests and changing environmental conditions is of utmost importance for ensuring food security.
3. Biodiversity provides ecosystem services: Ecosystems provide services essential for human well-being, such as clean air and water, pollination, nutrient cycling, and carbon storage. These services are often rather disregarded even though they hugely impact our daily lives (e.g. natural pollinators such as various insects – butterflies and bees are responsible for pollinating crops that provide us with food).
4. Biodiversity has economic and cultural value: it provides economic benefits such as tourism, recreation and bioprospecting for new medicines and technologies, and it also has cultural value, as it is often linked to the practices, beliefs and traditional knowledge of indigenous communities.
Understanding the importance of biodiversity is crucial to its conservation (Faster Capital, 2024). Recognizing its importance and adequately valuing the good (biodiversity) and services it provides (Bresnihan, 2017), we can take steps to protect it from the perils it faces.
Two ecological crises of the modern age are the loss of biodiversity and the climate changes caused by it. The collective choices in dealing with it can lead to significantly different outcomes for our planet and economy (Karolyi, & Tobin-de la Puente, 2023).
A global investment framework is necessary to slow down and stop the decades-long plunge in biodiversity. Meeting this goal requires a transformational change in how markets value nature, nature-based assets, and natural capital (Karolyi, & Tobin-de la Puente, 2023; Bril, & Schramade, 2023). In addition, innovative policies and financing mechanisms must be identified and implemented to mobilize the necessary financial resources for biodiversity

conservation and to reform policies resulting in biodiversity loss. Financial products and services focusing on biodiversity financing will require more creative thinking and innovative structuring to attract private capital as a vital part of the solution to preventing biodiversity loss. In their paper, Karolyi & Tobin-de la Puente (2023) stress that a greater focus on investing in natural infrastructure assets such as forests, wetlands, reefs and other natural systems that are natural habitats for diverse wildlife while providing valuable ecosystem services, including protection of watersheds and coasts, can also represent cost-effective infrastructure solutions and wiser investments.

Global biodiversity finance flows

Biodiversity is in severe decline due to a combination of conflicting private and public interests, incoherent policy and governance, and insufficient financing (UNDP, 2018). The latest analysis by a group of experts from The Paulson Institute, The Nature Conservancy, and the Cornell Atkinson Center for Sustainability (Deutz, et al., 2020) suggests that annual investments of between USD 722-967 billion are necessary on a global level to stop the decline in biodiversity by 2030 (Figure 2).

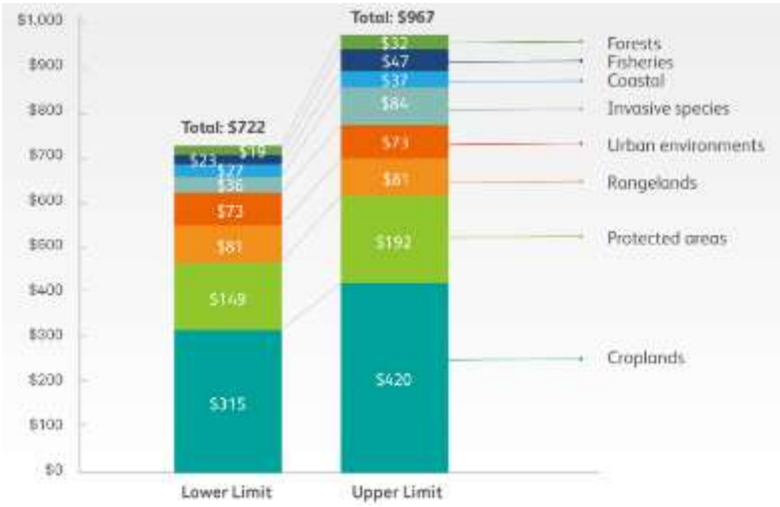

Figure 2. Global annual funding needs for biodiversity protection (USD billions) (Deutz, et al., 2020)

Fundamental changes are essential in all sectors that degrade nature to stop biodiversity loss. Due to the need to project future global annual financial investments for biodiversity protection, Deutz et al. (2020) grouped these sectors into three large groups, i.e. productive landscapes and seascapes, protected and urban areas. Thus, the cost of shifting agriculture, forestry, fisheries, infrastructure and other sectors that have an immense impact on biodiversity to more sustainable business practices is included in the estimate of annual investments to stop biodiversity decline by 2030 (Table 1).

Table 1. Sectorial analysis and objectives of financial investments for biodiversity protection

Sectors that degrade nature (affect biodiversity)		Global investment goal by 2030	Range of cost estimates (annual USD billions)
Sustainable management of productive landscapes and seascapes:	Croplands	Transitioning the agricultural sector to conservation agriculture practices in croplands.	315–420
	Invasive species	Minimizing and alleviating the impact of invasive species on biodiversity.	84
	Rangelands	Transitioning global rangelands to sustainable rangeland management practices.	81
	Fisheries	Transitioning the global fisheries sector to sustainable fisheries practices.	23-47
	Coastal	Restoration of damaged and endangered coastal ecosystems, such as saltmarshes, sea grasses, and mangroves, that provide multiple, and vital benefits for coastal communities.	27-37
	Forests	Transitioning the forestry sector to sustainable forest management practices.	19-32
Protected areas		Increase marine and terrestrial protected areas to 30% (Waldron, et al., 2020, p. 12).	149-192
Urban areas		Protection of biodiversity from the wastewater from urban areas because polluted water and untreated sewage affect water quality, and therefore, biodiversity in marine and river ecosystems downstream from cities. Biodiversity protection on the city peripheries because accelerated urban expansion by 2030 can potentially degrade 40% of strictly protected areas (or 290,000 km2 of natural habitats) located close to urban areas.	73
Total			**722-967**

Source: Elaboration of the authors according to Deutz, et al. (2020)

When considering the above projections, i.e. the global annual funding needed for biodiversity conservation (Figure 2) and the fact that global financial flows aimed at biodiversity conservation in 2019 amounted to 124 and 143 USD billion (Deutz, et al., 2020), we notice an average annual biodiversity financing gap of 711 USD billions (Figure 3).

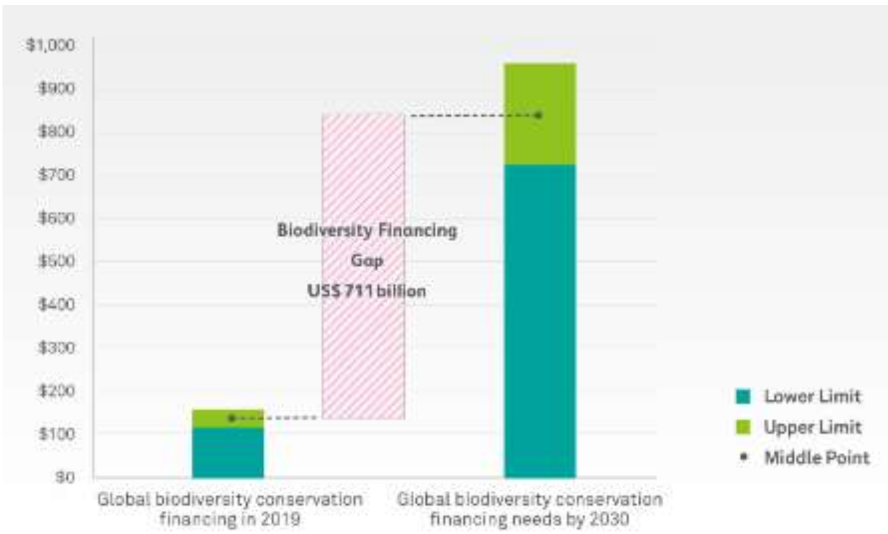

Figure 3. The global biodiversity financing gap (Deutz, et al., 2020)

Based on the presented projections (Figures 2 and 3), we conclude that the current global level of funding for biodiversity protection covers only 16-19% of the total funding needs. To overcome this problem, we need to make a shift towards a new investment paradigm that better includes the economic value and financial benefits of biodiversity and, at the same time, offer investors new financial instruments because it is clear that the financial sector and the companies are not investing adequately in biodiversity despite no shortage of liquidity in the world (UNDP, 2018).

Financial instruments for biodiversity

Biodiversity financing is the practice of collecting and managing capital that contributes and/or should contribute to the conservation, restoration and sustainable use of biodiversity (United Nations Development Program [UNDP], 2018; Organization for Economic Cooperation and Development [OECD], 2020). Capital for biodiversity financing can be collected from public and private sources through different financial instruments and mechanisms on the domestic and international markets. Financial institutions, private asset owners and managers appear most often as intermediaries, as shown in Figure 4 (OECD, 2020).

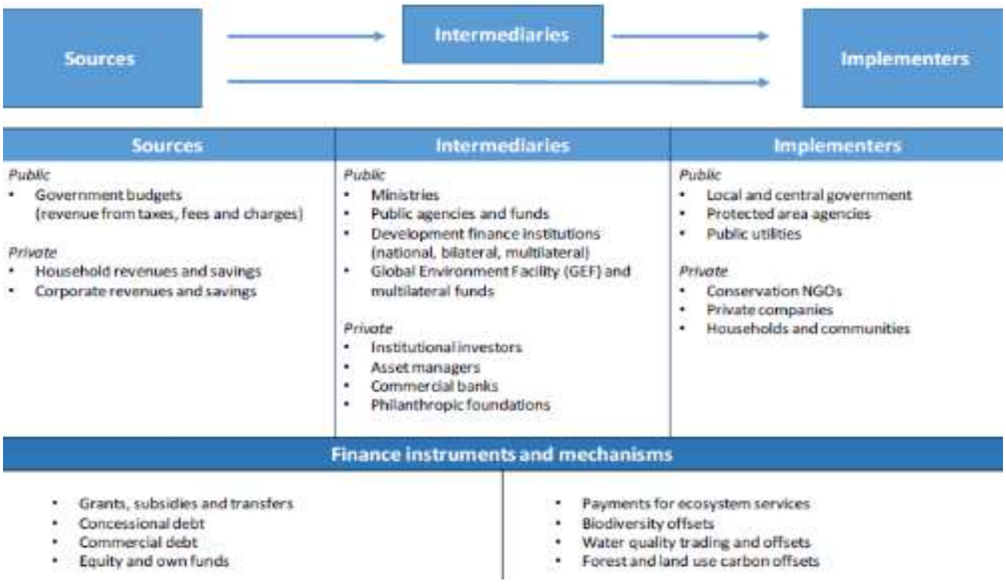

Figure 4. The biodiversity finance architecture (OECD, 2020)

On-going losses of biodiversity and nature are not formally or financially valued and are, therefore, often ignored. It is necessary to treat biodiversity and nature as goods to change that. It is possible to create financial instruments by understanding the actual value of biodiversity and natural assets and to find ways to invest in them because such investments are smarter and more profitable. Nevertheless, to fill the identified gap in biodiversity financing, public investments will have to mobilize private sector financing (European Investment Bank [EIB], 2023), primarily through innovative financial instruments – financial instruments for biodiversity.

Hence, in the continuation of the paper, we propose and consider four financial instruments for biodiversity suitable for attracting investments from individual investors due to their characteristics (stable cash flow and rated issuer). We have derived these instruments for biodiversity financing from a review of extensive scientific literature on sustainable financing innovations at the international level and positive practices of their application in financing biodiversity preservation. The proposed financial instruments for biodiversity and their characteristics are elaborated in Table 2 and explained in the rest of the paper.

Table 2. Proposal of financial instruments suitable for drawing private capital for preserving biodiversity

FINANCIAL INSTRUMENT FOR BIODIVERSITY OVERVEW					
Instrument	**Sector focus**	**Instrument Class**	**Investor market**	**Risk**	**Proof of Concept**
Green bonds	Multiple – across sectors	Debt – fixed interest bond	Multiple – individual and institutional investors	Stable cash flow & rated issuer	Established – examples internationally
Blue bonds	Multiple – oceans, freshwater, and land	Debt – fixed interest bond	Single/Multiple – institutional (less common individual) investors	Stable cash flow & rated issuer	Nascent stage – more frequent application at the international level since 2018
Eden bonds	Single – land use	Debt – fixed interest bond	Multiple – individual and institutional investors	Stable cash flow & government outcome funder	Concept stage
Biodiversity notes	Multiple – across sectors	Debt – fixed income	Multiple – individual and institutional investors	Stable cash flow & rated issuer	Established – examples internationally

Source: Authors' elaboration

● Green bonds have emerged as a significant financial instrument in support of biodiversity preservation (Faster Capital, 2024). Green bonds are fixed-income securities that raise capital for projects or activities with specific climate or environmental sustainability purposes (Golić, & Lalić, 2023). In other words, green bonds are financial instruments that finance sustainable projects dealing with environmental challenges, including biodiversity preservation (protection of forests, wetlands and ecosystems). They are structured the same way and with the same characteristics as standard bonds in terms of rating, seniority, execution process, and pricing, but with proceeds dedicated to biodiversity conservation projects (Rosembuj, & Bottio, 2016).

As an innovative way of mobilizing private capital for biodiversity preservation, green bonds have contributed to the implementation of numerous successful projects globally, some of which are listed below. The Mexican government's sovereign green bond issuance is one of the booming green bond projects. This bond raised USD 2.5 billion to finance environmental projects (conservation of protected areas, reforestation and sustainable agriculture), including biodiversity preservation. Another successful example of green bonds is the World Bank's Lion Bond, which raised $450 million to support African wildlife conservation, including lions, elephants and chimpanzees. The funds support biodiversity conservation efforts, including anti-poaching measures and restoration of the natural habitats of these wild animals (Faster Capital, 2024).

● Blue bonds are innovative financial instruments invented to support sustainable ocean, sea and fisheries projects. Even though some scientific literature classifies them as a subset of green bonds, we will treat them as a separate financial instrument in this paper.

It is a debt financial instrument issued by governments, development banks or other issuers to raise capital from institutional (rarely individual) investors to finance ocean and sea-based projects that have positive environmental, economic and climate benefits (World Bank [WB], 2018a). Investors are paid an interest rate on a fixed schedule and will receive their initial investment (principal) upon maturity. An example of international positive practice of this instrument application for biodiversity preservation is the Republic of Seychelles, which issued the first blue bond in 2018, thus raising 15 million USD for the expansion of marine protected areas, improved management of priority fisheries and the development of the blue economy of Seychelles. The bond was issued with a maturity of ten years and a coupon interest rate of 6.5% (WB, 2018b).

● Eden bonds represent a new class of long-term bonds created to return the land to its natural state because land degradation causes a quarter of all man-made carbon emissions (Boucher, 2019).

The issuer uses the bond proceeds to finance the land lease from the owner, which would be withdrawn from agricultural use and returned to natural ecosystems that create public environmental value (Boucher, 2019; Hall, & Lindsay, 2021). The government would buy that land at a fixed price in 10-30 years. The issuer simultaneously enters into a long-term performance-based payment agreement with the government or government agency that supplements the paradise bond interest payments, provided pre-agreed levels of land use change are achieved, i.e. the land remains uncultivated. These instruments are to finance large-scale land-use change and large-scale restoration of biological heritage (Hall, & Lindsay, 2021).

● Biodiversity notes appeared in the last few years as an instrument issued by a private entity that collects capital to finance biodiversity preservation projects (e.g. land restoration for the purpose of biodiversity conservation), offering the opportunity to invest in a growing market of impact investors. Repayment is not tied to revenue streams from the use of proceeds, but rather from the issuer's general business and/or fundraising activities. Thus, investors will assess the issuer's ability to generate revenues sufficient to cover the debt repayments on the notes, along with its operating expenses and other cash needs (Hall, & Lindsay, 2021). An example of international positive practice of the application of this instrument is Nature Conservation Note offered by Credit Suisse in partnership with investment specialist Althelia Ecosphere are a novel impact investing product designed to help reduce carbon emissions from deforestation and promote sustainable agriculture. The notes form part of the financing for the 101 million EUR Althelia Climate Fund. The notes were launched in 2015 and attracted 15 million EUR of investment from 50 individual investors including a foundations and small number family offices (Environmental finance, 2015).

Conclusion

Biodiversity and nature losses are often unjustifiably neglected and/or treated as problems whose solutions can linger because they have not been formally financially valued. Biodiversity is an asset – natural capital and should be treated as such in order to preserve it. Ecosystems that have more diverse natural assets are known to be more productive, resilient, stable and adaptive. When we compare it with a financial portfolio where diversity (securities or investment projects) reduces risk and uncertainty in terms of returns on invested funds, biodiversity preservation (biological diversity) also reduces risks and uncertainty within a portfolio of natural assets.

The analysis of the current state of the total global financial flows aimed at preserving biodiversity and what to invest in order to stop the decline of biodiversity by 2030 shown in this paper implies the existence of a global annual financial gap of over 700 USD billion. Public investments will have to mobilize private sector financing to overcome this financial gap in the following decade. When we refer to the above story about the portfolio, it actually means that individual investors should align their future investment portfolios with the preserving biodiversity goal, i.e. make them more sensitive and positive towards nature conservation. The main question is how this is possible. It is only possible through investments in innovative financial instruments focusing on biodiversity preservation. It is expected that private capital will be invested on a large scale in financial instruments with an emphasis on preserving biodiversity if the risk-adjusted returns on investments are competitive. We have proposed and considered four instruments in this paper - financial instruments for biodiversity, which we believe meet the stated criteria: green bonds, blue bonds, Eden bonds, and biodiversity notes.

We should not disregard the fact that well-targeted private investments have the potential to improve results in several aspects: first, to increase the volume of capital flows necessary to bridge the financial gap in financing biodiversity preservation; second, to bring seriousness and professional rigour to operational processes, project evaluation and biodiversity conservation impact assessment (UNDP, 2018). Nevertheless, a key challenge lies in the fact that investments in biodiversity are currently inherently out of alignment with conventional risk and return requirements for potential investors.

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CLIMATIC REGIONALIZATION AND RAINFED AGRICULTURAL PRODUCTION IN THE STATE OF MARANHÃO, BRAZIL

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Abstract

The specific objectives of this study were: a) to assess whether it is possible to divide the 217 municipalities in the state of Maranhão into 10 different climatic regions considering the rainfall and temperature series observed between the years 1901 and 2020; b) to assess the instability of rainfall and annual air temperatures in Maranhão between the years 1901 and 2020 with a breakdown for the 10 climatic regions into which the state was divided in this research; c) to show the aggregate behavior and adaptation of rainfed crops in these different regions. The data used was collected from NOAA (2022) and the IBGE's Municipal Agricultural Surveys (PAM) for the period 1974/2020, the years in which the IBGE provides information on crops. Dummy variables were used to test whether 10 climatic regions had different averages for both rainfall distribution and annual temperatures. To assess the suitability of rice, beans, manioc and corn crops for the regions created, the factor analysis method was used to generate weights in which harvested areas, yields, prices, rainfall and temperatures were aggregated at the level of 217 municipalities in the state of Maranhão. The results confirm that Maranhão has 10 regions with different annual rainfall averages. They also show that the same does not apply to average annual temperatures, which only differ in nine. With regard to aggregate agricultural production, it was observed that in five of 10 regions, crop productivity behaves differently from the others.

Keywords: *Climatic regions, Rural development, Land productivity, Rainfall, Northeast of Brazil.*

Introduction

Maranhão is one of the 9 states of the Northeast of Brazil. It is located in the extreme west of the Northeast of Brazil, bordering to the north with the Atlantic Ocean; to the east and southeast, it shares its limits with the state of Piauí; to the south and southwest, it borders the state of Tocantins, and to the west and northwest, with the state of Pará (Map 1). Its total area is 329,651,496 km², which corresponds to approximately 21.20% of the total area of the Northeast region, having the second longest coastline in the country, with an impressive 640 km. Maranhão is a state rich in natural resources, but has the poorest population in Brazil. It's population of 7,114,598 in 2020 had a GDP per capita of only USD 5,780.06, the lowest among all Brazilian states in that Year (Nascimento, Araújo, Campos, 2015; IBGE, 2022).
According to Montebeller (2007), in certain regions of the state the distribution of rainfall is conditioned by static-physiographic aspects such as latitude, distance from the ocean and orographic effects. In addition, the movements of air masses (dynamic factors) that are related to each other, characterize the rainfall rates of a defined region. According to Lemos (2020), in the eastern part of the state of Maranhão there are at least 45 municipalities that have semi-arid characteristics.

In Maranhão State, agriculture faces a high risk of crop loss due to the instability in the distribution of rainfall in time and space, in addition to the predominance of soils of limited efficiency for crops, due to the high process of devastation of the areas (Duque, 1980; Lemos, 2001). The use of techniques that disregard the specific conditions of each environment is a point that deserves to be highlighted, since it affects more intensely the small and medium rural producers, reducing the productivity of crops and their incomes.

It is understood that agriculture is one of the most relevant parts of the state's production chain, becoming dependent and vulnerable to climatic variability in view of the relationship between man and the environment. It is understood that soil and climate control the development and growth of crops and, therefore, environmental circumstances need to be properly analyzed before carrying out an agricultural activity.

In order to achieve better physical yields from the land and more economic returns, with regard to climate, it is known that each crop demands more favorable means throughout its development, that is, they require certain climatic specifications in their various stages of the production cycle, with a minimally adequate availability of water, also having a dry period in the maturation and harvest cycles. The systematization of information on climatic variables is what will make a certain region be seen as favorable for a particular crop and this also involves the risk of loss of plant production (Becerra; Bitencourt, 1999; INPE, 2016; Ramalho Filho and Motta, 2010; Silva *et. al.*, 1993).

Thus, this research seeks to understand how rainfall behaved in the state of Maranhão over a longer historical period (1901 to 2020) and how the distribution of these rains impacts the different areas of a heterogeneous state such as Maranhão. To this end, it has as specific objectives: a) To evaluate whether the climatic regions created for the state present differences in their respective rainfall and air temperature averages, considering the series of rainfall and temperature precipitations observed between the years 1901 to 2020 and b) To evaluate the instability of rainfall and annual air temperatures in Maranhão between the years 1901 to 2020 with unfolding for the 10 climatic sub-regions into which the state was divided; c) show the aggregate behavior of rainfed crops in these different regions.

Material and Methods

The work uses rainfall and air temperature information released by the National Oceanic and Atmospheric Agency (NOAA), for the periods 1901 to 2020, as well as the Sidra database, made available by the Brazilian Institute of Geography and Statistics (IBGE, various years) and in the IBGE Statistical Yearbooks. The period of data availability extends from 1974 to 2020 at the municipal level and at the state level we have the years 1933 to 2020. The crops that make up the series studied are: rice, beans, corn and cassava (IBGE, various years). The units of observations are the 217 municipalities of Maranhão.

The annual rainfall and temperature series covering the period from 1901 to 2020 will serve to make the climatic regionalization of the state. To evaluate the behavior of crops in each of the regions, the following variables are used: Harvested areas (ha), land productivity, which will be treated only as productivity ($kg.ha^{-1}$), prices ($USD.kg^{-1}$).

The original work of Menezes (2009), based on twenty years of rainfall observations gathered the 217 municipalities of the state of Maranhão in 10 climatic regions to. In this research, an attempt was made to evaluate whether those regions are maintained based on a series of rainfall precipitations covering the period from 1901 to 2020. According to the regionalization proposal, these areas are composed of municipalities with characteristics of convergent or similar rainfall conditions.

Table 1. Identification and number of municipalities included in the climate regions created for the State of Maranhão

Regions	Identification	Municipalities
R1	Litoral Ocidental (*Western Coast*)	42
R2	Itapecuru Mirim	25
R3	Baixo Parnaíba (*Lower Parnaiba*)	23
R4	Baixada Maranhense (*Maranhão Lowlands*)	24
R5	Cocais (*Babassu groves*)	18
R6	Alto Mearim e Grajaú (*Upper Mearim and Grajaú*)	24
R7	Chapada do Alto Itapecuru (*Upper Itapecuru Plateau*)	26
R8	Imperatriz	21
R9	Chapada das Mangabeiras (*Mangabeiras Plateau*)	7
R10	Gerais de Balsas (*General of Balsas*)	7
Total	Maranhão State	217

Souce: Menezes, 2009

Methodology used to achieve the first specific objective

In order to assess whether the climatic regions created for the state present differences in their respective rainfall averages and air temperatures, considering the series of rainfall precipitation and temperatures observed between the years 1901 and 2020, the model defined in equation (1) is used.

$$Y_{it} = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_3 + \beta_4 D_4 + \beta_5 D_5 + \beta_6 D_6 + \beta_7 D_7 + \beta_8 D_8 + \beta_9 D_9 + \varepsilon_{rt} \quad (1)$$

In this case, the variable Y_{it} can be either the rainfall or the annual temperature of the municipalities. The variables D_r (r = 1, 2, ..., 9) are dummies (binary) that assume the following values:

$D_1 = 1$, in R1, defined as Litoral Ocidental (*Western Coast*), or $D_1 = 0$ in the other regions;
$D_2 = 1$, in R2, defined as Itapecuru Mirim, or $D_2 = 0$ in the other regions;
$D_3 = 1$, in R3, defined as Baixo Parnaíba (*Lower Parnaiba*), or $D_3 = 0$ in the other regions;
$D_4 = 1$, in R4, defined as Baixada Maranhense (*Maranhão Lowlands*), or $D_4 = 0$ in the other regions;
$D_5 = 1$, in R5, defined as Cocais (*Babassu groves*), or $D_5 = 0$ in the other regions;
$D_6 = 1$, in R6, defined as Alto Mearim and Grajaú (*Upper Mearim and Grajaú*), or $D_6 = 0$ in the other regions;
$D_7 = 1$, in R7, defined as Chapada do Alto Itapecuru (*Upper Itapecuru Plateau*), or $D_7 = 0$ in the other regions.
$D_8 = 1$, in R8, defined as Imperatriz, or $D_8 = 0$ in the other regions.
$D_9 = 1$, in R9, defined as Chapada das Mangabeiras (*Mangabeiras Plateau*), or $D_9 = 0$ in the other regions.

When $D_1 = D_2 = D_3 = D_4 = D_5 = D_6 = D_7 = D_8 = D_9 = 0$. the linear coefficient of equation (1) will be the average rainfall of R_{10}, defined as General de Balsas (*General of Balsas*).

If some, or all, of the estimated values of β_r (r = 0, 1, 2. ..., 9) are statistically different from zero, it means that rainfall and temperature have different means. The random term ε_{rt}, by assumption, is a white noise. According to Wooldridge (2015) and Gujarati and Porter (2011) under thhis condition the linear and angular coefficients of equations (1) can be estimated using the Ordinary Least Squares (OLS) method.

Methodology used to achieve the second specific objective

To achieve this goal, the research uses the coefficient of variation (CV). The CV measures a percentage relationship between the standard deviation and an average arithmetic variable.

According to Gomes (1985), one can scale CV associated with a random variable according to the amplitudes of the variable in Table 2.

Table 2. Classification of coeficiente of variatin (CV) according to its amplitude

Classification of CV	**Range CV**
Low	CV < 10%
Medium	10% ≤ CV < 20%
High	20% ≤ CV < 30%
Very high	CV ≥ 30%

Source: Gomes, 1985.

Thus, the advantage of using CV in this evaluation model over other measures of variability is that it is independent of the units in which the variables are measured. Thus, it allows the comparison of homogeneities / heterogeneities or stabilities / instabilities between variables measured in different units of measurement (Allison, 1978; Garcia, 1989; O'Reilly; Caldwell; Barnett, 1989; Wiersema; Bantel, 1993; Punt, 2003; FAO, 2006).

Methodology used to achieve the third specific objective

In order to assess the association between yields and values per hectare, state and municipal variables related to agricultural production were selected. The data were aggregated using a weighted average. The determination of the weights to be applied in the weighting was conducted using the factor analysis method, with the technique of decomposition into principal components.

According to Fávero *et al* (2009), factor analysis is an interdependent technique that aims to summarize the relationship between a set of variables in synergy, in order to identify common factors of a phenomenon. The main objective of factor analysis is to simplify or reduce a number "n" of observed variables into a smaller "p" group of unobserved variables (p < n), called factors. Therefore, the interpretation and understanding of the dimensions obtained when performing factor analysis characterize the data in smaller quantities than the amount of original variables. And this is due to the correlation between the variables. In contrast, King (2001) and Hair *et al* (2005), portray that data reduction can be obtained by calculating the factor scores of each latent dimension and replacing the original variables with these factors that add, in smaller numbers, the information understood in the original variables.

Through the FA, linearly independent factor scores (FS) are generated, because the orthogonal rotation was performed, which has mean zero and variance one. Therefore, the FS exhibit positive and negative signs. It is these FS that are used to construct indices, which usually take on positive values. In order to generate positive indices, it is necessary to make a transformation of the EFs so that, without changing the distances between the observed values, they are standardized so that all values are strictly positive or zero. This is achieved by using the transformation shown in equation (2):

$$FE_{ijP} = \frac{(FEt - FEmn)}{(FEmx - FEmn)} \qquad (2)$$

For the construction of the productivity index that will capture the synergy between the variables, the geometric mean is used, in which the Productivity Index (PI) is defined using the averages, according to equation (3).

$$\text{PIj} = \sqrt[x]{\prod EF_{Pj}} \qquad (3)$$

So that PI_j refers to the composite productivity index associated with the j-th municipality (j = 1, 2, ..., 217) of Maranhão in year t (t = 1974, 1975, ..., 2020). Constructed in this way, the index will be contained between zero and one. To make it easier to understand, the index is converted into percentage values, generating the highest value equal to 100 and the others being molded as shown in equation (4).

$$PI_{j100} = \left(\frac{PI_j}{PI_{jMÁXIMO}}\right) \times 100 \quad (4)$$

Results and Discussions

Results found for the first objective

Between the years 1901 and 2020 the average rainfall in the state of Maranhão was 1,624.8mm, with a coefficient of variation of 17.4% which means medium instability, on the scale constructed by Gomes (1985). To analyze whether the climatic regions created in the research, based on this longer rainfall period (1901 to 2020) present differences in their respective rainfall averages, tests were performed by applying the linear regression analysis model that was shown in equation (1). From Table 3 which shows the results found to test the differences between the rainfall and air temperature averages estimated for the ten regions of Maranhão between the years 1901 and 2020, it appears that the hypothesis that there are 10 rainfall regions in Maranhão is confirmed. The same cannot be said for average temperatures, where the state is classified into ten regions.

Table 3. Results found for the test of differences of the average rainfall and temperatures of the regions created in the research in the period 1901 and 2020

	Yearly Rainfall (mm)		Yearly Temperature (ºC)		Regions		
Variables	**Est.**	**Sign.**	**Est.**	**Sign.**	**Regions**	**Rain. (mm)**	**Temp (ºC)**
D1	679.14	0.000	-0.078	<0.001	R1	2,075.00^{A}	27.08^{E}
D2	347.97	<0.001	-0.061	0.012	R2	1,743.83^{C}	27.10^{C}
D3	193.85	<0.001	-0.262	<0.001	R3	1,589.71^{D}	26.90^{I}
D4	392.23	<0.001	-0.160	<0.001	R4	1,788.09^{B}	27.00^{G}
D5	47.90	<0.001	-0.054	0.032	R5	1,443.76^{G}	27.10^{E}
D6	55.53	<0.001	0.021	0.390	R6	1,451.39^{F}	27.18^{B}
D7	-185.43	<0.001	-0.090	<0.001	R7	1,210.43^{I}	27.07^{F}
D8	122.28	<0.001	-0.199	<0.001	R8	1,518.14^{E}	26.96^{H}
D9	-194.55	<0.001	0.091	0.003	R9	1,201.31^{J}	27.2^{A}
Constante	1,395.86	0.000	27.157	0.000	R10	1,395.86^{H}	27.16^{B}

Sources of original data: NOAA, 2022.

Remarks: 1 - The adjusted R^2 for assessing differences in rainfall between regions was 0.391; the adjusted R^2 for assessing differences in temperature between regions was 0.02; 2 - The super-indices placed on the estimated medians of rainfall and temperature denote the following hierarchy: hierarquia: A > B > C > D > E > F > G > H > I > J.

This evidence confirms that the ten regions tested have different pluviometries. Regarding the regionalization of temperatures, it was observed that it was possible to prove the existence of nine regions with different temperature averages (Table 3).

Results found for the second objective

From the evidence shown in Table 2 it appears that the rainfall of the regions presented levels of instability, measured by the CV rescpectives, ranging from 16.72% in R10, therefore classified as medium on the scale proposed by Gomes (1985), to 26.22% in R3, therefore a high instability.

With regard to temperatures, it was observed that the variations are all less than 10% (Low), which shows greater levels of stabilities and similarities between the average temperatures of the climatic regions created in the research (Table 4).

Table 4. Coefficients of variation (CV) of rainfall and temperatures estimated for the ten rainfall regions of Ceará defined in the research.

Regions	**Rainfall (CV %)**	**Temperature (CV %)**
R1	19.58	2.44
R2	23.66	2.14
R3	26.22	2.54
R4	18.57	2.18
R5	22.17	2.29
R6	21.46	1.94
R7	23.39	2.28
R8	19.83	2.53
R9	19.81	2.02
R10	16.72	1.94

Sources of original data: NOAA, 2022.

Results found for the third objective

To answer this objective, the Productivity Index (PI) was created using the factor analysis method by the principal component decomposition technique. The results are shown in Table 5. From the evidence shown in this table it appears that the FA was possible to be applied to create the PI, considering that the correlation matrix between the variables is not an identity, as shown by the Chi-Square statistic of the Bartlet test, the KMO statistic = 0.711, as well as the total variance explained by the three estimated factors (73.98%), suggest the statistical robustness involved in the construction of the four orthogonal factors generated. Table 5 also shows that the 13 observed variables were reduced to four independent factors, given their orthogonal rotation.

Table 5. Results found with factor analysis (FA) for the estimation of IPD

Tests for Statistical Robustness in Factor Generation					
Bartlet test	Chi-Square = 4923.467		Degrees of freedom = 78		Sign. =0.000
KMO test =0.711			Explaneid Variance =73.98%		
	Factor loadings				
Variables	Communalities	F1	F2	F3	F4
Rice area	0.884	0.026	**0.939**	0.041	-0.006
Rice yields	0.602	-0.251	0.096	**0.716**	-0.132
Rice price	0.847	**0.884**	0.144	-0.097	-0.190
Bean area	0.736	-0.020	**0.857**	0.019	-0.020
Bean yields	0.759	0.419	-0.182	**0.636**	0.382
Bean price	0.839	**0.838**	0.156	-0.049	-0.332
Cassava Area	0.663	0.451	**0.556**	-0.256	0.292
Cassava yields	0.550	0.090	0.124	**0.715**	0.125
Cassava price	0.705	**0.767**	-0.216	0.076	0.255
Corn area	0.859	0.030	**0.911**	0.151	-0.072
Corn yields	0.692	-0.250	-0.041	**0.722**	-0.326
Corn price	0.890	**0.926**	0.064	-0.128	-0.109
Rainfall	0.591	-0.288	0.012	-0.063	**0.709**

Original data sources: IBGE/PAM (various years) and NOAA (2022).

The following are the results of the tests performed to verify whether the means of the PIs are statistically different between the regions already climatically defined in the survey. These results are shown in Table 4 and Map 4. The adjusted coefficient of multiple determination was 0.181. According to the evidence shown in Table 6, it appears that the ten (10) regions, that are different from an average rainfall point of view, are reduced to only five in terms of the productivity index (PI). Also, according to these results, the ten regions can be hierarchized as follows, taking into account the averages of the estimated PIs for each of them: R1> R4 > R6 > R10 > R2 = R3 = R5 = R7 = R8 = R9.

Table 6. Results for the test of differences of means of PI by homogeneous rainfall regions, means and coefficients of variation (CV) of the estimated PI for the regions.

Varibles	Estimate	Sign.	Region	Average PI	CV(%)
D1	**0.109**	**<0.001**	**R1**	**0.527**^{A}	17.48
D2	0.018	0.390	R2	0.436^{E}	15.14
D3	0.028	0.182	R3	0.446^{E}	14.45
D4	**0.079**	**<0.001**	**R4**	**0.496**^{B}	13.51
D5	-0.001	0.979	R5	0.418^{E}	15.76
D6	**0.071**	**<0.001**	**R6**	**0.489**^{C}	23.56
D7	0.010	0.629	R7	0.428^{E}	19.39
D8	0.026	0.209	R8	0.444^{E}	16.68
D9	-0.042	0.112	R9	0.376^{E}	25.90
Constant	**0.418**	**<0.001**	**R10**	**0.418**^{D}	25.14

Original data sources: IBGE/PAM (various years)
Note, the super-indices placed on the estimated rainfall and temperature averages denote the following hierarchy: A > B > C > D > E

From the evidence shown in Table 1 and Table 4, it appears that the regions with the highest average rainfall R1 (*Western Coast*) and R4 (*Maranhão Lowlands*) also had the highest IP. R6 (*Upper Mearim and Grajaú*) ranks as the sixth best in rainfall and has the third highest PI. These three regions border the state of Pará and have all the characteristics, including landscapes, of the Brazilian Amazon. Regions where food crops studied in the research have an even more relevant participation in the formation of monetary income, in the generation of occupation of the population and in the food security of families. R10 (*General of Balsas*), which has the fourth largest IP, is located in the agricultural frontier known as MATOPIBA (Agreement defining the joint agricultural border involving parts of the states of Maranhão, Tocantins, Piaui and Bahia), where capital-intensive with high productivity crops prevail, especially corn and soybeans, unlike the other regions of Maranhão where family farmers stand out. On the other hand, R9 (*Mangabeiras Plateau*), which presented the lowest PI, was also the region that had the lowest average rainfall over the period studied. Regions R2 (Itapecuru), R3 (*Lower Parnaiba*), R5 (*Babassu groves*), R7 (*Upper Itapecuru Plateau*) are located in the east of Maranhão, the part that borders the state of Piaui. A very significant part of the municipalities located in these regions has semi-arid characteristics, as demonstrated in the research by Lemos (2020). For these reasons they present low productivities. From the results shown in Table 4 it is also apparent that the highest instability estimated for PI through the respective CVs was observed in R9 (CV = 25.90). High in the classification of Gomes (1985). This region also presented the lowest mean value for PI (0.376). On the other hand, R1 (*Western Coast*) which presented the highest mean for PI had CV=17.48, classified as medium. The research showed that in 7 of the climatic regions defined in the research prevailed médium levels of instabilities whose CV ranged from 13.51% in R4 (*Maranhão Lowlands*) to 19.39% in R7 (*Upper Itapecuru Plateau*).

Conclusions

The present study was able to confirm that there are ten different regions in State of Maranhão, from a rainfall point of view. To reach this result, a rainfall series covering the period from 1901 to 2020 was used. The regions have statistically different rainfall averages, but all with medium or high instabilities.
These ten regions are not confirmed with regard to average annual temperatures. In this case only nine regions showed to have different average temperatures. The distributions of these temperatures are very homogeneous over the observed 120 years.
The survey showed that the productivity index (created in the survey) evaluated by the weighted average of harvested areas, land productivities and prices of the main food crops grown in the state (rice, beans, cassava and corn), has divergent averages in only five of the ten regions into which the state was divided in this survey.
It was also observed that the regions with the highest productivity indices (PI) are located on Maranhão's border with the Amazon, where rainfall is higher. On the other hand, the lowest productivity indices (PI) are mostly located on the eastern side of the state, where the municipalities with semi-arid characteristics are located, including in terms of part of the vegetation cover.
Thus, farmers and agents promoting research aimed at technical assistance and rural extension services now have a survey that shows in which parts of the state the crops are most suitable. The results found also serve to review crop planning, in search of understanding why in five of the ten regions studied the results were more promising. This raises the need to promote research that seeks to select cultivars that are suitable for the conditions of the other five regions of the state where productivity rates were lower.

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RURAL DEVELOPMENT VIA TRANSFORMING AGRO-INDUSTRIAL WASTE DERIVED FROM BIOMASS TO SUSTAINABLE BIOENERGY

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Abstract

Waste to energy practices and sustainable waste management could enhance the rural development of a country. For this reason, four wastes (olive stone OLS, extract olive pomace EOP, brewer’s spent grain BSG, and grape marc GM) from three different industries (winery industry, brewing industry, and olive oil production) were examined using several analytical methods for bioenergy production. More specifically, energy content analysis, proximate analysis, thermogravimetric analysis, ultimate analysis, and several other theoretical analyses, such as complete combustion index, were performed. The results showed enhanced energy (4,551 to 5,326 g/cal) and volatile content (71 to 80wt.%), almost zero ash content (<6%), very low sulfur content (3 of them <0.5 wt.% and BSG <1wt.%), of these wastes. Olive oil waste revealed the lowest nitrogen content (~1.2 wt.%) while beer waste revealed the highest (4.2wt.%). The comprehensive combustion index (CCI) revealed to be increased in the olive oil waste, while the grape marc sample showed the lowest CCI value among the analyzed samples. The thermogravimetric analysis showed that these alternative fuels revealed an increased maximum decomposition rate (BSG>EOP>GM>OLS). There is a huge number of these types of industries worldwide, producing huge quantities of waste. Until today, in most of the countries, these wastes have not been utilized with proper, sustainable, and eco-friendly management. The utilization of agro-industrial solid wastes, derived from biomass, may be an economically feasible and ecofriendly way to enhance rural development.

Keywords: *Rural development, management, agro-industrial wastes, waste to energy.*

Introduction

The energy crisis and the increased prices of oil and gas lead scientist to seek alternative sustainable fuels for energy security and rural development. Biomass is a clean source of energy with a zero-carbon footprint.

Brewer's spent grains (BSG), a solid biomass waste resulting from brewing industries, is mainly used as animal feed (~70%), whereas it should not be used as animal feed due to the high microbial load (Russ et al., 2005). Until today, there are very few (almost zero) studies (Bachmann et al., 2022) about brewer's spent grains for energy purposes (as an alternative sustainable fuel). It is an interesting waste, which is available throughout the year in large quantities, with a valuable chemical composition with the disadvantage of a high moisture content (~70%). Another industrial biomass waste that generates significant amounts of solid residues is grape marc produced by wine industries. These wastes cannot be used as animal feed due to the high amount of lignin and tannin they contain, as they are non-digestible substances and are therefore not a suitable nutritional supplement. On the other hand, corms have been used as an organic soil fertilizer but should no longer be used because of the high C/N ratio. Therefore, alternative sustainable ways of managing these wastes must be sought. Few studies have been carried out on the use of stems as solid biofuels (Basso et al., 2018; Khiari and Jeguirim, 2018; Gómez-Brandón et al., 2019).

Olive oil production in the Mediterranean countries (e.g. Spain, Italy, Greece) plays an important role in the economy of these countries.

Approx. 3373 thousand tons of olive oil were produced in 2016/17, worldwide (European Commission, 2020), while 1.95 billion hectoliters of beer were produced in 2017 worldwide (BrewersofEurope, 2018), while 248 mhl of wine production were produced in 2017 (OIV, 2020). So, there is a huge amount of waste that could be used as alternative biofuel for energy production.

The objective of the present paper is to study several agro-industrial wastes -brewer's spent grains, grape marc, olive stone and extract olive pomace- in order to provide several important quality characteristics of these alternative sustainable biofuels for rural development.

Material and Methods

The paper analyzed four agro-industrial wastes retrieved from three different types of industry:

1. Brewing industry (BSG: brewer's spent grain),
2. Wine industry (GM: grape marc), and
3. Olive oil industry, 2-phace process system (OLS: olive stone, EOP: extract olive pomace)

All samples were collected from Greece. The samples were air-dried and then dried in an oven at 80 ^oC for 24 hours, and ground to less than 1 millimeter.

Higher heating value (HHV) was determined via an isoperivol O_2 bomb calorimeter (leco ac500) by ASTM D5865-13 method. The proximate analysis, the determination of volatiles, ash, moisture, and fixed carbon content, was performed using a TGA instrument (leco 701) according to (Vasileiadou, 2024a). The thermogravimetric analysis was performed according to (Vasileiadou, 2023). Ultimate analysis was performed by using a FlashEA 1112 CHNS instrument by combusting about 4 mg of the samples at 900 ^oC according to (Vasileiadou, 2024b).

Apart from the above-mention analytical techniques, a theoretical method was performed by using the data of thermogravimetric analysis in order to calculate the comprehensive combustion index (CCI), ignition index (D_i), and combustion completion index (D_d) by using the following equations (Huang et al., 2019), Eq. (1), Eq. (2), and Eq. (3).

$$CCI = (-R_{max}) \cdot (-R_{aver})/(T_i{}^2 \cdot T_b) \quad (1)$$
$$D_i = -R_{max}/(t_i \cdot t_{max}) \quad (2)$$
$$D_b = (-R_{max})/(\Delta t_{1/2} \cdot t_{max} \cdot t_b) \quad (3)$$

where R_{aver} : is the average value of the mass loss, in %/min, t_i, t_b, t_{max} and $\Delta t_{1/2}$: is the ignition, ending times, the time when there is a maximum mass loss, and the time which refers to the half maximum mass loss $R_{max/2}$, in min. CCI index in $\%^2/\text{min}^2/^oC^3$, Di index, in $\%/\text{min}^3$, D_b index, in $\%/\text{min}^4$. The CCI index is used to compare combustion performance between different fuels.

Results and Discussion

Energy content (higher heating value, HHV) is one of the major properties of a fuel, as is the energy released by the fuel combustion. For instance, by using a fuel with a lower HHV than the designed HHV for a plant, in order to maintain the main steam production rate, a higher fuel feed rate is required. Figure 1 illustrates the energy content of the analyzed agro-industrial Greek wastes. The energy content of Greek lignite (Vasileiadou et al., 2023a) was used as a reference fuel. All biomass residue wastes revealed much higher HHV than the reference sample. More specificity, the olive stone sample showed the highest HHV (more than 5326 g/cal) of all analyzed samples, followed by EOP sample (~4690 g/cal), followed by GM and BSG samples (~4608 and 4550 g/cal, respectively). The results of olive oil industry waste, and wine industry waste are in accordance with literature (Toscano et al., 2013; Mediavilla et al., 2020).

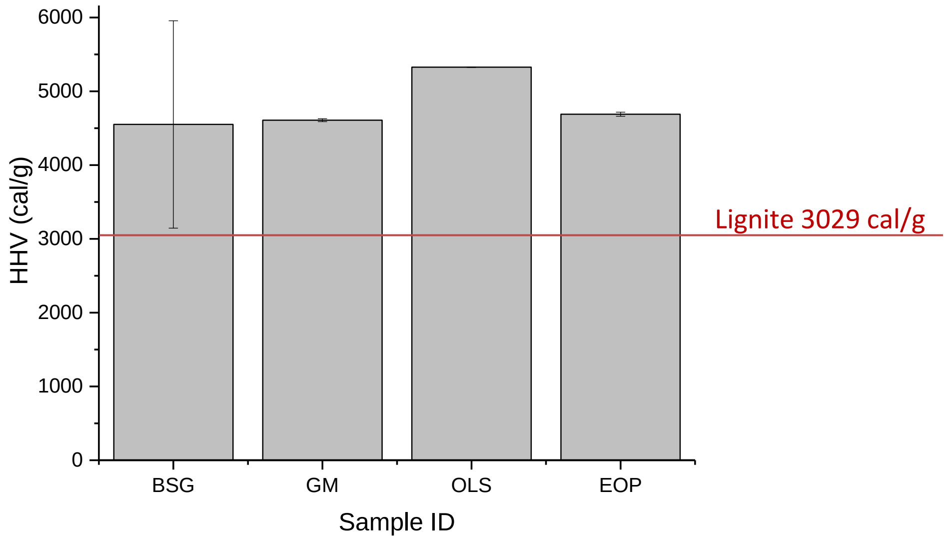

Figure 1. Energy content of the analyzed agro-industrial biomass-based samples [Data from previous works of the author (Vasileiadou, 2023; Vasileiadou et al., 2023b; Vasileiadou, 2024a)].

Figure 2a illustrates the results of proximate analysis. It can be observed that all analyzed biomass residue samples revealed high volatile content, with a range from 71 (GM) to 80 (OLS) wt.%. The Greek lignite volatile content is about 43wt.% (Vasileiadou et al., 2023a). So, the analyzed samples revealed almost double volatile content than the reference sample, which is translated to easily ignition and flame stability. High volatile content leads to high heating value. This fact is confirmed by the results of this study, (see Figure 2b). One more positive result from this study was the fact that the ash content of the analyzed wastes revealed to be very low (~5wt.%). From the other hand, the lignite -reference- sample has a significant amount of ash, ~39wt.%, (Vasileiadou et al., 2023a that causes several slagging and fouling problems in the combustion plant (Vasileiadou et al., 2023a). With the dry treatment of the analyzed wastes, the moisture content dropped to low levels (<6wt.%). So, the high moisture content of these wastes does not seem to be a problem if a proper dryer system is adopted to the plant. By reducing the content of organic materials, and/or reducing the moisture contained in the fuel, the HHV of the fuel increases. Several other authors used other wastes with high amount of moisture as alternative fuels (tomato residues, microalge, etc.) (Şen, 2022; Díaz et al., 2024; Pardilhó et al., 2024).

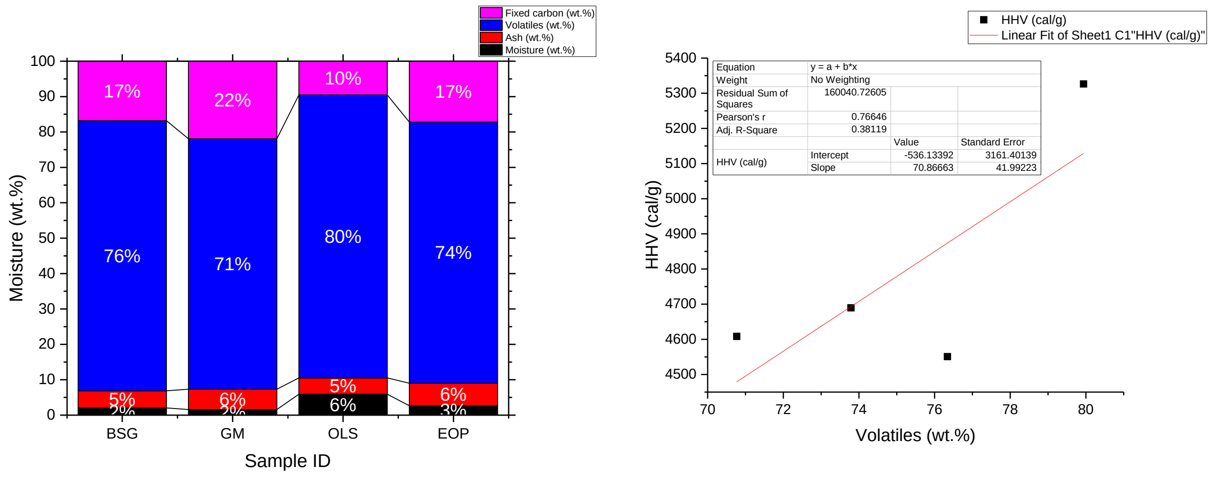

Figure 2. a. Proximate analysis results of the analyzed agro-industrial biomass-based samples [Data from previous works of the author (Vasileiadou, 2023; Vasileiadou et al., 2023b; Vasileiadou, 2024a)], b. Scatterplot between volatile content and higher heating value of the analyzed samples.

Figure 3 shows the thermogravimetric and the differential thermogravimetric profiles (curves) of the analyzed samples. The TG - DTG curves of a fuel are unic and represent the identity of a fuel. From the TG graphs, it can be concluded that OLS sample ignite earlier while EOP sample ignite latter. GM and BSG showed almost the same behavior. The second graph of figure 3, the differential thermogravimetric graphs (DTGs), showed that all samples revealed one significant peak among the temperatures 200 and 400 ^{o}C (devolatilization stage). The first small peak that is illustrated before 200 ^{o}C referred to the dehydration stage of the samples. The brewer’s spent grain showed the highest maximum rate of weight loss (5.5%/min), at lower maximum temperature (307 ^{o}C), followed by EOP sample, followed by GM while the lowest R_{max} revealed in OLS sample (R_{max}: 4.5%/min, T_{max} : 312^{o}C). The total weight loss of the samples is in accordance to the results of proximate analysis (ash content), see Figure 4).

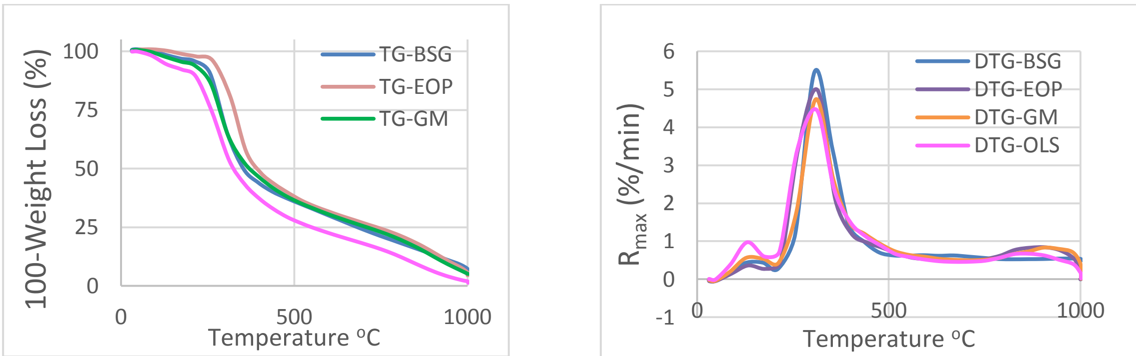

Figure 3. TG-DTG profiles of the analyzed agro-industrial biomass-based samples [Data from previous works of the author (Vasileiadou, 2023; Vasileiadou et al., 2023b; Vasileiadou, 2024a)].

Figure 5a presents the results of ultimate analysis of the industrial biomass residue samples. All samples revealed almost zero S content, <1% (BSG) and <0.5% (OLS, GM. EOP). Figure 5b presents the results of CCI, D_i and D_b indices. EOP and OLS showed tha highest value on CCI index (~8.5x10^{-8} %^{2}/min^2/C^3) while GM revealed the lowest value (~6.5 x10^{-8} %^{2}/min^2/C^3).

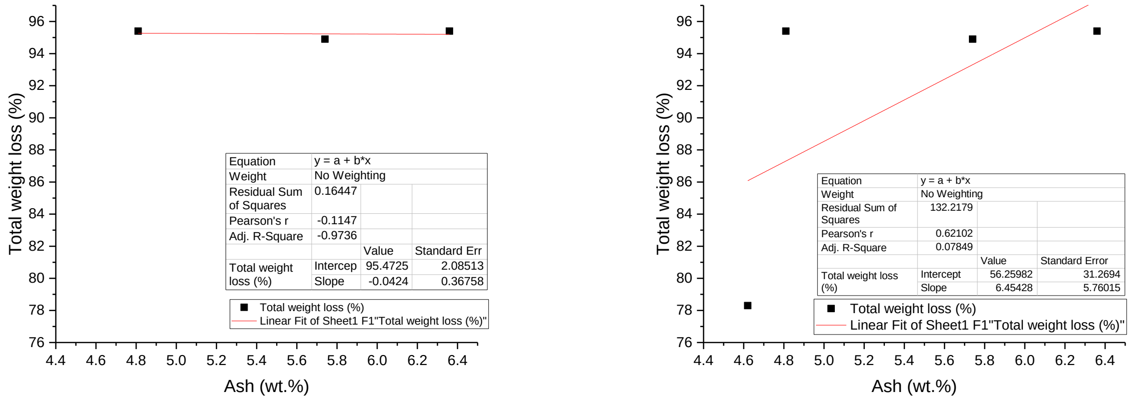

Figure 4. Scatterplot between the total weight loss and ash content of the analyzed samples. a. all samples except OLS, b. all samples.

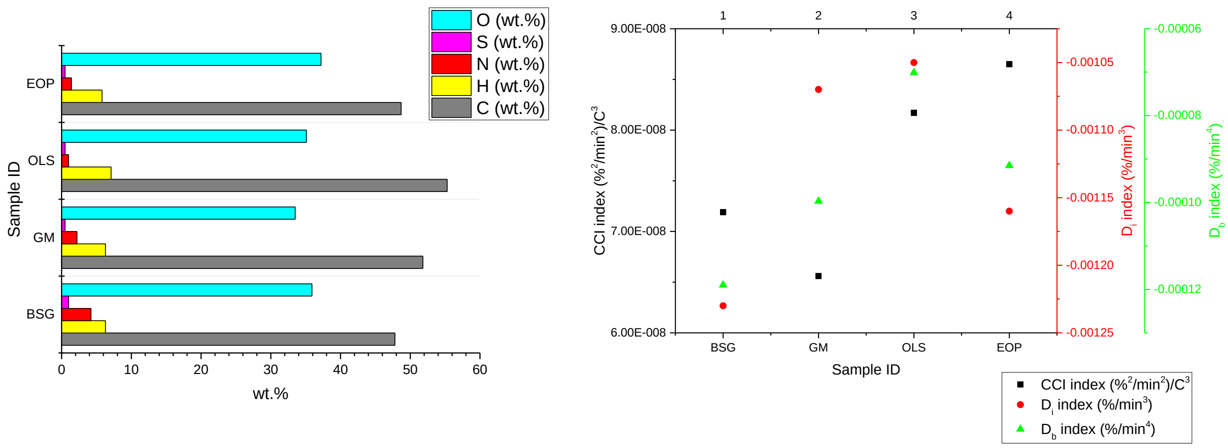

Figure 5a. Ultimate analysis results of the analyzed agro-industrial biomass-based samples [Data from previous works of the author (Vasileiadou, 2023; Vasileiadou et al., 2023b; Vasileiadou, 2024a)]. 5b. CCI, D_i and D_b indices of the analyzed agro-industrial biomass-based samples.

Conclusions

Rural sustainable development could be achieved by using several agro-industrial biomass origin wastes to energy production. Therefore, this paper focuses on the study of four different wastes produced by three different types of industries: wine production industry, olive oil industry, and beer production industry. The results showed enhanced fuel quality characteristics of the analyzed samples compared to low-quality reference sample (lignite). By using a low-quality fuel, the amount of organic material, ash, increases, where this translates into many problems, such as low plant efficiency, increase of primary and secondary air, problems of deposits, slag, etc. So, it is crucial to use high-quality fuels in order to reduce the above-mention issues. Using industrial-biomass origin- waste as a feedstock to energy production could be a promising, eco-friendly, and economically visible way for rural development.

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DEVELOPING TECHNOLOGY TRANSFER OFFICES TO SUPPORT INNOVATION: A CO-DESIGN PROCESS INITIATIVE IN BURKINA FASO AND NIGER

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Abstract

Until the 1990s, most national innovation systems operated on the belief that scientific discoveries and inventions would naturally drive economic development, thus advancing society. However, in the last two decades, a new approach has gained prominence. Research paths require validation among the public, aiming for positive societal impact and defined in collaboration with public input. At European level, this trend is emphasized through the adoption of the quadruple and quintuple helix system, considering innovation result of the collaboration between universities, the business world, policymakers, and local communities. This shift anticipates that involving various stakeholders will realign research paths with public preferences, resulting in more favorable and sustainable solutions. In modern rapidly evolving technological landscape, universities, research institutions, and industries continually strive to bridge the gap between groundbreaking research and real-world applications. One pivotal entity facilitating this transition is the Technology Transfer Office (TTO). TTO serves as a catalyst for innovation, acting as a liaison between academic research and commercialization opportunities. Its primary objective revolves around the transfer of intellectual property, scientific discoveries, and technological advancements from academic environments into practical applications. TTOs can be an invaluable resource for universities in Africa, contributing to their growth, innovation, and economic development. In SUSTLIVES project, starting from a need analysis made with all innovation actors and stakeholders, co-design workshops were organized to discuss with staff members of incubators and TTOs of Burkina Faso and Niger universities about the services to offer and procedures to put in place for entrepreneurial incubation, technology transfer to industry and support for the adoption of innovation within local SMEs.

Keywords: *Innovation, research, technology transfer, Burkina Faso, Niger.*

Introduction

Until the 1990s, the prevailing belief in most national innovation systems was that scientific discoveries and inventions would naturally drive economic development, thus advancing society. Research and development communities directed basic, applied, and industrial research, while the public primarily received innovations passively. However, in the last two decades, a new approach has gained prominence. Research paths now require validation among the public, aiming for positive societal impact and defined in collaboration with public input. This shift anticipates that involving various societal stakeholders and laypersons will realign research paths with public preferences, resulting in more favorable and sustainable solutions.

The Quadruple Helix model, originally conceptualized by Elias Carayannis and David Campbell as a spiral with four strands, clearly demonstrates that the four core components of an innovation system—academia, industry, government, and society—are not involved in unidirectional push-pull relationships, but rather in multi-layered, dynamic, bi-directional interactions (Carayannis and Campbell, 2009). This highlights the role of society as a major actor in national innovation systems as well as the importance of actively integrating the public into innovation projects.

Because greater public involvement entails greater demand for inter- and transdisciplinary processes, the scientific community is facing increasing pressure to elaborate and refine existing understandings of knowledge and the methods of its production and transfer. What is known as *mode 1* is a conventional method of generating knowledge; by focusing on knowledge production within academia, it leads to mono-disciplinary, homogeneous, primarily cognitive information, and organizationally hierarchical knowledge. The knowledge resulting from processes classified as *mode 2 (Gibbons et al, 1994)* or *3* is transdisciplinary, heterogeneous, and transient—it always emerges from interactions among diverse actors, and is therefore socially and economically applicable rather than universal.

There are many uses of the term “technology transfer”. Roessner, in his overview of technology transfer, defines the concept as “the movement of know-how, technical knowledge, or technology from one organizational setting to another” (Roessner, 2000). He further adds: “*the term has been used to describe and analyze an astonishingly wide range of organizational and institutional interactions involving some form of technology-related exchange. `Sources' of technology have included private firms, government agencies, government laboratories, universities, nonprofit research organizations, and even entire nations; `users' have included schools, police and fire departments, small businesses, legislatures, cities, states and nations.…Within single organizations such as large, research-intensive private firms, technology transfer has been used to describe the processes by which ideas, proofs-of-concept, and prototypes move from research-related to production-related phases of product development*”.

There is currently a growing global concern with the decrease in technological barriers between countries. Until relatively recently, technological advances were predominantly from developed countries and spread to the rest of the world.

In modern rapidly evolving technological landscape, universities, research institutions, and industries continually strive to bridge the gap between groundbreaking research and real-world applications. One pivotal entity facilitating this transition is the Technology Transfer Office (TTO). A Technology Transfer Office serves as a catalyst for innovation, acting as a liaison between academic research and commercialization opportunities. By facilitating the efficient transfer of knowledge and technology, this office drives economic growth, foster innovation ecosystems, and contribute to societal advancements. Its primary objective revolves around the effective transfer of intellectual property, scientific discoveries, and technological advancements from academic environments into practical applications in the marketplace.

In general, a TTO is a team whose goal is to identify research results susceptible to creating value added to society, protect these results through patents and licenses, as well as provide the necessary support to create spin-offs and other types of companies and, in general, linking researchers with enterprises. This definition is based on the idea that an entrepreneurship ecosystem is developed in an environment of dynamism and agility, composed of academics, students, and researchers involved with the transformation of knowledge developed in science to benefit society.

A TTO is an entity that helps institutions to innovate through its internal and external services to facilitate the launch of new business models based on emerging technologies. Recently, this theme has attracted much attention in different contexts, especially in academic ones. Academia is traditionally a primary source of new technologies and ideas, but valuable technologies or research findings are not effectively utilized or retained within the academic environment but rather diffuse into society. The TTO emerges as the necessary support, serving as a bridge helping to capture and re-integrate these technologies or findings back into the academic system. This way, the knowledge can be further developed, researched, or applied, ensuring that it contributes to academic advancement and innovation rather than being lost or underutilized.
TTOs are one of the most important paths for connecting scientific research to social needs. This is done by making technologies developed in academic institutions accessible to the market, from which society will benefit from these innovations.
The transition from universities to the market, and therefore the making of innovations available to society, passes through the world of businesses, which therefore play a strategic role in the activities of TTOs and decree their success. Facilitating the relationship between universities and companies, thus enhancing the results of academic research, is one of the crucial roles of the TTO teams. The growth of opportunities for relationship and collaboration between these two entities ensures the achievement of the goals of the third university mission because “the purpose of university technology transfer is to transfer university research results from the university out to businesses where the results are developed into new products and services that benefit society"(Hockaday , 2020).
But the alignment of business and academic interests can be problematic and requires mutual understanding and trust, a long process that requires great commitment on the part of the actors and mediators in charge, among whom the TTO is certainly privileged. There are several benefits from this relationship including: the development of medium and long-term initiatives such as strategic partnership agreements, shared infrastructure, negotiation of framework agreements for the continuing training of company employees, multidisciplinary approaches to research, development of new innovative products and services, the increase in reputation of companies and the possibility of new forms of financing for research and training by universities.
SUSTLVES[1], a four-year project funded by the European Union under the DESIRA programme, aims to strengthen the research and innovation capacities of actors in neglected and underused crop value chains (NUS) in Burkina Faso and Niger through two local universities (Joseph KI-ZERBO University (UJKZ) of Burkina Faso and the University Abdou Moumouni (UAM) of Niger). The project also aims to organise support activities for women and young people who wish to exploit entrepreneurship opportunities. The project among its activities has the task to organize a process of shared construction of the technology transfer model in each university to co-design services to offer and procedures to put in place for entrepreneurial incubation, technology transfer to industry and support for the adoption of innovation within local SMEs.
The young generation in Africa, including countries like Niger and Burkina Faso, represents a significant portion of the population and plays a crucial role in shaping the future of these nations. Both Niger and Burkina Faso have high birth rates, resulting in a substantial youth population (more than 60% of the population is under the age of 25). The youth in these countries face numerous challenges, including limited access to education, healthcare, employment opportunities, and basic amenities. Poverty, lack of infrastructure, political

[1] The project began in August 2021. The Italian Agency for Development Cooperation (AICS) is the project coordinator, and the Mediterranean Agronomic Institute of Bari (CIHEAM Bari) is responsible for the technical and scientific management.

instability, and climate change further exacerbate these challenges. Even access to quality education remains a significant concern. While efforts have been made to improve literacy rates and school enrollment, many young people in these countries still lack access to proper education due to factors such as poverty, distance to schools, and inadequate resources. Limited job opportunities, especially in urban areas, lead to underemployment and force many young people to seek informal work or migrate to other regions in search of better prospects.
Despite the challenges, the youth in Niger and Burkina Faso are increasingly becoming more vocal and active in advocating for their rights, participating in civic engagement, and pushing for positive change. Youth-led initiatives, organizations, and movements are emerging to address various issues affecting their communities. The younger generation in these countries is embracing technology and innovation. With increasing access to mobile phones and the internet, young people are leveraging these tools for education, entrepreneurship, and connecting with the global community. Efforts by both, local governments and international organizations, are underway to address the challenges faced by the young generation in these countries. Initiatives focusing on education, skills development, entrepreneurship, and creating opportunities for youth empowerment are being implemented to support and uplift the youth in Niger, Burkina Faso, and in general across Africa. One of the contributing factors to youth unemployment in Niger, Burkina Faso, and across Africa is the mismatch between the skills acquired through education and the demands of the job market. The education system often doesn't align well with the needs of the labor market, leaving many graduates ill-prepared for available job opportunities. There is often a significant difference in youth unemployment rates between rural and urban areas. Urban centers tend to have higher rates of unemployment due to the concentration of young people seeking limited formal job opportunities. Despite all these challenges youth have to face, the relationship between innovation and young people is dynamic and significant. Young people often possess a fresh perspective, creative thinking, adaptability, and a willingness to challenge the status quo, making them a driving force behind innovation. Every effort made to reinforce and improve institutions, like universities, that can support youth entrepreneurship and creativity is more than welcomed and international organizations are asked to answer to these needs.

Materials and methods

The activities carried on within SUSTLIVES project explored ways and opportunities to establish a TTO in each university (Joseph Ki-Zerbo University of Ouagadougou and Abdou Moumouni University of Niamey) to promote technology transfer and commercialization of research results. In an ever-changing world, where emerging markets and developing economies are positioned to reap the benefits of innovation, it is essential that Burkina Faso and Niger develop a visionary strategy and the institutions needed to thrive in the global knowledge economy.
The co-design process started with a workshop aiming at identifying the obstacles present in the territories and the shortcomings perceived by the actors of the quadruple helix (research, business, public administration, citizens) of Burkina Faso and Niger in relation to the valorization of research results.
Then, co-design workshops were organized with staff members of incubators and technology transfer offices of universities in Burkina Faso and Niger to discuss in detail about the services to offer and procedures to put in place for entrepreneurial incubation, technology transfer to industry and support for the adoption of innovation within local SMEs.
The shared construction process of the technology transfer model for each university followed different stages:

- Needs analysis, through specific questionnaires

- Local meetings with university technology transfer stakeholders
- Dedicated sessions during international training
- Follow-up sessions with international experts
- Model validation

The roadmap proposed is based on an analysis of the current state of the country and the university in terms of R&D and the national innovation system and its capabilities. We also drew on international best practices, particularly from emerging countries that have been successful in stimulating growth through innovative policies and effective technology transfer.

Results and discussion

TTOs can be invaluable resources for universities in African countries, contributing significantly to their growth, innovation, and economic development. The presence of a TTO in African universities can contribute in:

1. **Promoting Innovation and Research Commercialization:** TTOs play a crucial role in identifying, protecting, and commercializing innovative research outcomes and inventions developed within universities. They help transform academic research into tangible products, services, or processes that can be commercialized, creating opportunities for economic development.
2. **Supporting Entrepreneurship:** TTOs provide support and guidance to researchers and students interested in starting their own businesses or ventures based on university research. They offer resources such as business development advice, access to funding, mentorship, and networking opportunities, fostering an entrepreneurial ecosystem within the university.
3. **Facilitating Partnerships and Collaborations:** TTOs facilitate collaborations between universities and industries both locally and globally. These partnerships can lead to **joint** research projects, technology transfer agreements, and knowledge exchange, enabling universities to leverage external expertise and resources for further research and development.
4. **Intellectual Property Management:** TTOs manage the intellectual property portfolio of the university, ensuring that inventions and discoveries are appropriately protected through patents, copyrights, or trademarks. This protection not only safeguards the university's innovations but also creates opportunities for licensing or partnerships that can generate revenue.
5. **Economic Development and Job Creation:** By commercializing research outcomes, TTOs contribute to economic growth within the country. Successful commercialization can lead to the creation of new industries, employment opportunities, and the transfer of valuable technologies that can address local challenges.
6. **Capacity Building and Skills Development:** TTOs often organize workshops, training sessions, and educational programs aimed at building the capacity of researchers, students, and staff in areas related to entrepreneurship, intellectual property management, and technology commercialization.
7. **Funding and Grants Management:** TTOs aid researchers in identifying funding opportunities and managing grants to support further research and development. They play a crucial role in securing financial support for promising projects, enhancing their potential for successful commercialization.

For universities in African countries, establishing and strengthening TTOs can be instrumental in leveraging their research potential for socio-economic impact, fostering innovation, and contributing to the overall development of the country by translating academic knowledge into practical applications.

TTOs within universities in African countries can significantly contribute to job creation through various directions:

1. **Startup Ventures:** TTOs support researchers, students, and faculty in commercializing their innovations by starting their own businesses or startups. These new ventures often require a skilled workforce, leading to job opportunities for graduates and professionals with specialized expertise in fields related to the developed technologies.
2. **Industry Collaborations:** Collaborations between universities and industries facilitated by TTOs can lead to joint research projects, technology development initiatives, and knowledge transfer programs. Such collaborations often demand a workforce to execute projects, creating job opportunities for researchers, technicians, engineers, and other skilled workers.
3. **Supporting Local Industries:** Successful technology transfer and commercialization of university research can spawn new industries or bolster existing ones. For instance, if a university develops a new technology applicable in agriculture, it may lead to the creation of agro-processing facilities or agricultural technology companies, consequently generating employment in related sectors.
4. **Ecosystem Development:** TTOs contribute to building vibrant innovation ecosystems by nurturing a culture of entrepreneurship and innovation within and around the university. As startups and new businesses emerge from academic research, they often require a range of professionals, including managers, marketers, developers, and support staff, thus creating diverse job opportunities.
5. **Skill Enhancement and Training:** TTOs often organize training programs, workshops, and seminars focused on entrepreneurship, technology commercialization, and innovation. By equipping individuals with the necessary skills and knowledge, TTOs indirectly contribute to creating a workforce capable of driving innovation and employment opportunities.
6. **Social and Community Impact:** Job creation resulting from successful technology transfer doesn't only affect the university but also extends to the surrounding communities. New job opportunities can stimulate local economies, contribute to poverty alleviation, and improve living standards in the region.

It's important to note that while TTOs play a significant role in job creation through technology transfer and commercialization, the actual impact may vary depending on various factors such as the scalability of the developed technologies, market demand, and the support ecosystem available for startups and industries in the region. However, TTOs serve as catalysts, fostering an environment where research and innovation can translate into tangible economic and employment opportunities for the society at large.

In the particular case of Burkina Faso and Niger universities, the co-design process highlighted the following main problems :

- Poor adherence of university training to the needs of companies
- Lack of management of intellectual property by universities
- Legal framework for TTOs to be improved and lack of creation of spinoffs
- Poor constructive dialogue between universities and companies
- Limited access and poor promotion of research results
- Spaces and equipment dedicated to TT activities to be improved
- Lack of funds for the development of innovative projects

The result of the co-design process that was undertaken through the above-mentioned workshops and discussions with the beneficiaries of the SUSTLIVES project has been the development of specific user-centric services when it comes to stimulating entrepreneurship

and finding appropriate applications for technologies developed as part of the efforts of university research.
The proposed vision is to build an autonomous and dynamic innovation ecosystem both in Burkina Faso and Niger that takes advantage of internal and external technologies and knowledge and adapts them to the local context, promoting economic growth and social development.
The mission of the TTO would be to promote and facilitate the transfer of innovative technologies to stimulate the economic and social development of the country through the creation of strong partnerships between research and industry actors, to promote research results and support the emergence of innovative companies.
To achieve the above vision and mission objectives, the co-design activity identified six time-phased intervention levers:

1. Strengthening the management of intellectual property
2. Establishing an appropriate governance
3. Building skills: Raising awareness, training and developing team and ecosystem capacities
4. Implementing a monitoring strategy and mobilization of funds
5. Strengthening the university's 3rd mission, identifying and setting up projects with the private sector
6. Promoting the creation of the technologically innovative startup

Conclusion

In conclusion, the relationship between innovation and young people is symbiotic, with their creativity, adaptability, willingness to take risks, and passion for change driving forward new ideas and advancements across various fields. Encouraging and supporting the innovative potential of young minds is essential for fostering a culture of innovation and driving progress in society. This process need the establishment of specific supporting structures and an enabling environment including all the relevant actors of the innovation ecosystem: the TTO within the university has a crucial role in achieving this goal.

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AGRI-FOOD RESEARCH IN CHAD

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Abstract

The primary sector still plays a vital socio-economic role in Chad, a landlocked and Sahelian country in Central Africa. The country faces several challenges, such as food insecurity and poverty. Research is needed to foster the transformation of the agri-food system, which is necessary to address such challenges. However, a comprehensive analysis of the research landscape is lacking. Therefore, this review provides a bibliometric analysis of the scholarly literature on agriculture and food in Chad. It draws upon a systematic review of 94 eligible articles retrieved through a search conducted in April 2024 on the Web of Science. The analysis highlights several weaknesses in the research field, such as a low annual output of articles, a focus on biological and environmental sciences, and about three-quarters of the papers, despite dealing with Chad, not involving any Chadian scholar. Moreover, many prominent organizations are based outside Chad, particularly in Europe. Only two of the top 25 affiliations are Chadian and the most relevant funding agencies are based abroad. The research field primarily focuses on the crop production agriculture subsector and production stage of the food chain. However, there has been an increase in the number of publications, indicating a growing interest in research on agri-food, and the research field is multidisciplinary. To address the multiple challenges faced by Chad and to facilitate the transition towards sustainable and resilient agri-food systems, promoting agri-food research is crucial.

Keywords: *Sahel, Central Africa, Agronomy, Food system, Bibliometrics.*

Introduction

Chad, named after Lake Chad, is a landlocked country at the crossroads of North and Central Africa bordered by Libya, Sudan, the Central African Republic (CAR), Cameroon, Nigeria, and Niger. A Sahelian and continental country located in the heart of Africa, Chad covers an area of 1,284,000 km^2, making it the fifth-largest country in Africa and the twentieth-largest nation by area in the world (Central Intelligence Agency, 2024; INSEED, 2013). It had a population estimated at 16,344,852 inhabitants in 2020 (of which about 1.6 million live in the capital and largest city of N'Djamena), with an average annual growth rate of 3.6% and an average density of 8.6 inhabitants per km^2 (République du Tchad, 2021). The United Nations Human Development Index (HDI) ranks Chad 189 out of 195 countries (only Niger, CAR, South Sudan and Somalia perform worse than Chad) (UNDP, 2024). The gross domestic product (GDP) per capita was estimated at just US$ 1,724 in 2022 (International Monetary Fund, 2023).
Agriculture (subsistence farming and livestock rearing) continues to be a crucial source of food security and livelihood for the Chadian population (Central Intelligence Agency, 2024). Indeed, the primary sector (agriculture, forestry, and fishing) still makes a significant contribution to the country's GDP (22.6% in 2022) (World Bank, 2024a) and employment

(69% in 2021) (World Bank, 2024b). Chad has several climatic regions: the Sahara desert in the north, an arid, Sahel zone in the center, and a more fertile Sudano-Savanna zone in the south (INSEED, 2013). Chad has significant agro-sylvo-pastoral and fishing potential whose exploitation represents both the main activity of the population as well as the engine of growth and diversification of the economy. Cultivated areas represent only 13% of the potential 39 million ha. The use of agricultural resources faces several cumulative problems, including the repercussions of insecurity, the pandemic of COVID-19, climate change, poorly controlled population growth, and economic and financial crises (République du Tchad, 2021). At the level of local production, family farming is the main source of food for the population and provides most of the income of rural people. But, family farms are for the most part poor and poorly equipped. They use few inputs and rely on archaic agronomic practices. Production systems are inefficient and depend heavily on rains while groundwater and surface water resources are abundant (République du Tchad, 2021). Additionally, West Africa and the Sahel are highly vulnerable to the effects of climate change. Rain-fed agriculture, in particular, is susceptible to variations in climate (Sultan & Gaetani, 2016). Rising temperatures, shifting rainfall patterns, and increasing extreme weather events threaten food production and disrupt traditional farming methods. Chad's vulnerability is exacerbated by its high level of poverty and dependence on sectors that are sensitive to climate change, such as agriculture (World Bank, 2021). Moreover, the country still faces significant challenges in terms of food insecurity and malnutrition. Indeed, the prevalence of undernourishment in the total population remains high, 31.4% over the period 2020–22 (FAO et al., 2023). The causes of food and nutrition insecurity are varied and complex, and include, among others, the incidence of high poverty, insufficient food production and access to drinking water, repercussions of climate change, low coverage of basic social services, inadequate hygiene and sanitation conditions, and limited alternative sources of income (République du Tchad, 2021).

There is an urgent need to transition toward a sustainable and resilient food system in Chad to address food insecurity, poverty, and climate change challenges. Many activities within the food system must be transformed to enable sustainability while taking seriously environmental considerations associated with the impact of climate change. The National roadmap for the transformation of the Chadian food system (République du Tchad, 2021), whose purpose is to eradicate hunger and malnutrition in the country, is structured around five action tracks: (i) strengthening the resilience of households and communities most vulnerable to crises and disasters; (ii) promoting nutritious and healthy diets for all; (iii) improving productivity and sustainability of agricultural and food systems; (iv) empowering youth and women in food systems; and (v) ensuring the capacity building of stakeholders and inclusive food system governance. Research is crucial for transforming the food system. For that, promoting research results, acquired knowledge, and good practices from development projects and programs are considered among the levers for implementing the National roadmap (République du Tchad, 2021). However, there is no recent overview of the state and contours of the research system in Chad. In this context, the present review provides a bibliometric analysis of the scholarly literature on agriculture and food in Chad.

Methods

This article is based on a thorough examination of all documents indexed in the Web of Science (WoS) Core Collection and adheres to the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Moher et al., 2009; Page et al., 2021). In April 2024, a search was conducted using the following string: *(agriculture OR agro OR food) AND Chad*. Therefore, one limitation of the study is that papers referring to Central

Africa or the Sahel without Chad might not have been retrieved through the search. To be included in the review, documents had to meet three eligibility criteria: they had to be geographically relevant (i.e., they had to deal with Chad), thematically focused (i.e., they had to deal with research on agriculture and/or food), and of a specific document type (i.e., only journal articles, book chapters, or conference papers were considered; editorial materials such as letters to editors, commentaries, notes, and reviews were excluded). Only documents that met all three criteria were deemed eligible and included in the review.
The search conducted on WoS resulted in 185 documents (Table 1). However, during the initial screening process, 31 documents were excluded based on their titles as they did not pertain to Chad. The articles/documents that covered a broader geographical area like the Sahel, Central Africa, Lake Chad, Sub-Saharan Africa, or those that did not specify the geographical scope in their titles were kept for further scrutiny. Upon reading the abstracts, another 41 documents were excluded as they did not meet at least one of the inclusion/eligibility criteria. For example, CHAD refers to Conventional Hot-Air Drying, Convective Hot-Air Drying, Community Health Awareness of Diabetes, and Consolidated Human Activity Database. Lastly, the analysis of full texts led to the exclusion of 19 documents, including 10 reviews. Therefore, the systematic review included 94 documents (84 articles and 10 proceeding papers).

Table 1. Selection of eligible documents to be included in the review.

Selection steps	Number of potentially eligible documents	Number of excluded documents and reasons for exclusion
Search on WoS	185	--
Screening of documents based on titles	185	31 documents were excluded because they deal with countries other than Chad e.g., Cameroon, India, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, and Sudan
Screening of documents based on abstracts	154	41 documents excluded: 18 documents that do not deal with Chad 23 documents that do not address research on agri-food
Scrutiny of full-texts	113	19 documents excluded: 8 documents that do not deal with Chad 1 document that does not address research on agri-food 10 reviews
Confirmation of eligibility and inclusion in the systematic review	94	--

The chosen articles were subjected to a bibliometric analysis that concentrated on sources/journals, research areas, Sustainable Development Goals (SDGs), authors, affiliation organizations, affiliation countries, and funding agencies. Additionally, analyses of agriculture subsectors (including crop production and the primary crops addressed, animal/livestock production, and fisheries/aquaculture) and food chain stages (viz. production, processing, distribution/retail/marketing, and consumption) were performed. All analyses were carried out using the methodology outlined in the work of El Bilali and Ben Hassen (2023).

Results and Discussion

Based on the analysis of the chosen documents, it can be inferred that the initial article dealing with agri-food in Chad, indexed in WoS, was published in 1991 (Delisle et al., 1991).
The *number of articles* produced annually varies greatly from one year to another but is generally quite low. Considering the period 1991 – 2024, the number of articles ranges from zero in various years (viz. 1992, 1996, 2001, 2002, 2003, 2004) and just one article in other years (viz. 1993, 1994, 1997, 1998, 1999, 2006, 2008, 2009, 2010, 2011) to a maximum of 12 in 2023. However, overall, there has been a rise in the number of publications, which may suggest an increase in interest in research on agri-food in Chad.
As for *sources* (Table 2), the most significant number of articles were published in the Food and Nutrition Bulletin and Scientific Reports (3 articles each). However, there were a total of 87 journals and sources that published research on agri-food in Chad, which suggests that there are no specific publication outlets.
Out of the selected articles, the majority can be linked to *research areas* of Environmental sciences – Ecology (25 articles, 26.60%) followed by Agriculture (12 articles, 12.77%) and Nutrition – Dietetics (10 articles, 10.64%). Nevertheless, the selected 94 documents can be classified into 48 research areas (including anthropology, biochemistry, biotechnology, business economics, cell biology, chemistry, development studies, evolutionary biology, food science, genetics, geography, geology, meteorology, pediatrics, veterinary sciences), which suggests that agri-food research in Chad is multidisciplinary. However, it can be argued that while environmental and biological sciences are adequately addressed, social sciences and economics are generally overlooked.
The various sectors and disciplines involved in the field of research are reflected in the *SDGs* it addresses. Twelve SDGs have been related to the selected articles, with the most prominent being
SDG 13 – Climate action (32 documents, 34.04%), SDG 03 – Good health and well-being (29 documents, 30.85%), SDG 02 – Zero hunger (26 documents, 27.66%), SDG 06 – Clean water and sanitation (24 documents, 25.53%), SDG 01 – No poverty (22 documents, 23.40%) and SDG 15 – Life on land (12 documents, 12.77%). Additionally, marginal SDGs include SDG 05 – Gender equality (2 documents), SDG 11 – Sustainable cities and communities (2 documents), SDG 08 – Decent work and economic growth (1 document), SDG 09 – Industry, innovation and infrastructure (1 document), SDG 10 – Reduced inequalities (1 document) and SDG 16 – Peace, justice and strong institutions (1 document). A document can be associated with one or several SDGs.
The analysis of the selected publications shows that France Begin (Montréal University), Stefano Corsi (Milan University), Hélène Delisle (Montréal University), Anastasia Marshak (Tufts University), Erik Nilsson (Lund University), and Edgar Wabyona (World Food Programme), with just 3 articles each, are the most prominent and productive *authors* in research on agri-food in Chad. Nevertheless, the 94 selected documents have been authored by 388 scholars. That means that 382 scholars have authored just 2 or 1 article. This finding, in turn, suggests a lack of consistency in the research field. In other words, even researchers working on agri-food in Chad seem to do so sporadically rather than systematically. This may be due to the absence of long-term research projects or programs in Chad. Therefore, it is no surprise that the National roadmap for food system transformation (République du Tchad, 2021) calls for significantly increasing the resources allocated to agricultural research and extension.
The analysis of *affiliation countries* suggests that Chad is the most active country in the research field with 24 articles, accounting for 25.53% of the total. However, this also means that about three-quarters of the selected papers (74.47%) dealing with Chad do not involve

local scholars. This, in turn, suggests that the research field in the country may have some weaknesses. The prominent affiliation countries are mostly located in Europe (e.g. France, Italy, England) and Northern America (e.g. USA, Canada).

The 94 selected documents have been authored by researchers and scholars affiliated with 217 *organizations*, but most are based outside Chad and even Africa. Many notable organizations working in the research field are based outside of Chad, with a significant presence in Europe (e.g., *Centre National de la Recherche Scientifique* – France, INRAE – France, *Université de Montpellier* – France, *Institut de Recherche pour le Développement* – France, University of Milan – Italy, CIRAD – France, European Commission Joint Research Centre, Institute of Tropical Medicine – Belgium, Lund University – Sweden, Ghent University – Belgium), Northern America (e.g., Johns Hopkins University – USA, *Universite de Montreal* – Canada, Tufts University – USA) and, to a lesser extent, in Africa (e.g., University Joseph Ki-Zerbo – Burkina Faso, University of Ngaoundéré – Cameroun, *Institut Togolais de Recherches Agronomiques* – Togo). Prominent organizations also include some international ones such as the Food and Agriculture Organization of the United Nations (FAO), CGIAR (Consultative Group for International Agricultural Research), and ICRISAT (International Crops Research Institute for the Semi Arid Tropics). The top 25 organizations dealing with research on agri-food in Chad include only two Chadian ones namely the University of Ndjamena and ITRAD (*Institut Tchadien de Recherche Agronomique pour le Développement*). These findings suggest a network of researchers working on agri-food in Chad from different countries. Still, they may also denote a weakness in the country’s agricultural knowledge and innovation system (AKIS).

Table 2. Bibliometrics of the scholarly literature on agri-food research in Chad.

Journals/sources (a*)	Research areas (b*)	SDGs (c*)	Authors (d*)	Countries (e*)	Organizations (f*)
Food and Nutrition Bulletin (3)	Environmental sciences – Ecology (25)	SDG 13 – Climate action (32)	Begin F. (3)	Chad (24)	*Centre National de la Recherche Scientifique* – CNRS (6)
Scientific Reports (3)	Agriculture (12)	SDG 03 – Good health and well-being (29)	Corsi S. (3)	France (21)	FAO (6)
Berichte Uber Landwirtschaft (2)	Nutrition – Dietetics (10)	SDG 02 – Zero hunger (26)	Delisle H. (3)	USA (20)	*Institut de Recherche pour le Développement* – IRD (6)
Cahiers Agricultures (2)	Food science – Technology (8)	SDG 06 – Clean water and sanitation (24)	Marshak A. (3)	Italy (16)	University of Ndjamena (6)
Faseb Journal (2)	Geology (7)	SDG 01 – No poverty (22)	Nilsson E. (3)	Cameroon (10)	CGIAR (5)
Frontiers in Sustainable Food Systems (2)	Meteorology – Atmospheric sciences (6)	SDG 15 – Life on land (12)	Wabyona E. (3)	England (9)	INRAE (5)
Hydrology and Earth System Sciences (2)	Science technology – Other topics	SDG 05 – Gender equality (2)	Atchozou E. A. (2)	Canada (5)	*Université de Montpellier* (4)

Journals/sources (a*)	Research areas (b*)	SDGs (c*)	Authors (d*)	Countries (e*)	Organizations (f*)
	(6)				
Journal of Arid Environments (2)	Public environmental occupational health (5)	SDG 11 – Sustainable cities and communities (2)	Becker P. (2)	Belgium (4)	University of Milan (4)
Science of the Total Environment (2)	Biochemistry – Molecular biology (4)		Birkett C. M. (2)	Burkina Faso (4)	CIRAD (3)
	Pediatrics, Remote sensing (4)		Crétaux J. F. (2)	Germany, Nigeria, China, South Africa, Switzerland (4)	European Commission Joint Research Centre (3)

* Figures in brackets refer to the number of documents/publications by a journal (a), a research area (b), an SDG (c), an author (d), an affiliation country (e), or an affiliation organization (f).

The analysis shows that the most significant *funding agencies* include the European Union, the European Commission Joint Research Centre, the Crafoord Foundation (Sweden), the Leverhulme Trust (United Kingdom), the Swedish Research Council, and the National Natural Science Foundation of China (NSFC). This suggests that a considerable portion of the funding for research on agri-food in Chad comes from outside, mainly from Europe. As a result, this highlights the lack of domestic funding, which poses a risk and may obstruct the development of long-term domestic research programs.

Several of the chosen articles discussing agriculture and food in Chad do not specify a particular *agriculture subsector*. This is particularly true for studies focusing on food security, diets, consumption patterns, and livelihoods. Similarly, articles discussing climate change’s impacts usually do not mention a specific subsector. Articles that discuss a particular subsector usually focus on crop production while animal production/livestock (Elshahawy et al., 2021; Özcelik et al., 2023) and, especially, fisheries are often overlooked. Many of the articles that discuss crop production cover topics such as water scarcity (Neukum et al., 2023; Nkiaka et al., 2024), pest management (Coop et al., 1991), and irrigation (Abdoulaye et al., 2021). They also refer to alternative agricultural systems, such as agroforestry (Foundjem-Tita et al., 2021). The crop production articles analyze various crops, including pearl millet (Naino Jika et al., 2017), sorghum (Naoura et al., 2019, 2023; Rai et al., 1999), sesame (Corsi & Filippini, 2023), cotton (Cherif et al., 2019) and shea (Zhang et al., 2018). Most studies concentrate on staple crops, cereals, and a few export, cash crops (e.g., cotton).

The focus in the *food chain* is mainly on the production stage, but some papers discuss marketing and distribution (Fenn et al., 2021; Moussa & Yilmaz, 2023) and consumption (Gassara et al., 2023; Kang et al., 2023). Meanwhile, processing seems wholly overlooked in the existing literature. Some authors take a broader perspective and cover different stages of the food chain in their analysis, thus referring to value chains (Orsi et al., 2017) or supply chains (Corsi & Filippini, 2023). For example, Ganry (2009) sheds light on the status of the production and consumption of fruits and vegetables in francophone African countries (including Chad) and explores the associated health impacts.

Conclusions

This paper provides a comprehensive overview of agri-food research in Chad by analyzing the bibliometrics of the research field. The analysis reveals that the annual output of articles varies significantly but remains generally low. There has been an increase in the number of publications, indicating an upsurge in interest in research on agri-food in Chad. The number of research areas and SDGs addressed suggests that agri-food research in Chad is multidisciplinary. However, the overemphasis on environmental sciences, at the expense of social sciences and economics, indicates the need for a more balanced and complete approach to exploring agricultural and food systems in the country. Most authors have only one or two articles, which might indicate a lack of consistency in the research field. The analysis of affiliation countries suggests that about three-quarters of the selected papers, despite dealing with Chad, do not involve any Chadian scholar/author. This might indicate the weaknesses of the research field in the country. Many prominent organizations in the research field are based outside Chad, especially in Europe. The top 25 affiliations include only two organizations from Chad: The University of Ndjamena and ITRAD. This result might be considered a further indicator of the weakness of the Chadian AKIS. The literature mainly addresses crop production in agriculture subsectors. The research in crop production focuses primarily on staple crops and cereals. Production is the most-addressed stage of the food chain.

It is of utmost importance to support research on agri-food in Chad to tackle various challenges like food insecurity, poverty, and climate change. This will help to establish a sustainable and resilient agri-food system. Research in this field is also crucial to harnessing the potential of the agri-food sector and transforming it into a key contributor to inclusive growth and sustainable development in Chad. In order to strengthen research on agriculture and food in Chad and make it instrumental for the development of the agri-food sector in the country, it is appropriate to consider many of the recommendations and suggestions provided by scientists and researchers to foster food system transformation in the country (République du Tchad, 2021): establishing a competitive-based research program; encouraging the reform of AKIS to support agroecology and other innovative approaches; promoting the integration of research, extension/dissemination/education/capacity building in an inclusive approach; encouraging transdisciplinary research to ensure healthy, nutritious and sustainable food; supporting applied research and promoting the transfer of innovative technologies; paying attention to innovation platforms serving transdisciplinary research and encouraging mutual exchanges between producers and researchers; and promoting the sharing of mutual experiences between researchers and stakeholders, through the periodic organization of scientific events.

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CIRCULAR AGRI-FOOD SYSTEMS: INTEGRATING NEXT-GEN TECHNOLOGIES FOR ZERO-WASTE AGRICULTURE

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Abstract

The increasing focus, on agriculture has led to the adoption of farming methods like technology, bioreactors and precision farming to achieve zero waste objectives. This research delves into how these technologies are incorporated into systems and their impact on sustainability and environmental results. By examining existing literature and surveying 120 companies the study reveals insights into the effectiveness, obstacles and economic implications of these innovations. The results indicate that these systems could slash waste by 40% and boost resource efficiency by 50%, although initial expenses and the demand for expertise pose challenges. The study highlights the potential of these systems in promoting sustainability while stressing the importance of investments in education, policy adjustments, and stakeholder engagement. Overcoming these hurdles is crucial to empower systems to enhance practices.

Keywords: *Circular Agri-Food Systems, Zero-Waste Agriculture, Next-Generation Technologies, Sustainability, Resource Efficiency.*

Introduction

In agriculture, we find ourselves at a juncture on the path to progress, where age-old practices must adapt to keep pace with the ever-evolving needs of a dynamic world. Embracing cutting-edge technologies has never been more imperative. Circular agri-food systems present a game-changing strategy emphasizing self-sustaining processes that recycle and repurpose resources. By integrating tools like intelligence, bioreactors, and precision agriculture techniques, these systems strive for zero waste while significantly boosting sustainability efforts and minimizing environmental impact. This shift goes beyond advancement; it resonates with global sustainability goals, especially in conserving our environment and optimizing resource efficiency.

Central to farming are principles rooted in recycling, reusing, and efficient resource management. These principles establish a closed-loop system that minimizes waste generation and maximizes resource utilization. This approach directly addresses agriculture's challenges, from effective waste disposal to addressing resource scarcity and combating environmental harm stemming from conventional farming practices.

Implementing cutting edge technologies, into these systems offers a way to greatly enhance farming methods ensuring they are efficient and eco-friendly (Pearce, D. W., & Turner, R. K., 1990). This research delves into the framework of agri food systems emphasizing their potential to revamp landscapes into more sustainable and productive settings. The study highlights the role of following gen technologies in propelling these transformations. Provides practical approaches to boost the effectiveness and sustainability of farming practices (Sauvé, S., Bernard, S. & Sloan; P., 2016). Furthermore, it examines the impacts of these innovations on development, community welfare, and environmental conservation. By analyzing instances from regions, the study showcases the broad relevance and advantages of embracing circular agricultural models (Ellen MacArthur Foundation, 2019).

The imperative to shift towards systems is emphasized by the increasing global food demand, the severe effects of climate change, and the urgent necessity to preserve natural resources. These systems tackle both economic dilemmas while paving the way for future farming methods that are sustainable and technologically progressive (Food and Agriculture Organization of the United Nations [FAO] 2019).
This article summarizes these systems and supports their use as an important step toward promoting sustainable farming practices and enhancing rural growth.

Materials and Methods

Data Collection and Sampling

Review of Existing Data: We conducted a thorough review of existing literature, focusing on sources such as academic journals, industry reports, and white papers published between 2015 and 2023. Key publications from journals like the Journal of Sustainable Agriculture, Renewable Agriculture and Food Systems, and Technology in Society were specifically targeted. Our search spanned multiple databases, including Scopus, Web of Science, and Google Scholar, using keywords such as "agriculture," "agri-food systems," and "technology in agriculture." This review was crucial in identifying trends in innovative technologies and highlighting areas where circular principles are underutilized in agri-food systems.
Gathering Primary Data: Primary data collection involved distributing questionnaires to 120 agricultural business leaders and technology experts across Northern and Western Europe, regions known for their pioneering efforts in sustainable agriculture. Participants were carefully selected based on their active involvement in projects focused on waste reduction and the enhancement of sustainable practices. The sample size was determined to ensure representativeness and statistical robustness in our analysis.
Questionnaire Design: The questionnaire was meticulously designed to capture a broad spectrum of information:

1. Demographic Information: This section included questions on age, educational background, and professional roles within their organizations.
2. Implementation of Technologies: Participants were asked about the tools and technologies they employ, such as eco-friendly materials, automated waste sorting systems, and AI-powered resource management solutions.
3. Adoption of Circular Practices: This section explored the extent to which participants had implemented circular practices, including regenerative farming techniques, renewable energy usage, and closed-loop water systems.

Facilitators and Barriers: Participants identified key barriers to and opportunities for embracing circular economy principles, along with factors supporting these practices' integration.
Evaluation of Impact: The questionnaire also assessed the environmental and social impacts of adopting circular practices, measured through key performance indicators (KPIs) such as waste reduction rates, energy conservation, and return on investment (ROI) in sustainable technologies. The questionnaire was pilot-tested with a small group to ensure clarity, relevance, and effectiveness in capturing the necessary data.
Data Analysis Methods: Quantitative data were analyzed using SPSS software to perform various inferential statistical tests, including chi-square tests, t-tests, and regression analysis, to identify correlations and trends related to adopting technological innovations. Qualitative feedback was analyzed using NVivo software, involving data coding, theme identification, and validation to extract deeper insights into the challenges and strategic approaches within circular agricultural food systems.

Results and Discussion

Integration and Use of Technology: The section on Results and Discussion highlights the integration and utilization of technology in practice. The survey data shows an incorporation of technologies in agricultural operations, indicating a growing emphasis on sustainable methods. Tools like precision agriculture equipment and bioreactors promote sustainability, with most participants supporting these innovations. This aligns with the findings of Refsgaard, K., and Magnussen, K. (2021), who observed an increasing adoption of technologies as drivers in modern agriculture.

Notable Technologies Used:

1. Bioreactors for Organic Waste Composting: Bioreactors, utilized by 70% of respondents, are essential for converting waste into compost, thereby reducing waste from agricultural activities. The use of bioreactors not only aids in waste reduction but also boosts soil health by providing nutrient-rich compost. This discovery is consistent with Tonini, D., Martinez Sanchez, V., & Astrup, T. F. (2021), who emphasized the effectiveness of bioreactors in waste management.

2. AI-Driven Systems for Resource Management: AI-driven systems have been adopted by 65% of surveyed farms to optimize water and nutrient usage, ensuring resource allocation and minimal wastage.

These systems play a role in precision farming, where making informed data-based decisions can significantly enhance productivity and sustainability. Klerkx, L., Jakku, E., & Labarthe, P. (2019) also highlight the growing significance of AI in optimizing methods.

Economic and Environmental Impact: Decrease in Waste and Resource Consumption: The enhanced processing techniques enabled by these technologies have resulted in a 40% decrease in waste production. Moreover, 80% of participants have reported a 50% energy and water efficiency enhancement, highlighting the effects of embracing these technologies. These outcomes align with the research conducted by Geissdoerfer, M., Savaget, P., Bocken, N. M. P., & Hultink E. J. (2017) and Song, M., Fisher, R., & Wang, J.L. (2021) which also observed enhancements in resource efficiency and waste reduction through integration.

Financial Performance: Sixty percent of companies have seen increased profitability due to reduced resource costs and the ability to command prices for eco-certified products.

The economic feasibility of these technologies is further backed by Bocken, N. M. P., & Geradts, T. H. J. (2021), who argue that embracing practices can result in financial gains by tapping into the growing demand for eco-friendly products.

Challenges in Implementation:

1. Initial Costs: 75% of participants highlighted the considerable upfront expenses associated with implementing technologies as a barrier to widespread adoption. Pieroni, M. P., McAloone, T. C., & Pigosso, D. C. A. (2019) also address the challenges posed by these technologies, suggesting that the high initial costs could deter businesses from embracing them.
2. Skill Shortages: 65% of farm managers pointed out a significant issue as a lack of workers with the technical skills to manage these advanced systems effectively. This skills gap is challenging since implementing these technologies hinges on specialized expertise and training. Goldsmith, P. D., & Martin, L G. (2021) stress the importance of bridging this gap to capitalize on the benefits of advancements in agriculture.
3. Regulatory Obstacles: 50% of respondents identified inconsistent regulations as a hindrance to the adoption of technologies. The absence of a framework creates

ambiguity and may deter investment in new technologies.
Smith, L., Cubbage, F. & Balmford, A. (2020) suggest that aligning regulations across regions is crucial to promote the adoption of sustainable farming methods.
Trends in Consumer Behavior and Market Dynamics: Shifting Consumer Preferences: Many companies have noticed increased consumer demand for goods produced through practices. This trend reflects a move towards sustainability, where consumers prefer products that resonate with their environmental values. According to Luchs, M. G., Brower, J., & Chitturi, R. (2021), this heightened consumer awareness allows businesses to set themselves apart.
Market Expansion: About half of the companies surveyed have entered markets by leveraging their commitment to sustainability. This expansion highlights the possibilities of agricultural and food systems as companies seize on the increasing desire for sustainable goods to enter and succeed in fresh markets. Zhu, Q., & Dou, Y. (2020) also stress the importance of sustainability credentials in supporting market expansion and gaining an edge.

Conclusions

Moving towards food systems integrated with state-of-the-art technologies offers a holistic approach to addressing various sustainability issues in modern farming. Embracing these advancements can significantly boost the sustainability of the agriculture sector, leading to an environment and a secure food supply for the future.

Suggestions

1. Strategic Investments: Essential investments in technology, training, and infrastructure are vital to harnessing the potential of agricultural and food systems.
2. Policy Support: Governments and international bodies should implement policies that promote practices while offering technical and financial assistance to farmers.
3. Stakeholder Engagement: The active participation of communities and stakeholders in designing and implementing food systems is crucial to ensuring that these innovations meet needs and gain widespread acceptance.
4. Continuous Research: Continuous research into enhancing bioreactor efficiency, AI-driven resource management, and other technologies is vital to keeping up with trends and ensuring the resilience and adaptability of agricultural and food systems.

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ANALYSIS OF PRODUCTION AND EXPORT OF MONTENEGRIN WINE WITH PARTICIPATION OF SMALL PRODUCERS

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Abstract

The importance of the research of wine is imposed due to the fact that it is one of rare products which has been on the list of export agricultural products of Montenegro for many years. Wine production traditionally represents an important segment of Montenegrin economy as it has, in addition to North Macedonia, among Western Balkan countries, a positive foreign trade exchange balance. Subject of this paper is the analysis of production, import and export of wine in Montenegro for the period 2016-2021, with the analysis of share of small wine producers. Biggest import countries of Montenegrin wine are presented as well as biggest wine export countries whose wine is imported in Montenegro. Data in the paper show that, apart from Montenegro registering surplus in wine trade exchange for many years, the share of small wine producers is small. Apart from production volume, small wine producers are faced with various problems and challenges, most prominent one being the sales of wine on domestic as well as international markets (export). This is a common problem typical for other small wine producers in Western Balkan countries. Through the survey, it has been determined that majority of Montenegrin small wine producers sell their products on domestic market in their vineyards.
The objective of the paper is to determine the reason for lower representation of small wine producers in the export and to point out to the possibility of their larger promotion on both domestic and international markets considering that this product has had a considerable share in foreign trade exchange for many years resulting in significant financial influx.

Keywords: *export, import wine, production, small wine producers.*

Introduction

The world grape and wine market is exceptionally developed and highly competent, and apart from European countries, there are so-called "new wine" countries that are considerably represented. Based on OIV (2022) data, Italy had the most extensive wine production in 2017–2021, with an average production of 48,8 million hl annually. France was second with an average annual production of 42,4 million hl, whereas Spain was third with an average annual production of 37,4 million hl. These three countries had 47.4% of the world's total wine production in the period mentioned. These countries are also at the top of the list regarding world wine exports.
Due to favourable climatic conditions and geographic position, Montenegrin viticulture and wine production has a long tradition and is based on autochthonous types Vranac and Kratošija (Ulićević, 1966; Pejović, 1988). In vineyard structure in Montenegro, dominant autochthonous types for producing white wine are Chardonnay and Krstač, whereas main grape types for producing red wine are autochthonous types Vranac and Kratošija (Pajović-Šćepanović et al., 2016)
In last few years, apart from the increase in number of Vranac wine producers, there were a lot of investments in modernization of wine production technologies which resulted in

increase of Vranac wine offers (Pajović et al., 2013). Grape and wine production has a long tradition in Montenegro. Its potential is not used to its utmost considering that a large number of small wine producers exists in this agricultural production segment (Perović et al., 2024). Research on distribution of wine products as well as their promotion with the emphasis on small and medium producers were carried out by the following authors (Perović et al., 2024; Savić, 2011; Jelić et al, 2020; Simonović, 2019).
Out of total wine production and export of Montenegro, over 95% is done by big wine producers, whereas small wine producers’ percentage is low. The significance of this research is that the wine as agricultural product has been on Montenegrin list of export products with excess in foreign trade exchange. The subject of the research is the analysis of grape and wine production, import and export for the period 2016-2021.
The aim of this paper is to research and point out to the way small wine producers in Montenegro can improve and increase the distribution of their wines both on domestic and international market.

Materials and methods

The paper analyses vineyard areas of Western Balkans with the emphasis on Montenegro, grape and wine production, import and export as well as the overview of the biggest countries into which Montenegro exports its wine as well as overview of countries from which Montenegro mostly imports wine for the period 2016-2021.
For the research, secondary data used are from the National Statistics Bureau (MONSTAT, 2022), State of the world wine and wine sector 2021. (OIV 2022), Report for the viticulture and wine sector in the Western Balkans: 2022: Standing working group for regional rural development (Basha et al., 2022), as well as the overview of relevant literature with application of standard statistical and mathematical methods. The most important changes are presented in tables and graphs.

Results and discussion

Western Balkans - Wine Production
The location of the Western Balkans on the trade routes between Central Europe and Asia Minor contributed to the spread of grapes along the main trade routes (Hudelson and Promises, 2014). Subsequently, a new generation of Balkan winemakers has strengthened small-scale production, paying more attention to quality and expression (Abramishvili, 2023). The Western Balkan wine region includes Albania, Bosnia-Herzegovina, Kosovo, Montenegro, North Macedonia, and Serbia.
By the available data from 2020 study, in Western Balkan, the total area under vineyards is 71,004 ha (Figure 1), and the average annual grape quantity is 682,632,429 kg (Figure 2), i.e. 136,023,071 litres of wine (Figure 3) (Bare et al., 2022).

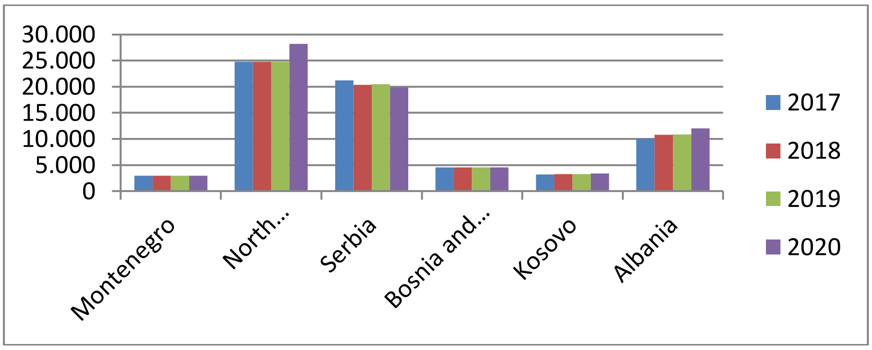

Figure 1. Total area under vineyards (ha) of the Western Balkan countries

Further, this study shows that in the observed period 2017-2020., out of all Western Balkan countries, Northern Macedonia has the highest grape production. In 2020. it is 244,603,000 which is 34.95% out of total grape production in the Western Balkan countries (Figure 2) (Bare et al., 2022).

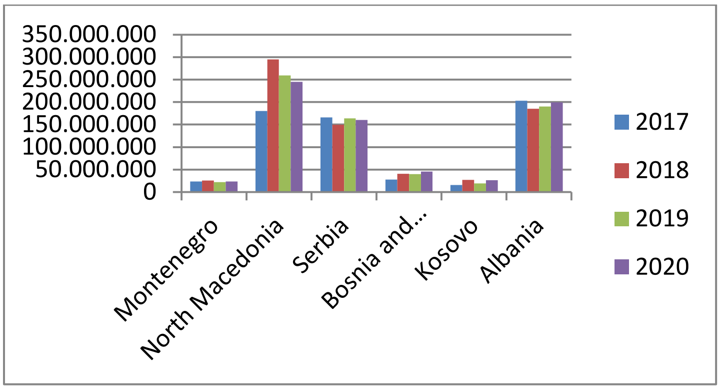

Figure 2. Total produced grapes (kg) of the Western Balkan countries

When total wine production is concerned, for the observed period 2017-2020. (Figure 3), the same study shows that slightly more than half (58%) out of total wine production in Western Balkan countries, the biggest production is in Northern Macedonia. Remaining 42% of total production is in remaining Western Balkan countries. Montenegro, for the same period, with its 7.5% or 10,205,950 l of total wine production has the third place. (Bare et al., 2022).

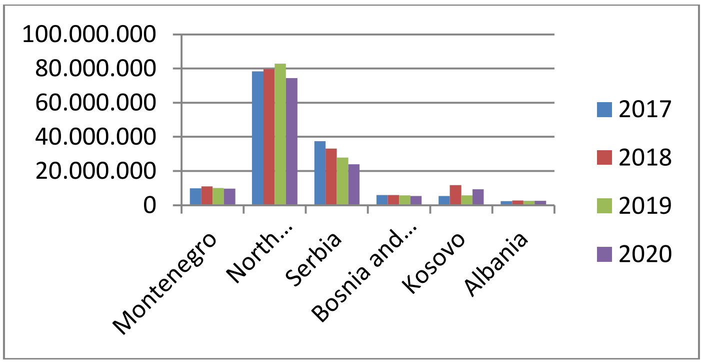

Figure 3. Total produced wine (l) of the Western Balkan countries

Wine Production in Montenegro

The sustainable development of wine tourism in the Western Balkans and Montenegro was due to wine production that depended on the presence of varied temperatures, isolated valleys, different soil types, and orography, which created a variety of habitats that, combined with its historical and geographical background, contributed to the development of a large variety of grapevines (Maraš et al., 2014; Maraš, 2019; Maraš et al., 2020).

As already mentioned, Montenegro has a long tradition in vine cultivation and wine production which dates to B.C. ages. This is in large a result of favorable climatic and land conditions typical for coastal area and Skadar Lake basin. In Montenegro, total area under vineyards until Second World War was 1,145 ha (Pejović, 1988). Since then up to today, Montenegro registers a considerable increase of vineyard area as well as the trend of increase of wine production with small producers (Pajović-Šćepanović et al., 2016). Precisely this increase of vineyards and grape production in last few decades resulted in the increase of types and numbers of Montenegrin wines. Thus, for production of most famous Montenegrin

white wines, there are autochthonous types Krstač and Chardonnay, whereas for red/black wines, these are Kratošija and Vranac.
According to Monstat (2023), for 2022 (Table 1), the total area of vineyards in 2021 was 2.834,9 ha, of which the production area was 2.787,3 ha. Grape production for the observed period (2016–2021), depending on meteorological conditions in a specific year of production, was at an overall minimum in 2019 of 21.865t and an overall maximum in 2016 of 28.925 t. In 2021, total grape production was 24.405,7t, approximately 7.5% more than in 2020, or 1.3% more than the 6-year period average (2016–2021), which was 24.091,6 t.

Table 1. Grape production in vineyards

Year	Total area ha	Producing area ha	Number of producing vines	Total production t	Production yield t/ ha	Production yield in kg per vine
2016	2860.4	2781.9	11010517	28925.4	10.4	2.6
2017	2850.0	2804.2	10634011	22201.9	7.9	2.1
2018	2837.9	2790.2	10629873	24440.6	8.8	2.3
2019	2880.0	2825.4	10614620	21865.0	7.4	2.0
2020	2888.0	2831.9	10884796	22711.1	7.5	2.0
2021	2,834.9	2787.3	10563430	24405.7	8.3	2.2

Company Plantaže AD Podgorica confirms its leadership position in the region with its production of approximately 22 million kg of wine and table grapes per year, as well as with annual sales of more than 16 million bottled products in more than 40 countries worldwide. Their vineyard in Ćemovsko polje is declared the largest vineyard in one complex in Europe, with 2310 ha of plants in one complex and approximately 10 million vines (13. Jul Plantaže, 2023). The remaining production comes from smaller producers.
For years, one of the most significant agricultural products exported in Montenegro has been wine. The percentage of wine in total exports is 3.3%, whereas in total exports of agricultural products it is 25.8%. According to Monstat data as of 2022 (Table 2), the value of exported wine in 2021 was 12.217.662 €, which is 11.8% more in comparison to 2020, whereas the obtained export value for 2021 in the amount of 7.695.327€ is higher by 30.6% in comparison to the previous year when it was 5.891.659€.

Table 2. Overview of foreign trade exchange of wine for the period 2016-2021.

Foreign trade exchange balance for wine in the period 2016-2021 (in €)						
Foreign trade exchange	2016	2017	2018	2019	2020	2021
Total exports	14.804.591	13.863.202	13.235.033	13.631.493	10.928.434	12.217.662
Total import	5.353.837	5.835.499	6.915.058	7.029.170	5.891.659	7.695.327
% coverage	276,5%	237,6%	191,4%	193,9%	158,5%	158,8%

Considering all the analysed years, the lowest export value was in 2020, when the entire world was impacted by the crisis caused by COVID-19, which temporarily resulted in decreased exports in Western Balkan countries and worldwide (OIV, 2022).
The significance of wine research is also because it was continuously on the list of exported agricultural products in Montenegro, according to which there was an influx of specific foreign currency (Perović, 2013).

Wine is a product with a foreign trade exchange surplus, i.e., year by year, the value of export wine is almost double that of imported wine. In the last two years covered by the analysis, there was the lowest foreign trade balance. In contrast, in 2021, coverage of imports by export was 158.8%, which, except for 2020, was the lowest in the observed period, and the imported value of the wine was the highest in the last observed year (Table 2).
Table 3 presents the most significant export countries for Montenegrin wine, whereas Table 4 presents the largest wine import countries to Montenegro from 2016–2021. These tables show that Western Balkan countries are considerably represented among the most significant wine producers, such as France, Italy, and Germany.

Table 3. Overview of the largest export countries for Montenegrin wines in the period 2016-2021 (in €)

Countries	2016	2017	2018	2019	2020	2021
Serbia	6.580.704	6.507.365	6.358.185	6.378.180	4.431.220	5.848.135
Bosnia and Herzegovina	2.357.881	2.802.694	2.230.366	2.651.835	3.302.592	2.169.957
China	1.340.142	1.765.805	1.833.416	1.519.039	1.205.770	1.672.168
Kosovo	839.645	786.737	836.902	825.163	503.401	633.740
Germany	358.727	279.492	320.961	346.331	248.579	298.128
Russia	1.660.434	50.811	-	11.025	11.060	-
Other	1.667.058	1.670.298	1.655.203	1.899.920	1.225.812	1.595.534
Total	**14.804.591**	**13.863.202**	**13.235.033**	**13.631.493**	**10.928.434**	**12.217.662**

Table 4. Overview of the largest wine import countries in Montenegro in the period 2016-2021, (in €)

Countries	2016	2017	2018	2019	2020	2021
Serbia	1.693.773	1.770.887	1.738.481	1.694.057	1.240.932	1.411.970
Croatia	982.971	902.711	1.082.980	929.935	789.975	840.364
France	784.037	791.631	1.064.582	1.148.132	1.493.694	1.645.366
Italy	785.241	795.333	1.015.836	1.444.358	1.289.619	1.631.159
Northern Macedonia	755.463	1.139.336	1.190.493	1.200.368	632.013	1.554.553
Other	352.352	435.601	822.686	612.320	445.426	611.915

Conclusion

As already mentioned, the biggest grape and wine producer in Montenegro and one of the biggest in Western Balkans region is the winery “13.jul Plantaže” AD Podgorica. In terms of export, this company is also in the top with 95% of the total export with the remaining percentage being small and medium producers. According to the research and the findings of the authors (Perović et al., 2024), when considering the wine export of small producers, out of the total number of interviewed, only 24,2% export their wine and only 10-20% of its total annual production. One of the reasons they gave for lower export is the small quantities they produce which in turn is mostly sold under their own brand in their own wineries. They also cite an insufficient and limited budget for the promotion of their wines as one of the reasons. Precisely because of this, small wine producers in Montenegro should more use of those promotional activities (digital marketing) that require smaller financial resources and give greater results in placement.

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PERCEPTUAL ANALYSIS OF MOUNTAIN ZONE GOVERNANCE: CASE OF THE TICHOUKT MASSIF - MIDDLE ATLAS, MOROCCO

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Abstract

This article studies the cognitive aspects which guide public and private actors in the conception of development plans for mountainous areas in Morocco. Our ambition is to understand how local actors perceive the specificities of the development of mountainous areas in Morocco. We used a combined method to collect and analyse data in the Tichoukt massif; a mountainous area located in the Moroccan Middle Atlas. The results of the strategic analysis using the Mactor method were submitted to a panel of experts for confirmation (Delphi method). Our results suggest that the absence of a reference framework that guides local actors in the conception of development plans for mountainous areas of Morocco hinders the convergence of collective interventions. The complexity of the problem is linked to the difficulties inherent in evaluating the cognitive convergence of multisectoral interventions. The overlapping of objectives, the multiplicity of interventions and the fragmentation of their areas are limiting factors. This study is a contribution to the study of development problems in rural areas in Morocco. The results obtained can be used to deepen investigations into the cognitive factors of the governance of public policies in developing countries.

Keywords: *Territorial governance – Mountainous areas – Local actors – cognitive framework – Tichoukt massif.*

Introduction

Advanced regionalization represents an operational response to the disparities that have characterized Morocco's development since independence, as well as to the limitations of top-down approaches to social and economic policymaking. The territorial approach aims to ensure that public policies are better tailored to the specific needs of each territory, by establishing new conditions likely to involve local stakeholders in the development of their living space (Pecqueur,2014).

Morocco's mountainous regions represent a paradoxical situation. On the one hand, high levels of poverty coexist. On the other, the presence of potential for transformative prosperity. Because of their specific geographical features and the scale of the disparities that characterize them, these areas are at the top of the agenda for Morocco's political decision-makers[2]. Moreover, the specific nature of these difficult areas, which need to be addressed, calls for the mobilization of knowledge, the use of adaptive approaches, the design of opportunities and the finding of special means. Directly through the territorialization of public policies, through this reflection, we want to apprehend the cognitive aspects that orient actors in the governance of mountainous areas in Morocco. Our objective is to understand how these actors perceive the specificities of the development of mountainous areas in Morocco.

[2] Economic, Social, and Environmental Council Report. "Rural Development: Space of Mountainous Areas." Rabat, Morocco (2017).

As a case study, we chose the Tichoukt massif, based on its characteristic features of mountainous areas in Morocco. This geographical area is subject to the same paradox of all mountainous areas in Morocco, namely the presence of a poor population in an environment rich in natural and cultural resources[3]. Indeed, poverty rates are high compared to national and regional averages with strong manifestations of vulnerability and exclusion at all levels[4].
We adopt an interpretivist approach structured in two phases. The aim is to understand and interpret the behaviours of actors involved in governance in Morocco's mountainous areas. In other words, what are the factors, reasons and intentions behind a given decision. We began with a prospective analysis, based on the "Mactor Method", of the positions of the actors in our field of study, questioning their perceptions of the problems of the massif, the effectiveness of the actions undertaken and the prospects for the development of these specific areas. The proposals formulated were then submitted, in the form of a questionnaire, to a panel of experts for confirmation by using the confirmatory Delphi method.

Materials and methods

Our study is divided into two interdependent phases following an exploratory approach, which is warranted by the complexity of our issue. The objective is to enhance our comprehension of the existing connections between the cognitive frameworks of territorial governance stakeholders and the development of Morocco's mountainous regions. To achieve this goal, we conducted a strategic analysis of stakeholder interactions in our research area using the Mactor method. Subsequently, we presented the obtained results as a proposal to experts for validation, aiming to identify both convergences and divergences through the Delphi Argument Method.
The aim of the first phase of our work is to explore local stakeholders' perceptions of the specificities of the development of the Tichoukt massif, highlighting convergences and divergences in the positions of stakeholders. The choice of an actor-based approach as the unit of analysis (structures are represented by their respective managers) is a particular response to the composite nature of our research object. The territorial governance being a field of interaction in which the objectives, strategies and actions of actors condition the coherence of their joint actions. Based on their roles in local governance, we were able to identify the most influential actors based on the municipalities' organic laws[5] and an interview with the presidents of the municipalities of Boulemane, Sekoura and El Mers. Following this, in-depth research on the selected stakeholders enabled us to determine their objectives and the structure of their interactions.Once the stakeholders had been identified, a questionnaire accompanied by an institutional letter was sent to them. In practice, the questionnaire is perfectly suited to collecting quantitative data from large samples whose individuals have divergent objectives, interests, and strategies. At this level, it is important to point out that while the quantitative approach is commonly linked to verification, the questionnaire can also serve as an exploratory tool under specific conditions (Thiétart, 2014)[6].In our case, we proceeded in two stages, following a sequential analytical logic. First, we opted to explore the perceptions of local stakeholders directly involved in the governance of mountain development policies, before submitting the results of our survey to a panel of experts in the field of mountain development in Morocco. Based on our exploratory phase, we formulated an initial list of proposals that was submitted to the panel of experts to assess the levels of

[3] Economic, Social, and Environmental Council Annual report,2015 on https://www.cese.ma/docs/rapport-annuel-2015/.
[4] The poverty rate at the level of the massif is 15.5%, it reaches 32.5%, or three times the national average in the municipality of Elmers, followed by Sekoura 11.9%, while it does not exceed in the Boulemane region 1.9% (HCP, 2014).
[5] Organic Law No. 111-14 relating to regions, No. 112-14 relating to prefectures and provinces and No. 113-14 relating to municipalities.
[6] This mainly involves ensuring that the questions formulated guarantee a large margin of freedom in the reaction of the targeted population. This is why the use of scaled and semi-open questions is often recommended.

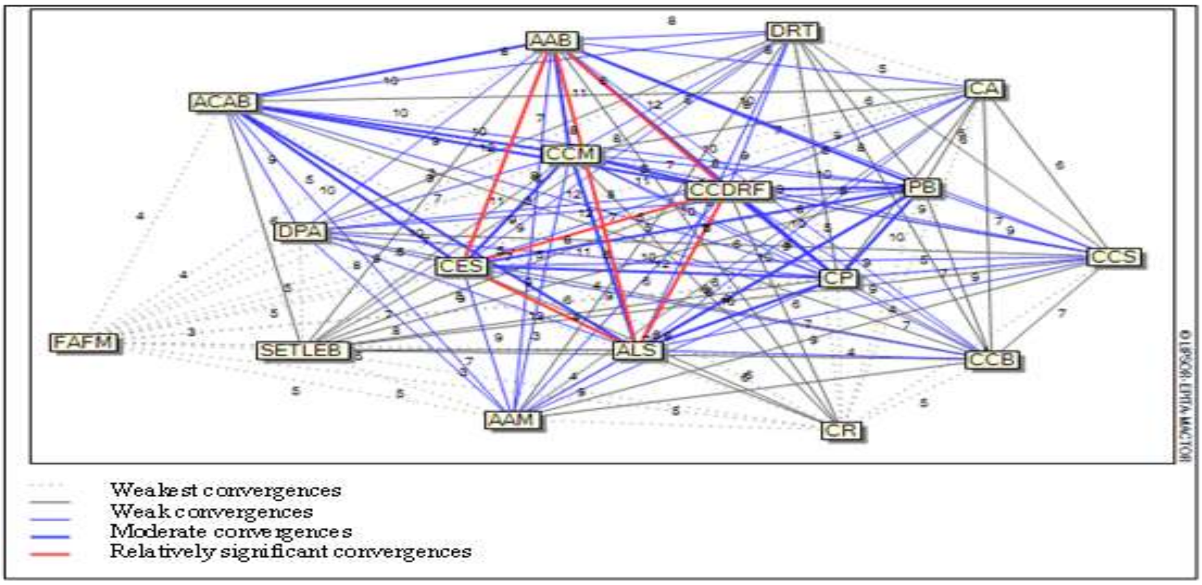
convergence of positions on a "five-level Likert scale" (Cherkaoui, a., & haouata, 2017). This list contains affirmative and prospective questions formulated to provoke debate and produce responsive arguments or opinions. After designing the questionnaire, we proceeded with three rounds of data collection by sending the questionnaires in Word format to the designated experts with a response time of two to three days.

Results and discussion

The results of our exploratory study with local governance actors in the Tichoukt massif and our consultations with a panel of experts fill the need to appreciate the coherence of the cognitive framework of territorial governance actors and to apprehend its impact on the development of these areas of specific character. To better assess the actual mobilization of stakeholders around the objectives, we analysed the results of the matrix (3MAO), which enables us to better visualize the real or effective importance of each stakeholder by integrating the balance of power between stakeholders. Actor/objective analysis considers the weighted evaluated positions and the number of consensuses on each development objective. As shown in the figure below, stakeholder mobilization rates around the massif's development objectives are relatively high.

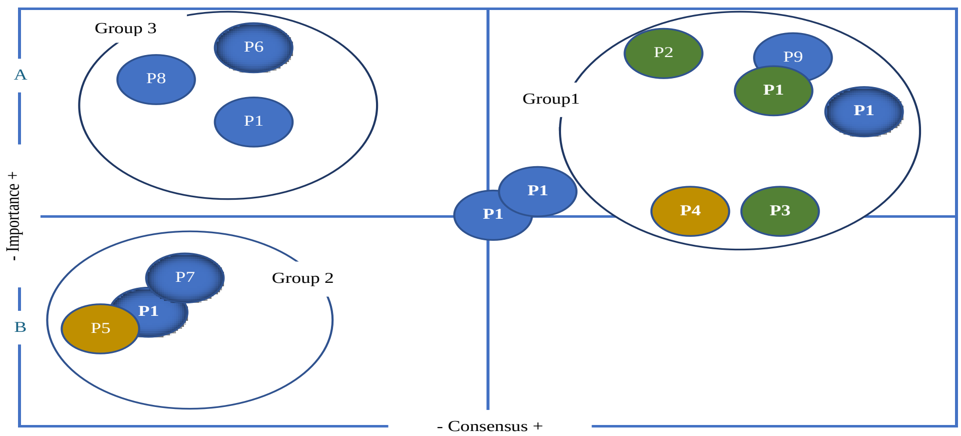
The results show that the institutions formally involved in all the objectives are in fact at relatively moderate levels of mobilization. While the design and implementation of the regional development program (PDR) guides local actors in the design of the CAP, their levels of mobilization across all objectives remain low. In addition to this, the actors with high levels of mobilization either do not have an influential formal position in territorial governance or are sectoral institutions whose objectives are linked to their respective areas of specialization. This is the case for representatives of civil society, the Chamber of Crafts, or the Provincial Department of Agriculture.The matrix of simple convergences provides us with a description of the structure of institutional stakeholder interactions by visualizing the number of consensuses on the massif's development objectives. While the sum of consensus for each stakeholder reflects the weight of the dominant actors within the system of joint action, analysis of the matrix data for each pair of actors has more important connotations.

Figure 1: Graph of simples' convergences (1 CAA)



In practice, the graph of simple convergences (1CAA) enables us to present, for each pair of stakeholders, the number of objectives on which the stakeholders express the same positions (for or against), i.e. the number of potential alliances within the territory studied. By simply reading the graph above, we can distinguish five levels of convergence, from the strongest to the weakest. The results obtained (Figure 1) confirm the existence of low levels of convergence of positions between the main Actors in governance at massif level, mainly between the Regional Council and the Province and Provincial Council, bearing in mind that the latter two play a decisive role in coordinating actions at territorial level. This is also true of interactions with external sectoral services. In addition, we note the low levels of convergence between representatives of civil society, a sign of the lack of integration of the roles of these institutions despite the large number of associations and cooperatives in the massif, which are supposed to play the role of mediator in transferring the demands and needs of residents to decision-makers .At the massif level, it is advisable to take into account the balance of power between stakeholders and the structure of their interactions. Once we had processed the expected questionnaire data in the second phase of our empirical approach, we centralized them in a single file for comparative purposes. The figure below (Figure 3) shows the results of our survey of experts on the development of Morocco's mountainous areas, according to the degree of importance of the proposals submitted and the degree of convergence of the positions identified.

Figure 3: Result of the survey among the experts surveyed



Based on the experts' positions on the proposals put forward and the analysis of their comments and suggestions, we can distinguish three homogeneous groups according to the criteria explained above:

● Group 1: The experts' positions converge on eight proposals qualified as important to very important. Firstly, the participants in our survey are in complete agreement on the high territorial resource potential of Moroccan mountain regions, on the low levels of cognitive convergence among local governance Actors, and on the shortcomings of coordination mechanisms between the decentralized services of sectoral departments. There is also agreement on the low levels of human development indicators in these difficult, high-potential areas, which have not received the same attention in the country's development policies, and on the malfunctioning of local coordination and participation mechanisms, which have a direct impact on the population of these geographical areas with their specific needs. natural resources for subsistence purposes.

● Group 2: Proposals noting the involvement of civil society and the weak position of the municipality as a relay player in the local governance of development strategies in mountainous areas are deemed unimportant and lack consensus.

In commenting, our experts assert that it is the mode of governance adopted that marginalizes the role of civil society, penalized by the lack of conditions favouring its participatory function and reinforcing the convergence of its interventions. In practical terms, territorial governance is a complex system fed by the interactions of public and private Actors, of which civil society is one, with relatively inadequate resources to accomplish its missions.

Conclusion

By way of conclusion, the results of this research have paved the way for us to explore certain dimensions of territorial development in Morocco in the light of the implications of the advanced regionalization plan, which embodies the desire for a transition towards a decentralized approach to correcting spatial inequalities. In principle, this approach encourages local Actors to take greater responsibility for promoting their territories. Regarding the specific features of mountainous areas, the cognitive aspect is decisive insofar as local Actors need to start from a shared diagnosis, with a common definition of problems and needs, to be able to coordinate their actions and design appropriate responses in line with the objectives set. The cognitive aspects defining the specificities of development in their territories. This dysfunction stems primarily from the low levels of involvement of civil society representatives in the design, implementation, and evaluation of development policies for these rural areas. In addition, the lack of formalism in coordination mechanisms has a negative impact on consultation and harmonization processes, aggravating divergences that are not limited to interactions between local political-administrative Actors and civil society representatives but are also observed within these two groupings. At the level of politico-administrative actors, the absence of a reference framework dedicated to the development of mountainous areas in Morocco fuels uncertainties and leads to a disaggregation of local actors' interventions and a waste of territorial resources. In addition to this, other factors inherent in the profiles of elected officials and their logic of action have a negative impact on the apprehension of the constraints linked to these difficult areas. Among others, the heterogeneous nature of the associative fabric and the shortcomings felt at the level of skills and coordination and participation mechanisms mean that the actions undertaken lack coherence.

The results suggest that local development is not simply a matter of decentralizing powers without the financial means to meet the needs of local stakeholders. It is above all a set of mechanisms centred on the relationship that stakeholders forge with their territories. In fact, stakeholders must base their actions on a shared vision of the specificities of their area, which is a framework that clarifies the cognitive aspects of common actions and the mechanisms for their implementation, monitoring, and evaluation.Ultimately, the development of mountainous areas depends on the operationalization of the advanced regionalization plan as a framework for adapting sectoral programs and strategies to the requirements and specificities of each geographical area. A strong coordination between territorial governance Actors can help reinforce the convergence of individual actions.

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DYNAMICS OF OASIS AGRICULTURE IN MOROCCO: BETWEEN INSTITUTIONAL GOVERNANCE AND CLIMATIC CHALLENGES

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Abstract

Oasis agriculture in Morocco, primarily anchored in the practice of date palm cultivation, faces a series of obstacles resulting from various cumulative factors. Among these are the effects of public policies characterized by excessive centralization, which have exacerbated geographical inequalities, while degrading climatic conditions, fueled by contemporary climate change, have brought significant modifications to the socio-economic and environmental landscape of the oasis areas, leading to substantial rural population migrations. In response to this growing deterioration, government authorities have implemented agricultural policies such as the Green Morocco Plan (GMP) and Generation Green (GG), with a territorially tailored approach, aiming to preserve the existing agricultural heritage and promote the emergence of a modern, competitive sector adapted to local realities. A field survey was conducted to assess the impact of the GMP agricultural policy on the socio-economic development of the oasis areas in the Drâa-Tafilalet region, known for its high concentration of oases, covering 88% of its territory. This study also aimed to examine the role of this policy in preserving the oasis ecosystem as a whole. In our survey, a representative sample of 700 farmers specializing in date palm cultivation was selected. The results reveal the persistence of a top-down decision-making pattern, as well as a disconnection between the agricultural policy orientations and the local contextual realities. In light of this disparity, our recommendations urgently highlight the need to strengthen governance mechanisms to effectively coordinate local stakeholders and better address the specific challenges unique to each region.

Keywords: *Phoeniciculture, Agricultural policies, Climate change, Morocco*.

Introduction

In a contemporary context marked by significant climate changes, the planet is experiencing detrimental consequences globally on ecosystems, particularly harmful in fragile areas such as mountains and oases. These regions have undergone environmental degradation, altering biodiversity and impacting local populations (IPCC, 2014; Daly, 2014; Sala et al., 2000). The Drâa-Tafilalet region exemplifies this situation with multiple challenges, including a desert to semi-desert climate, and a centralized institutional management model favoring the development of coastal urban areas at the expense of rural and inland regions, resulting in significant disparities and negative consequences for these territories.

In response to these challenges, Morocco has embarked on a transition from a centralized governance model to a decentralized one (Faguet, 2014; Bergh, 2017), aiming to better address the socio-economic issues of various regions, particularly Drâa-Tafilalet (Jafari & El Moujaddidi, 2016). This transition is supported by large-scale projects such as advanced regionalization, which seeks to promote balanced and sustainable development by taking into account local specificities, notably in the date palm sector, a major economic pillar for the region. Simultaneously, the territorial adaptation of the Green Morocco Plan (GMP) into

Regional Agricultural Plans (RAP) aims to integrate territorial particularities and position agriculture as a driver of regional economic growth (Amiri et al., 2021).
This approach allows for the consideration of regional particularities and fosters more effective and locally adapted agricultural development, given that date palm cultivation holds a predominant place in the agricultural landscape of the Drâa-Tafilalet region, accounting for 90% of the national date production. This crop forms the backbone of oasis agroecosystems, currently generating 60% of the agricultural income in these oases and providing 4 million workdays for over two million people. The average annual production of the region is estimated at over 105,000 tons, contributing approximately 1.84 billion Moroccan dirhams in value added, or 43% of the agricultural gross domestic product (GDP) of the Drâa-Tafilalet region (ROAD, 2024).
This paper will focus on evaluating the impact of the GMP agricultural policy and its territorial adaptation, addressing two main aspects. First, the reconsideration of the centralization of public policies, and second, the analysis of the GMP's contribution to addressing ecological issues by focusing on four essential aspects: soil conservation, adoption of resilient crops, efficient water use, and capacity building for date palm cultivators.

Material and Methods

To reveal the institutional and ecological impact of the GMP project in the Drâa-Tafilalet region, we adopted a quantitative approach based on the Structural Equation Modeling (SEM) method, renowned for its in-depth use in analyzing causal relationships between the relevant variables of our study (Hair et al., 2019). To ensure the robustness of our results and the validity of our model, we performed data purification through Principal Component Analysis (PCA).
The variables selected for our analysis are divided into three distinct spheres. The first sphere includes variables related to the date palm sector, covering all aspects of the value chain, from planning and supply to production, valorization, marketing, as well as advice and support for date palm cultivators. The second sphere encompasses variables related to territorial development, including space structuring, regulation establishment, territorial governance, as well as the valorization and sustainability of the oasis heritage. As a third sphere and mediating variable, we considered the socio-economic level of the date palm cultivators. However, this last sphere was not examined in this work, as it was already the subject of a previous study (Chiabri et al., 2023).
The data required for our study come from two distinct sources: secondary data obtained from the subdivisions of the Regional Office for Agricultural Development (ROAD) of Tafilalet and Ouarzazate, and primary data collected through field surveys. These surveys were conducted over a period of more than four months, from November 2021 to March 2022, covering the four oasis provinces of the region, namely Errachidia, Ouarzazate, Zagora, and Tinghir. Given the substantial number of date palm cultivators in our study area, totaling 62,472 individuals, sampling was conducted to ensure data representativeness across the four provinces. The selected sample included 700 date palm cultivators. The data collected from the cultivators initially focused on their identification and living conditions. Subsequently, the study paid particular attention to the agricultural process, allowing an in-depth analysis of the sector from upstream to downstream. Finally, the study focused on the governance of the sector and its expansion within the oasis area.

Results and Discussion

It has been determined that the agricultural policy of the GMP and its territorial adaptation have a positive impact on mitigating the devastating effects resulting from centralized decision-making and harsh climatic conditions in our study area.

The first axis of our research examines the transition from centralization to decentralization, characterized by a participatory approach aimed at engaging all regional stakeholders. Specifically, the Moroccan Interprofessional Federation of Dates, which includes actors from the sector such as cooperatives and economic interest groups, seeks to integrate into a decision-making process that reflects the local and regional realities of the date palm sector.

We have also observed that this transition to decentralization has created a complex network of relationships among actors, where interactions play a crucial role in territorial structuring, particularly through proximity and cooperation among stakeholders. This dynamic is notably evident in the coordination between date palm growers and state agencies. However, while this participatory approach is intended to facilitate collaboration between date growers and territorial state agencies, it remains at a preliminary stage and does not fully meet the expectations of date farmers, as clearly illustrated in the following table.

Table1. The percentage of date palm farmers benefiting from decentralized state services

The relevant decentralized agency	The percentage of date palm growers benefiting from decentralized state services
Regional Agricultural Development Office	37,8%
National Agricultural Advisory Office	16%
National Agency for the Development of Oasis Zones and Argan Tree	5%
National Office for Food Safety	0,6%
Others	0,1%

Source: Author's elaboration based on the questionnaire survey results.

The second axis focuses on assessing the ecological dimension, requiring a thorough analysis of the four aspects previously mentioned: soil conservation, adoption of resilient crops, efficient water use, and capacity building for date palm growers.

Regarding the first aspect, focused on soil conservation, our study reveals that over 42% of farmers have observed significant benefits resulting from the localized initiatives of the Green Morocco Plan (GMP). These benefits include better access to communal lands, maintenance of plots, establishment of wells and hydro-agricultural infrastructures, as well as the installation of solar pumping systems. Such interventions have played an important role in extending cultivated areas within desert regions, which in turn has generated several positive outcomes. These outcomes include effective soil erosion prevention, enhancement of soil fertility, and the creation of a favorable microclimate that supports the well-being of both inhabitants and livestock. This microclimate has proven to be beneficial in mitigating the harsh effects of the desert environment, thereby promoting sustainable agricultural practices.

However, the implementation of improvements in new farms has been limited, with only 4.6% of farmers benefiting from communal lands experiencing such advancements. This limitation is primarily due to the complexity of administrative procedures required for land development, as well as persistent family conflicts regarding the equitable distribution of land shares. These conflicts often stem from historical claims and the intricate nature of land inheritance, which impede collaborative efforts for land improvement.

Additionally, among those who have benefited from communal lands, concerns emerge regarding the current strategies for land management. A major issue is the predominance of date palm monoculture, which poses significant risks to soil health and biodiversity. The traditional three-tier agricultural system, which historically optimized the use of resources through diversified cropping, has been neglected. This traditional system included the cultivation of various crops at different canopy levels, enhancing soil conservation, water retention, and overall ecosystem resilience. The move away from this diversified approach towards monoculture undermines the resilience of agricultural systems, making them more vulnerable to environmental challenges such as diseases and climate fluctuations (Baker et al., 2023).

In terms of existing farms, which account for 85.6% of the cases studied, the rehabilitation of old plots has been undertaken in only 15% of these farms. This low rate of rehabilitation is attributed to several factors. One major factor is the predominance of small-sized plots, with nearly 73.3% of these plots being less than 4 hectares, and 46.9% not exceeding 2 hectares. The small size of these plots limits the scalability of agricultural interventions and makes the implementation of comprehensive land improvement plans more challenging. Furthermore, the spatial dispersion of these small plots adds another layer of complexity, as it hinders the coordination and efficiency of rehabilitation efforts. The logistical difficulties in reaching dispersed plots, coupled with limited resources, constrain the ability of farmers to fully benefit from the proposed soil conservation measures and other agricultural enhancements.

For the second aspect, which concerns the adoption of resilient crops, the state has committed to providing vitroplants to 56.6% of date farmers. These farmers have expressed satisfaction with the quality and variety of the plants offered, which have been specifically selected for their resistance to Fusarium wilt. This selection is crucial as Fusarium wilt poses a significant threat to date palm cultivation, and the provision of resistant varieties is a strategic measure to ensure crop resilience and productivity.

However, despite these efforts, more than 30% of farmers have expressed distrust towards the vitroplants provided by the local agricultural development offices (ROAD Ouarzazate/Tafilalet). This distrust stems from past incidents involving inadequate varietal mixes, which have led to complications in cultivation and reduced trust in the provided resources. These incidents highlight the need for increased vigilance and stringent quality control measures by the responsible authorities.

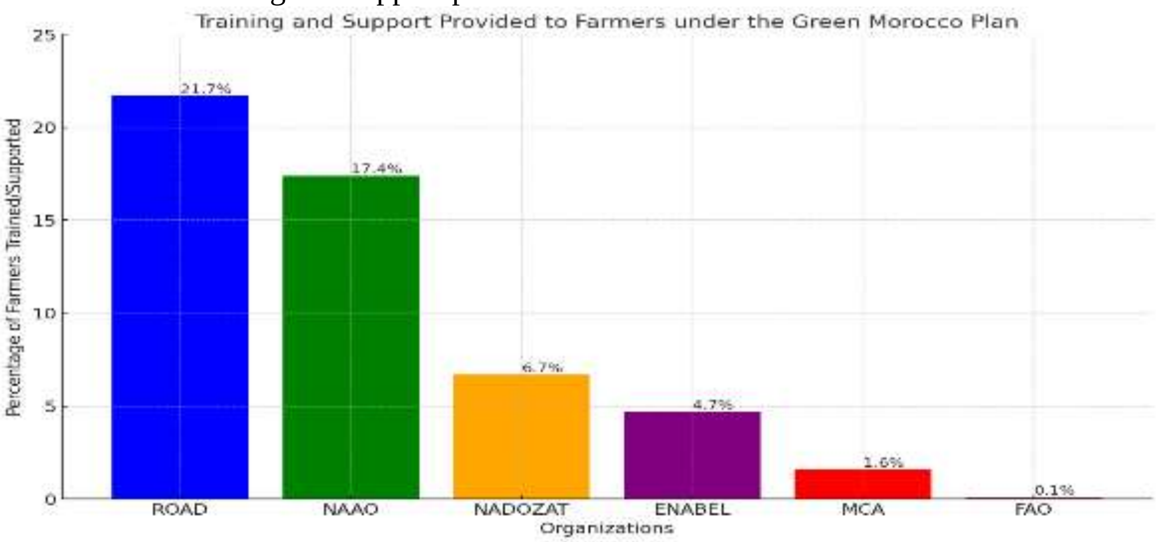
To address these concerns, it is essential for the authorities to implement comprehensive measures. These should include proper identification and labeling of the vitroplants to ensure traceability and transparency. Geographical isolation of different varieties can prevent cross-contamination and maintain varietal purity. Additionally, continuous quality monitoring and control are necessary to uphold the standards and build confidence among farmers. Such measures, recommended by the FAO (2018), are vital for fostering a robust and resilient agricultural system, ensuring that the benefits of the Green Morocco Plan are fully realized and sustained over the long term.

Regarding the third aspect, irrigation, our study confirms the predominance of gravity-fed techniques among farmers, with localized irrigation methods being utilized to a lesser extent. This reliance on gravity-fed irrigation is largely attributed to the fragmentation of agricultural land, which poses significant challenges to the implementation of more efficient irrigation systems. Gravity-fed irrigation, while traditional and widely practiced, is often less efficient in terms of water use and distribution compared to localized irrigation techniques such as drip or micro-sprinkler systems. These modern methods are designed to deliver water directly to the plant roots, minimizing evaporation and runoff, thereby enhancing water use efficiency and crop productivity.

The limited adoption of localized irrigation is a concern that necessitates a concerted effort from all stakeholders, including farmers, agricultural development agencies, and government

authorities. Addressing the fragmentation of land is crucial, as it complicates the logistics and infrastructure required for the widespread implementation of advanced irrigation systems.
To overcome this challenge, it is essential to foster collaboration and coordination among the various stakeholders. This could involve the consolidation of smaller plots into more manageable units, the provision of technical support and training to farmers on the benefits and operation of localized irrigation systems, and the development of supportive policies and incentives to encourage the transition from traditional to modern irrigation methods.
Additionally, investments in infrastructure and technology are necessary to facilitate the adoption of localized irrigation. This includes the installation of water distribution networks, the availability of affordable irrigation equipment, and the establishment of maintenance and support services to ensure the sustainability of the systems implemented.
The fourth aspect, which focuses on strengthening farmers' capacities and raising local awareness about the importance of preserving the oasis ecosystem, has been addressed through continuous training and awareness programs organized by various regional institutions. Specifically, the Regional Office for Agricultural Development (ROAD), the National Agricultural Advisory Office (NAAO), and the National Agency for the Development of Oasis Zones and the Argan Tree (NADOZAT) have conducted these initiatives.
These training programs are designed to equip farmers with the knowledge and skills necessary to adopt sustainable agricultural practices, enhance productivity, and preserve the delicate oasis ecosystem. The training sessions cover a wide range of topics, including soil conservation, efficient water use, pest management, and crop diversification.
Moreover, under the Green Morocco Plan (GMP), efforts have been made in cooperation with international organizations to further enhance the capacities of local actors in both agricultural and ecological fields. Notably, the Belgian agency ENABEL has provided training to farmers focusing on advanced agricultural techniques and sustainable farming practices. The Millennium Challenge Account (MCA) has offered support sessions aimed at improving farmers' technical skills and knowledge. Additionally, the Food and Agriculture Organization (FAO) has organized training workshops for farmers, highlighting the importance of sustainable agriculture and environmental conservation. The next illustration visualizes the percentages of the beneficiaries of all these sessions.

Illustration 1: Training and support provided to farmers under GMP



Source: Author’s elaboration based on the questionnaire survey results.

These collaborative efforts are essential for building the capacity of local farmers and ensuring the long-term sustainability of agricultural practices in the region. By participating in these training programs, farmers can gain a deeper understanding of the ecological challenges facing the oasis ecosystem and learn practical solutions to address them. This, in turn, contributes to the overall resilience and sustainability of the agricultural sector, fostering a more productive and environmentally conscious farming community.
Furthermore, the involvement of international organizations brings valuable expertise and resources to the local context, enabling the transfer of knowledge and best practices from different parts of the world. This global perspective enriches the local training programs and provides farmers with a broader understanding of sustainable agriculture and ecosystem management.

Conclusion

The territorialized GMP agricultural policy has positively impacted the diversification of the date palm sector in the Drâa-Tafilalet region, highlighting positive outcomes in developing agricultural practices, particularly concerning modern management and the adoption of resilient crops adapted to the regional climate. The efforts to support farmers in this process have also been notable.
Moreover, the decentralized approach of the GMP has strengthened the trust of the region's farmers in state policies, encouraging them to invest more in this cultivation to preserve the oasis ecosystem, a central element of the kingdom's diversity.
However, the optimal success of the territorialized GMP implementation faces several challenges that require deeper engagement and awareness from all stakeholders. This includes genuine territorial governance, prioritizing the major issues of the region, such as land management. The fragmentation of agricultural lands constitutes a major obstacle to any rehabilitation action and the creation of new farms, complicating territorial development and investment policies. To overcome these challenges, it is necessary to organize farmers within more coherent and structured professional organizations, allowing them to rationally manage the land platform and water resources.

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THE ECONOMIC AND ENVIRONMENTAL COST OF CULTIVATING OIL PALMS IN PAKISTAN

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Abstract

Pakistan plans to cultivate oil palms to achieve self-sufficiency in edible oils. However, the 1994 land suitability survey is outdated, necessitating a new suitability map and cost-benefit analysis. This paper presents an updated suitability map and examines the economic and environmental impacts of oil palm cultivation. Land, climate, soil, and irrigation data were collected and analysed using QGIS. Furthermore, calculations were performed for water consumption of competing crops, future palm oil imports, and carbon stocks. It was found that 1.08 million hectares of land in eight coastal districts were suitable for oil palm cultivation showing an increase of 0.58 million hectares of suitable land as compared to the 1994 survey. The analysis also revealed that competing cash crops – banana, sugarcane, and rice – generated \$437 million on cropping land of 0.35 million hectares and consumed 14.2 km^3 of water per year. In comparison, oil palms have the potential to produce 2.8 million metric tons of palm oil with a value of \$2.5 billion and consumption of 13 km^3 of water per year if grown all over 1.08 million hectares of suitable land. It was also calculated that oil palm could provide 0.1 to 0.36 million employment opportunities at the farm level. Such massive cultivation can also store up to 83 million metric tons of carbon over 30 years. Looking through the carbon market perspective, these can generate earnings from \$0.4 billion to \$2.3 billion. Overall, cultivating oil palms in coastal Sindh offers economic benefits, efficient land and water use, and contributes to edible oil self-sufficiency.

Keywords: *Edible oil, Self-Sufficiency, Palm oil, Water security, Pakistan.*

Introduction

Pakistan's edible oil imports share surpassed the local production share of edible oil in the total supply of edible oil in 1975-76 (Haq, 1991). Currently, the country's second-largest imported good is edible oil, and imports of palm and soybean oil cost \$4 billion to the national exchequer (State Bank of Pakistan, 2022). In 2022-23, Pakistan's edible oil demand was 4.247 million metric tons, of which 3.7 million metric tons of edible oil was imported and the remaining 0.5 million metric tons of edible oil was produced locally. Within these imports, 3.065 million metric tons of edible oil was palm oil (Pakistan Oilseed Development Board, 2023). Therefore, Pakistan's edible oil demand is majorly fulfilled by imported palm oil. The per capita edible oil consumption in Pakistan has also been increasing and was 9.95 kg in 1981-82 which jumped to 13.06 kg in 1991-92 and then 24 kg in 2021 (Haq, 1991; Rehman, 2021). Considering the increasing demand for palm oil in Pakistan, the government carried out a feasibility survey in the coastal areas of Sindh in 1994 to see, whether or not, the country could become self-sufficient in edible oil supply (Rashid & Nizami, 1994). The survey identified 95,415 hectares of land in coastal Sindh to be moderately well-suitable land for oil palm cultivation. The federal and provincial governments tried several times to be self-sufficient in producing palm oil locally but they mainly failed and today, only three oil palms farms on several acres of land exist and all three are in the district Thatta with one managed

by the Sindh Coastal Development Authority, one by Dalda Foods Ltd., and one jointly by Dalda Foods Ltd. and Sindh Agriculture University, Tando Jam.
However, oil palms require annual rainfall of 2000-2500 mm of equally distributed making it one of the most water-intensive crops in the world (Corley & Tinker, 2016; Hartley, 1988). Conversely, Sindh receives an average of 160 mm of rainfall per year which is far below the minimum rainfall requirements of oil palm (Government of Sindh, 2022). But, during our visit to two of these farms, we found that the oil palms at these farms are irrigated regularly. Thus, these oil palms are not dependent on rainwater only, particularly the monsoons. Though water-intensive, oil palm plantations have eventually moved to drier areas across the world (Carr, 2011). Additionally, oil palms can thrive better in the presence of a reliable irrigation system (Affandi, 2022; Carr, 2011; Corley & Tinker, 2016; Goh et al., 2011). Fed by irrigation and monsoons, the growth of oil palms, fresh fruit bunches, and the quality and quantity of oil content are evidence that oil palms can be grown on a large scale in coastal Sindh (Sindh Coastal Development Authority, 2021; Saleem et al., 2010).
However, such large-scale conversions of land into oil palm plantations could backfire and there is little debate over what could be the possible economic and environmental outcomes of this. For example, Pakistan is one of the largest net exporters of blue virtual water through its rice exports while, on the other hand, it is a net importer of green virtual water through its palm oil imports (Ali et al., 2019; Hoekstra & Mekonnen, 2012). Therefore, if Pakistan starts cultivating oil palm domestically, it will result in a reduction in the importation of green virtual water. Principally, this shift could raise local agricultural water consumption, but this is one of the objectives that this paper looks into. Also, replacing the current three major cash crops – banana, sugarcane, and rice – could also lead to employment fluctuations, and importantly, massive oil palm production could also lead to a greener environment and may probably increase carbon stocks in the coastal region of Sindh. Some studies say that oil palm can be a threat to animal species through deforestation. For example, oil palm plantations and expansion pose a threat to 321 species while also promoting invasive species in the tropical forests of Malaysia, Indonesia, and Thailand (Meijaard et al., 2020). In Coastal Sindh, there are no such forests, however, there are a few reserved areas where some species are under threat due to human activities. On the economic side, oil palm cultivation positively influences various aspects of farmers' lives, including their households' education, nutrition, dietary quality, general living conditions, and asset ownership (Chrisendo et al., 2022). Furthermore, it also serves as a source of employment and proves economically advantageous for small-scale farm holders (Ahmad et al., 2023). Thus, the above discussion can prove that there could be both positive and negative outcomes of growing oil palms in the coastal region of Sindh.
However, despite previous setbacks in growing oil palms on a larger scale, the government, encouraged by the substantial import expenditure and the favourable conditions of Pakistan's coastal area for oil palm cultivation, has shown its intent to pursue industrial-scale oil palm cultivation in the coastal regions. This intention aims to address the domestic demand for edible oil (Saleem et al., 2010; Siddiqui, 2022). There is no holistic study as of yet that could answer questions such as what could be the possible economic, and environmental costs and benefits of large-scale oil palm plantations in coastal Sindh, to help decide whether Pakistan should opt for cultivating oil palms on a larger scale in coastal Sindh or whether it should not. This research will provide policymakers with the latest oil palm suitability map and also present post-plantation economic and environmental aspects of oil palms in coastal Sindh to help policymakers choose the best policy.

Materials and Methods

The study area included eight coastal districts with a total area of 3.25 million hectares: Badin, Hyderabad, Matiari, Mirpur Khas, Sujawal, Tando Muhammad Khan, Tando Allahyar, and Thatta. The methodology of this research was exploratory and was divided into two parts;

1- The Suitability Map Analysis
2- Comparative Economic and Environmental Calculations

The suitability analysis was carried out using twelve variables from different sources. First, the data layers were cut to fit the study area. Then, through the Weighted Overlay Method, a suitability map was generated using QGIS. The details of the variables are shown in the following table.

Data	Variable	Format	Period	Year	Source
Climate	Mean Monthly Minimum Temperature	Raster	1970-2000	2020	World Clim
	Mean Monthly Maximum Temperature	Raster	1970-2000	2020	World Clim
	Solar Radiation	Raster	1970-2000	2020	World Clim
	Wind Speed	Raster	1970-2000	2020	World Clim
	Relative Humidity	Raster	2002-2021	2023	Pakistan Meteorological Department
Soil	Soil Texture	Raster	-	2013	Harmonized World Soil Database v1.2 (FAO)
	Soil Salinity	Raster	-	2013	Harmonized World Soil Database v1.2 (FAO)
Land	Drainage Class	Raster	-	2013	Harmonized World Soil Database v1.2 (FAO)
	Land Elevation	Raster	-	2008	SRTM (CGIAR-CSI)
	Crop Area	Raster	-	2022	Sentinel-2 10m land use/land cover Data (ESRI)
Water	Irrigation System	Vector	-	2023	Sindh Irrigation Department
Environment	Reserved Area	Vector	-	2023	Protected Planet (UNEP-WCMC)

Source: Authors

For the remaining analyses, further data was acquired from both primary and secondary sources. For example, the labour data was acquired during the field visits while the cultivated land data was gathered from the government surveys. The table below shows the variables and their purpose.

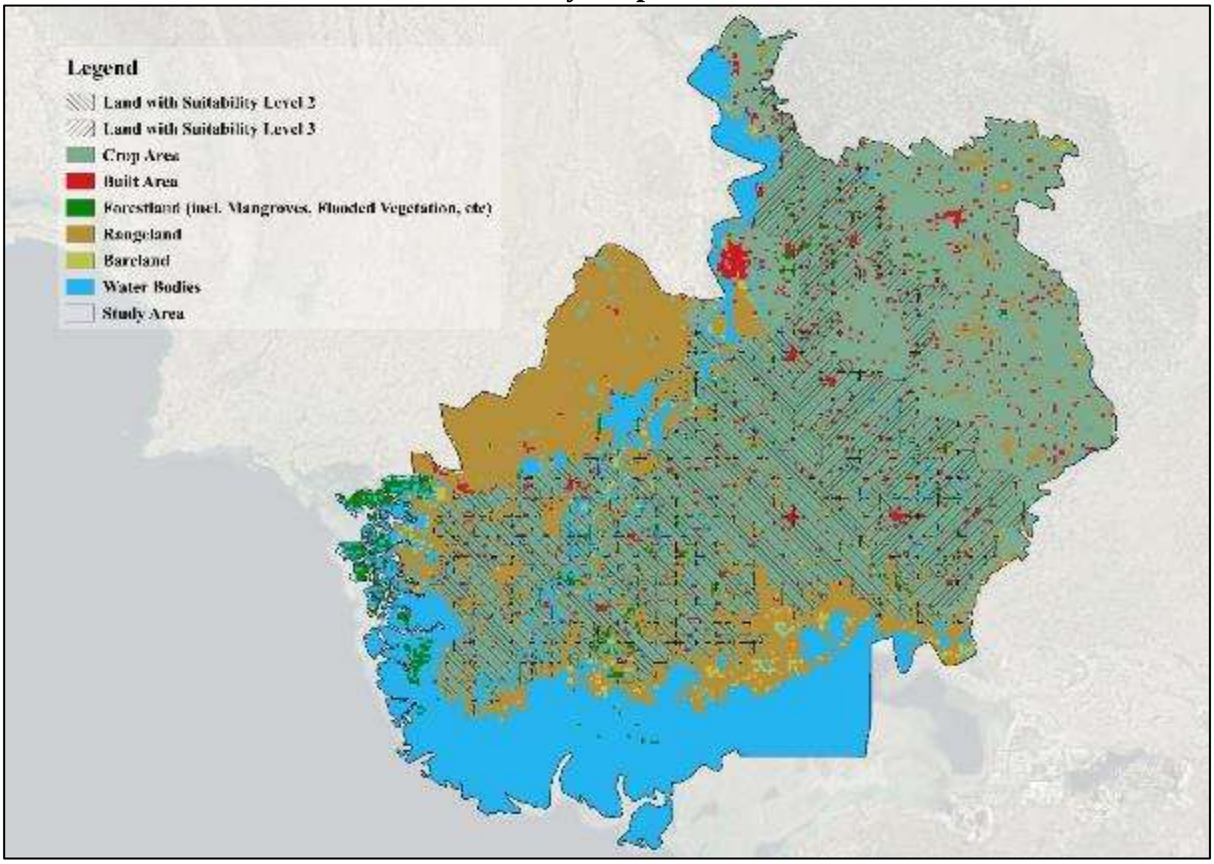
Sections	Variables/Data	Purpose
Economic Analysis	Edible oil Import Data, Water Consumption Data, Cultivated Land Data, Crop Data, Crop Value, and Labour Data.	To Calculate the Competing Crop Values, Employment Data, and Post-Oil Palm Plantations Future Scenarios on Import Substitution of Palm Oil.
Environmental Analysis	Carbon Emissions Data, Endangered Species Data, and Environmentally Protected Areas.	To Calculate the Carbon Sequestration, Impacts on Reserved Areas, and Post-Oil Palm Plantations Scenario.

Source: Authors

Results and Discussion

The land suitability analysis showed that of a total study area of 3.25 million hectares, only 0.68 million hectares are moderately well suited (70-80%) or have suitability level 2, and about 0.4 million hectares are moderately suited (60-70%) or have suitability level 3. Our map shows an increase of 0.58 million hectares of suitable land as compared to the 1994 survey conducted by the National Agriculture Research Centre, Islamabad. The suitability map is shown below.

Oil Palm Suitability Map for Coastal Sindh



Source: Authors

Based on the suitability map, future productivity was calculated based on hypothetical yields per hectare.

Suitability Level	Available Land	Average Yield	Total Yield	Total Value[7]
	Mha	MT/ha/yr	MMT/yr	m (USD)
Moderately Well (S2)	0.68	2.8	1.9	1719.58
Moderately (S3)	0.40	2.3	0.9	845.34
Total	**1.08**	**-**	**2.8**	**2564.92**

Source: Authors

Based on the average hypothetical yield of 2.5 Mt/ha/yr, 70% of Pakistan's current palm oil imports can be substituted locally. With a higher productivity of 3.5 Mt/ha/yr, Pakistan can fulfil 100% of its palm oil demand locally. Our analysis showed that Pakistan would be importing nearly 7 million metric tons of palm oil in 2050. By planting oil palms on all suitable land with a productivity rate of 2.5 Mt/ha/yr, Pakistan could meet 40% of its palm oil demand. Increasing the productivity rate to a maximum of 3.5 Mt/ha/yr could allow Pakistan to substitute 56% of its palm oil needs locally.

[7] The value of per ton of palm oil is based on the average imports of Pakistan from 2019-2022 with each year's imports in rupees adjusted for average USD-PKR exchange rate. In this way, we calculated the average imports that is USD 2,784.46 million and then divided the value in USD by the average imports for 2019-2022 that is 3.08 million metric tons to get average per ton value for palm oil equal to USD 903.45.

Similarly, our calculations show that competing crops – sugarcane, rice, and banana - have a combined monetary value of only USD 437.36 million per year, with a total water consumption of 14.27 km³ per year. In comparison based on our hypothetical values, we concluded that oil palms, in total have the potential to produce palm oil of up to 2.83 million metric tons, valued at $2.5 billion with a maximum water consumption of 13.01 km³ per year. The production value roughly equals 88.1% of the country’s average palm oil imports per year from 2013-2023. Therefore, it suggests a significant saving of foreign exchange reserves in the future while simultaneously fulfilling 88.1% of the local palm oil demand. On the employment side, it was calculated that oil palm cultivation at a suitable area can generate 0.1 to 0.36 million employment opportunities at the farm level.
According to Statista (2022), the total annual carbon emissions of Pakistan from fossil fuel and industrial sectors amount to 199.33 million metric tons, while concurrently, the country's forest area accounts for approximately 4.8% of its total land area (World Bank, 2021). At maximum capacity, oil palms on the suitable land could contribute an additional 1.08 million hectares of forested land to the existing national forest area of 3.68 million hectares. Being such massive plantations, they can also store up to 83 million metric tons of carbon over 30 years. Looking through the carbon market perspective, these can generate earnings from $0.4 billion to $2.3 billion over 30 years. However, these plantations may destroy some of the environmentally important areas such as the Runn of Kutch Wildlife Sanctuary, Kinjhar Lake, and Hadero Lake Wildlife Sanctuaries.

Conclusion

Pakistan’s palm oil imports are expected to grow in the future and therefore, to be self-sufficient in edible oils, Pakistan can opt for growing oil palms in coastal Sindh. Our analyses show that oil palms in coastal Sindh could have positive economic and environmental impacts. The suitable area of 1.08 million hectares of land could be sufficient to fulfil the local palm oil demand while also utilizing the scarce water and land resources efficiently. However, starting from scratch would not be an easy task and oil palms are new to this region and the farmers as well. Therefore, the government should allocate sufficient funds for training the farmers, and development of scientific labs specially focused on oil palms and their tissue culture. Also, the government needs to provide financial and administrative assistance to the farmers and investors. Lastly, the government should ensure the sustainability of plantations and the production processes.

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SETTLEMENT OF YOUNG FARMERS: IDENTIFICATION OF MAIN CONSTRAINTS FROM THE RESULTS OF A SURVEY

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Abstract

Young farmers are fundamental for the generational renewal of agricultural holdings, their modernization and implementation of innovations, but the installation rate success is very low. The objective of the study was to better understand the main constraints to the installation of young farmers. The survey methodology was used, applied to young farmers supported by rural development programs, between 2007 and 2020 (PRODER and PDR2020 programs). A total of 445 young people were interviewed (4% of the total settled young farmers financially supported by PRODER and PDR2020). Most of the young farmers had some activity linked to agriculture before the settlement. The main motivations were to obtain additional income or maintain the family farm. Almost a third had higher education training. One of the main restrictions for young farmers to settle was access to land (68% explore family land). Almost two thirds reported difficulty in accessing credit, mainly due to difficulties in approval by banking institutions. However, the main difficulties expressed were with the contraction of labor (lack of available workers) and with marketing (low production prices). Positive factors for success: technical monitoring of the farm, family support and farmers' associations; the existence of agricultural land available in the family; the choice of production, considering the market, technical knowledge, and the adaptation of crops to soil and climate conditions; and a love for farming, some experience, persistence and dedication. Negative factors: little care in preparing the business plan and lack of market knowledge; inappropriate choice of crops; lack of technical support and monitoring in the agricultural and commercial areas.

Keywords: *agricultural policy, Portugal, success factors, young farmers.*

Introduction

Young farmers are fundamental for the generational renewal of agricultural holdings, their modernization and implementation of innovations, but the installation rate success is very low, despite support policies under the Common Agricultural Policy. The so-called "young farmer problem" in Europe has been studied both at national level (Eistrup et al., 2019; Kerbler, 2012; Kontogeorgos et al., 2014; Oliveira et al., 2021) and at the European Union level, with the apparent shortage of young farmers occurring in countries where small-scale holdings are more prevalent, particularly Portugal, Italy, Romania and Greece (Zagata and Sutherland, 2015).
Portugal has 290,229 agricultural holdings (INE, 2021), with 13.7 hectares on average. The Utilized Agricultural Area (UAA) occupies 3,963,945 hectares, 43% of the national territory, and more than half is occupied by permanent grassland. The majority of agricultural holdings are managed by individual producers (94.5%), but agricultural companies manage 36.7% of UAA. In 2019, the average Economic Size of farms was 23.3 thousand euros of Total

Standard Output (TSO), with great regional variability. There has been a reinforcement of the specialization of Portuguese agriculture, in which 3/4 of farms are specialized (more than 2/3 of the TSO comes from one activity only), namely berries, subtropical fruits and almond groves, olive groves, vineyards, cattle, and sheep livestock, and represents 88.4% of the TSO.
The farming population and farm managers are very aged with few young people. The sole holders are on average 64 years old, 46.3% have only completed the first level of basic education and 53.0% have exclusively practical agricultural training. The agricultural enterprise managers are 13 years younger than the sole holder and have high academic and professional qualifications (INE, 2021). In Portugal, only 3.1% of farm managers are less than 35 years old (data of 2020), and the EU average is 6.5% (Pordata, 2023). The weight of young people in single producers is even smaller. In the group of individual producers, only 0.3% are under 25 years old and 10% are under 45 years old (INE, 2021). In the group of agricultural managers, the age group under 25 years old represents 0.7% and managers under 45 years old represent 32%.
Policies to encourage the installation of young farmers have not been able to promote the rejuvenation of Portuguese farmers (Cunha, 2021; Oliveira et al., 2021). Although the issue of rejuvenation is a main concern in the Common Agricultural Policy, in the Portuguese context this relevance is accentuated given the low number of young farmers and the failure of policies. Several studies have already been carried out, namely on success and failure factors and comparative study of policies with other countries (Alberto, 2004; Cordeiro, 2008), the characterization of young farmers and motivations for their installation as agricultural producers (Mateus, 2022; Soares, 2013). Eistrup et al. (2019) also addressed the barriers to the installation of young farmers, but it is important to go deeper, from a more comprehensive perspective, at the national level and the constraints. The present study aims to identify the main bottlenecks, the main mistakes to avoid, and ways to tackle the main constraints to young farmers' settlement success. The Portuguese case is a good case study because the farmer's regeneration is a policy objective that has been a priority in the programming cycles of the Common Agricultural Policy, but Portugal has the lowest generational renewal in agriculture, with a trend to decline

Material and Methods

The paper is based on primary data collected by structured questionnaires carried out between November 2019 and January 2020, with 445 Portuguese young farmers, settled between 2007 and 2019, and supported by a Start-Up Aid for Young Farmers payments, from the Rural Development Programme, Common Agricultural Policy.
The young farmers interviewed were selected from a survey of 12,130 applications for the first installation of Young Farmers, approved under the development programs, between 2008 and 2019 (PRODER and PDR2020), with the geographic identification of the investment. The interviewed farmers were distributed across the five regions of the Portuguese mainland (NUTS II, Nomenclature of Territorial Units for Statistics). The random sampling method stratified by the NUTS II region was used, with distribution being made by the number of projects approved in the two rural development programs. The sample of young farmers interviewed represents 4% of the total number of young farmers supported in the period under study. The surveys were carried out by technicians from the Portuguese Association of Young Farmers (AJAP, in Portuguese).
The inquiry addressed several issues regarding the young farmer characteristics, attitudes and motivations, the settlement process, constraints and farm structure: the process of preparing the application which is often referred to as one of the potential challenges that young farmers have to face in their installation, namely due to the complexity and bureaucracy associated

with the process; motivations that led the young person to decide to settle on an agricultural holding, including their employment situation before submitting the application; issues related to the installation of young people as farmers, from access to land, which is usually mentioned as one of the main difficulties, but also access to credit, academic qualifications and professional training, and the investments made in the first installation; characterization of the productive structure in which the young farmers established their first installation; and the main difficulties experienced by young farmers throughout their first installation process (from application to the date of the survey).
The results of the surveys were analyzed with the IBM® SPSS® Statistics software. In a second phase, on July 12th, 2021, a meeting was held, with various stakeholders, to delve into the main restrictions on the installation of young farmers, and present political recommendations to face these constraints. The main results of the survey were presented, with discussion topics being launched by the facilitators. The participants intervened, with comments on the topic, possible success factors and difficulties in establishing young farmers, how to resolve problems, and recommendations within the scope of public policies. Given the context of the COVID-19 pandemic, the meeting was held via videoconference, with the focus group facilitators based at AJAP headquarters and the stakeholders participating via videoconference. The remote meeting (due to pandemic restrictions) did not allow us to discuss, deepen and analyze the survey results as we would have liked, but it is highly unlikely that this introduced any bias in the focus group results. The pandemic had no effects on the survey, as the interviews were conducted prior to the implementation of movement restrictions, which only began in March 2020.

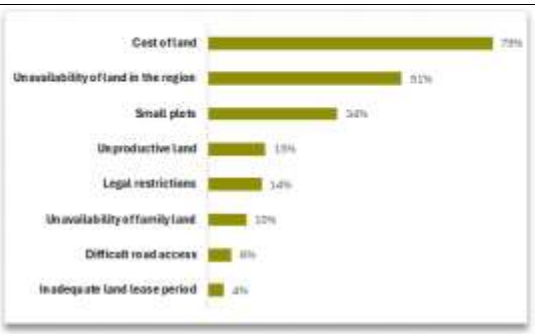
Results and Discussion

Most of the young farmers had some activity linked to agriculture before the settlement (55%), with some differences between regions (at the NUTS II level). The main motivations to become a farmer were to obtain additional income (42%) or maintain the family farm (40%). Only 27 percent said they wanted to set up their own business and 15 percent said they were unemployed. Most of the respondents were employed, either on their account or as an employee, before setting up as young farmers. These results confirm that the main motivation for young Portuguese people to invest in agriculture may be the search for a complementary income to another main activity (Soares, 2013), while the influence of family tradition is also very important (Borec et al. 2013).
One of the main restrictions for young farmers to settle was access to land (EC, 2021), as had been pointed out by other studies (FAO et al., 2014; Zmija et al., 2020). The 86% of young farmers reported difficulties in accessing land, the main factors being the price of land (74%) and the lack of land in the region where they intend to settle (51%). The vast majority (68%) exploit family land and 22% have resorted to renting land. Municipalities could play a key role in the issue of access to land because they are closer to economic agents and have a better understanding of local specificities. One possible mechanism for mobilizing the land factor would be to identify farmers who are no longer able to exploit their land and make it available to young farmers. It is currently not possible to regulate the price of land rents, which would introduce rigidity and greater scarcity into the market. In Portugal, there is an attempt to collect farmland rental prices, but it is a very opaque market, with a great lack of information, which makes it difficult to use this resource more efficiently. In the focus group, the problem of smallholdings and fragmentation of property was also mentioned, and the possibility of attributing benefits, in shares by succession, to those who want to set up farming was raised.
Access to capital is one of the main barriers to entry into the farming sector (EC, 2021), mentioned in various studies (Beckers et al., 2018; Meyer, 2015). Almost two-thirds reported

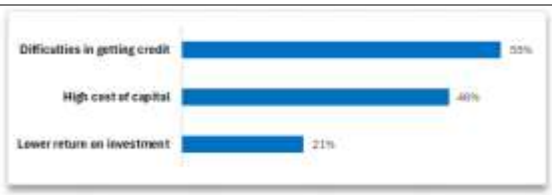
difficulty in accessing credit, mainly due to difficulties in approval by banking institutions (55%) or due to the high cost of capital (46%). Some young people have no banking history or guaranties, and this makes it difficult to access credit. One way of trying to resolve the situation would be to set up mutual guarantee societies. Another factor mentioned in the focus group was the importance of paying the reimbursements of eligible expenses in the installation project approved for the young farmer's installation on time, as a delay in this reimbursement could jeopardize the fulfillment of the bank loan payment obligations.
However, the main difficulties expressed, in 91% of cases, were related to the contraction of labour (lack of available workers) and marketing (low production prices). These results have not been adequately addressed in studies on the establishment of young farmers, although the issue of marketing is directly related to the problem of low profitability and is one of the reasons why young people not become involved in agriculture (FAO et al., 2014). Regarding the problem of commercialization, the main issue is low prices of the products. Low negotiating capacity (in 40% of cases), late payments (27%), lack of contracts, quality requirements, low differentiation, and quantitative requirements are also mentioned. Farms run by young farmers are more profitable and market-orientated (Oliveira et al, 2021) but commercialization is a critical issue and should be the starting point for analyzing an agricultural start-up project. The focus group meeting emphasized the importance of identifying possible ways of placing production on the market, the need for training in this area, the importance of sharing experiences and associating to gain market power, and also to access technical support and better conditions for purchasing inputs. The young people surveyed pointed to a lack of knowledge about markets and a lack of training in the commercial sector as mistakes they had made when setting up as agricultural producers and which they would not repeat.

Table 1. Main constraints for settled young farmers

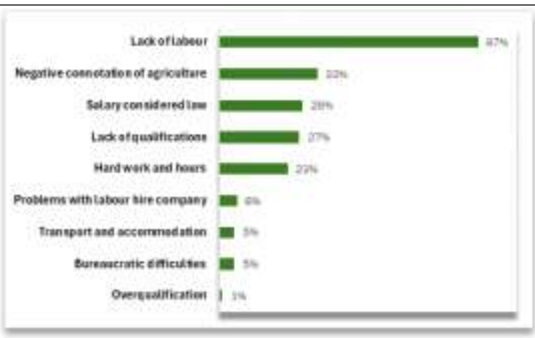
Difficulty accessing land: 86%



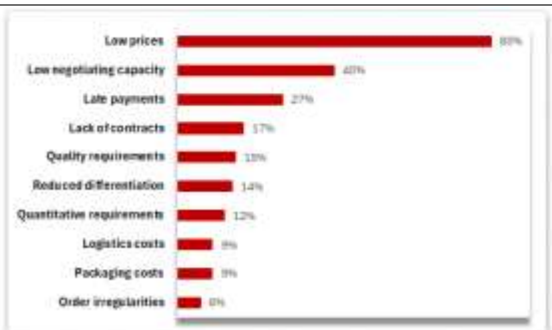
Difficulty accessing credit: 62%



Difficulties in hiring labour: 91%



Difficulty in marketing products: 91%



*Source: Author’s elaboration based on the questionnaire survey results

The problem of a lack of labour was identified by 87% of those surveyed, which is higher than other factors mentioned in studies on the establishment of young farmers (Zmija et al., 2020), such as the negative connotation of farming (in 33% of cases), the level of the salaries (28%), the lack of qualifications (27%) or the harshness of the work and the hours worked (23%).
This labour shortage has been filled by foreign workers, who already account for 40% of employees in the agriculture and fisheries sector (Banco de Portugal, 2024). This restriction has not been addressed in the various studies on the installation of young people in agriculture, but there is a concern about the capacity to create jobs (EC, 2021; Soares, 2013).
Access to knowledge is a crucial issue for the success of young people setting up in agriculture (EC, 2021; FAO et al. 2014). Almost a third of the young farmers surveyed had higher education training not related to agriculture or forestry (30%), and only 11% had education in agriculture or forestry sciences (around one-quarter of the young farmers with high education). We also found that almost half (45%) had exclusively practical experience and a fifth had no experience or professional agricultural training. These results show a generation that is highly qualified, but not in farming. We also asked the young people about their training after setting up as farmers. Almost half (48%) had training in the application and handling of pesticides (because it's compulsory), but only 43 percent had training in agricultural production techniques and only 32 percent in management or accounting. Despite these results, young Portuguese farmers have more academic and agricultural training than other farmers, have more economically viable farms, and make greater use of technology and more sustainable practices (Mateus, 2022). A study on innovation in agricultural production found that young people have a high innovative potential in the agricultural sector (Reis, 2013). In the focus group meeting, the importance of training and mentoring to accompany young people during their installation was emphasized. Access to knowledge and advice is still insufficient, and the CAP generational-renewal measures are not well adapted to support the entry of young farm managers with no family background in farming (EC, 2021).

Conclusions

The study confirmed the main motivating factors for young people to take up farming: looking for a supplementary income or maintaining the family farm. The restrictions due to difficulties in accessing land and capital were also confirmed, but it should be noted that the respondents gave more weight to difficulties in accessing markets and labour (although this is not a specific restriction for young farmers). Contrary to what was expected or desirable, the young people did not emphasize the problem of access to knowledge, nor did they show much appetite for training. As positive factors for success: technical monitoring of the farm, family support and farmers’ associations; the existence of agricultural land available in the family; the choice of production, considering the market, technical knowledge, and the adaptation of crops to soil and climate conditions; and a love for farming, some experience, persistence and dedication. On the negative factors, it should be stressed: little care in preparing the business plan and lack of market knowledge; inappropriate choice of crops; lack of technical support and monitoring in the agricultural and commercial areas.
In conclusion, motivating young people to take up farming and the success of their setting up must go beyond financial support for setting up, as it is crucial to analyze the situation carefully and realistically before setting up and to provide technical and tutorial support during the initial phase of farming. An integrated approach is needed, both in terms of implementing CAP (national strategic plans) and integrating them with regional and local development policies.

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STRATEGIC OBJECTIVES OF THE DEVELOPMENT OF ORGANIC AGRICULTURE IN THE REPUBLIC OF SERBIA

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Abstract

Agriculture represents a very important economic activity that significantly affects the overall economic activity of our country. In agriculture, the use of land and the cultivation of plants and animals produce primary products of suitable plant or animal origin. The development problems of Serbian agriculture can be solved with an active approach that must be based on an effective policy for the development of this social activity. That approach should be based on significant engagement of the state and economically rational activation of the overall development potential of domestic agriculture. Due to the specificity of the production cycle, slow turnover of capital, low profitability of production, i.e., lack of own sources of financing, constant financial and credit support is necessary for agriculture. In order to develop highly productive agricultural production, it is very important to build a stable, efficient and sustainable agricultural sector that will contribute to economic growth and be competitive on the international market. In this sense, it is necessary to ensure the stabilization of the internal agricultural market, along with structural adjustments of agricultural production in accordance with the demand on the world market, so that the agricultural sector can also become a strong export sector, primarily products of higher stages of processing, regardless of market barriers and problems. with protectionist measures in many developed countries.

Keywords: *Agriculture, economic growth, economic development, agricultural market*.

Effects and measures of agricultural policy

Agriculture represents an important component of the overall economy of the Republic of Serbia. In agriculture, by using land, growing plants and animals, primary products of appropriate plant or animal origin are obtained.
Agricultural policy is a set of measures and activities undertaken by competent authorities in accordance with the constitution and the law, which achieve the goals of agricultural policy. According to agricultural policy measurement tools, all agricultural policy measures are grouped into three main pillars:
– market support measures and direct support measures for producers,
– structural measures and rural development measures,
– general measures related to agriculture (Volk et al., 2015).
The first pillar, Market support measures and direct support measures for producers, includes only those measures that affect the income of agricultural producers and are generally not related to any restrictions regarding production techniques or the location of the agricultural holding. At the next level, the first pillar measures are divided into two groups: market support measures and direct producer support measures. Budget expenditures for market support measures include measures through which the policy affects supply and demand on the domestic market, and thus indirectly affects the prices of agricultural products (Volk et al., 2015).

Budget expenditures related to these measures are divided into three groups, namely: export subsidies, market interventions and consumer support.
Direct support measures for producers are further divided into two larger groups.
The first group, - Direct payments and subsidies for variable inputs, contains all forms of regular direct payments to producers, which are further divided in relation to implementation criteria (production, area - animals, fixed criteria, other criteria) and subsidies for variable inputs divided according to the type of input (seeds, fuel, fertilizer, insurance, etc.).
The second group, - Accident payments and other benefits to producers, contains only payments that producers can apply for on specific occasions. These payments are made mostly on an ex- post basis, while the first group of payments is planned in advance and paid regularly (Volk et al., 2015).
The second pillar, - Structural and rural development measures, is divided into three main groups: Improving the competitiveness of the agricultural sector, Improving the environment and rural areas, and Supporting the rural economy and population. The first group (Improving the competitiveness of the agricultural sector) was divided into three subgroups of measures in the first step, where the main criterion for the division was the target group for which the support is intended. The second group consists of measures aimed at improving the environment and rural areas, while the third group contains measures to support the rural economy and population.
The third pillar, General measures related to agriculture, covers measures aimed at supporting public services related to agriculture, such as research, development, advisory and expert services, food safety and quality control (veterinary and phytosanitary measures, quality policy, etc.) and other general support measures that exist in agriculture (Volk et al., 2015).
When it comes to agricultural policy in the Republic of Serbia, the Department for Agricultural Policy performs tasks related to: monitoring domestic and global production, consumption, processing, prices and trade in primary agricultural products, organic products, fishery products and processing products from the mentioned products; preparation of professional bases for drafting regulations in the field of agricultural policy and fisheries policy, as well as their harmonization with the legal acquis of the European Union (EU), rules, principles and agreements of the World Trade Organization (WTO) and standards of international organizations and international agreements; drawing up a balance of basic agricultural products; participation in the drafting of strategic documents in the field of plant and animal production, organic production, processing and quality of agricultural products, as well as in the field of agricultural statistics; making analyzes of the situation and giving projections of the development of the food industry; preparation of proposals for the distribution of budget funds for incentives in agriculture and rural development; proposing agricultural and fisheries policy measures, as well as monitoring and analyzing the achieved effects of the measures; monitoring and analysis of customs policy in the field of agricultural and food products and proposing measures to improve foreign trade exchange with agricultural and food products of the Republic of Serbia; participation in the preparation of information and documents and for cooperation at the bilateral level and negotiations and cooperation with the EU, WTO and international organizations, as well as participation in the preparation, coordination and implementation of projects from EU funds and other sources of financing in areas within the competence of the Sector; analysis of the effects of climate change on the agricultural sector and the impact of agricultural production on climate change; proposing measures for adapting and mitigating climate change important for agricultural policies, as well as monitoring and analyzing the achieved effects of the measures; other tasks in this area (Law on Agriculture and Rural Development).

Common agricultural policy of the European Union

The European Union is the largest exporter and importer of agricultural and food products in the world. Taking into account that the Common Agricultural Policy is one of the most generous policies of the European Union, the importance of observing the effects of the measures of this policy on the internal market, as well as considering the impact it has on the economies of countries outside the borders of the Union, is relevant.

The foundations for the establishment of the Common Agricultural Policy were provided by the Treaty of Rome (1957) and the Conference in Stresa, Italy (1958). At the aforementioned Conference, three principles of functioning were established, namely: the single market, the primacy of the Union and financial solidarity. Internally, the single market means the flow of goods free from customs and non-customs barriers. Externally, it implies a uniform level of customs duties in the EU that apply to imported products. The aim of the Union championship is stability in supply and avoidance of market disturbances, as well as assistance in placing products on foreign markets, and financial solidarity means the participation of all members of the Union in the financing of common policies, although the share is not equal for all (Guide to EU policies - agriculture, European movement in Serbia, 2011). From its foundation until today, the Common Agricultural Policy strives towards the same goals, while the modalities of their achievement have changed form through the numerous reforms it has undergone. The objectives of the Common Agricultural Policy are as follows (Živadinović and Milanović, 2011):

– support to farmers and improvement of agricultural productivity, ensuring a stable supply of food at affordable prices;

– enabling EU farmers to earn acceptable wages;

– help in the fight against climate change and sustainable management of natural resources;

– maintenance of rural areas and landscapes across the EU;

– maintaining the rural economy by promoting jobs in agriculture, agro-food industries and related sectors.

Today, the Common Agricultural Policy has three groups of interrelated measures to achieve defined goals, which are organized into two pillars of the Common Agricultural Policy. The first pillar consists of direct payments and measures for market regulation and they are financed from the European Agricultural Guarantee Fund (EAGF). The second pillar consists of rural development measures financed from the European Agricultural Fund for Rural Development (EAFRD).

Direct payments are the main source of income support for agricultural producers, which is based on the hectares they cultivate. Therefore, they represent a stable source of income that does not depend on market fluctuations.

Market measures serve to regulate the sudden destabilization of the market, which can be caused by e.g. excessive supply. In such cases, the European Commission can activate allocated funds (on average about 5% of the CAP budget) in order to stabilize the market.

Rural development measures are designed to address the economic, social, and environmental challenges faced by EU rural regions. Rural development policy has three long-term goals: encouraging competitive agriculture, sustainable management of natural resources and implementation of climate measures, and achieving sustainable development of rural regions, including the creation and maintenance of jobs.

Agricultural policy in the light of the Agriculture Development Strategy of the Republic of Serbia 2014 - 2024

In an effort to chart the direction of future reforms of the agricultural sector, as clearly as possible, as part of external and internal activities to restore and activate the development potential of rural areas, in 2014, the Government of the Republic of Serbia adopted the Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2014-2024. The strategy defines:
– directions of future development of agriculture and food industry,
– a support model that would lead to accelerating the development of the agri-food sector,
– the direction of future agricultural policy reforms, the adoption and full implementation of the legislative framework that provides a legal basis for the implementation of the strategy itself and the harmonization of national legislation with EU legislation - Acquis communautaire (institutional reforms).
The strategy contains a brief analysis of the state of the sector in rural areas with reference to the measures from the previous period, the structure of the agricultural budget according to the pillars of support for the measures and their effects (Strategy of Agriculture and Rural Development of the Republic of Serbia for the period 2014-2024, "Official Gazette of RS", No. 85/2014). The document establishes the following six strategic development goals:
– production growth and producer income stability;
– increase in competitiveness with adaptation to the requirements of the domestic and foreign markets and the technical and technological improvement of the agricultural sector;
– sustainable resource management and environmental protection;
– improving the quality of life in rural areas and reducing poverty;
– effective management of public policies and improvement of the institutional framework for the development of agriculture and rural areas
– modernization of organs and organizations and adaptation of agricultural policy to the EU Common Agricultural Policy model.

In order to achieve these goals, the strategy planned interventions in priority areas of agricultural policy action in accordance with the set strategic goals such as:
– stabilization of the income of agricultural producers;
– financing of agriculture and rural development and risk management;
– efficient land management and increasing the availability of land resources; improving the state of physical resources;
– improvement of the knowledge transfer system and development of human resources – adapting to and mitigating the impact of climate change;
– technological development and modernization of agricultural production and processing;
– development of market chains and logistical support for the agricultural sector;
– protection and improvement of the environment and preservation of natural resources;
– diversification of rural economy and preservation of cultural and natural heritage;
– improvement of social structure and strengthening of social capital;
– modernization and adaptation of legislative bodies and organizations;
– improvement of product quality and safety (Strategy of agriculture and rural development of the Republic of Serbia for the period 2014-2024, "Official Gazette of RS", No. 85/2014).

From all of the above, it can be concluded that if Serbia wants to preserve the national economy and mitigate the dramatic socio-economic consequences of the situation in which its rural areas, rural population, rural economy and the agricultural sector as such are, then it would have to establish an agrarian and rural policy much more in order of priority, in the socio-economic and political, that is, development sense.

Objectives of the development of organic agriculture

In today's conditions, organic production is gaining special importance, which represents a system of agriculture based on ecological principles and implies the use of exclusively natural substances and the complete elimination of the use of pesticides, artificial fertilizers, growth regulators and any chemically processed substances. Organic agriculture is a production management system that promotes the preservation of ecosystems by combining biodiversity and biological cycles while emphasizing the use of methods that exclude the use of inputs for off-farm production (Simić, 2021). Organic agricultural production particularly emphasizes the importance of food quality and safety, while avoiding the use of synthetic mineral fertilizers, plant protection agents, growth regulators in livestock and feed additives. An organic product is the result of organic production that takes place with the application of agrotechnical measures that exclude the use of synthetic-chemical agents, and as such differs from conventional agricultural production. Conventional agriculture uses chemical fertilizers, and organic agriculture uses natural fertilizers to feed the soil. Conventional farmers use pesticides against insects and plant diseases, and organic farming uses natural methods, such as natural plant barriers and predators that destroy insects. Conventional farmers destroy weeds using synthetic (artificial) herbicides, while organic farmers cultivate the land by digging weeds by hand and covering them with straw (mulch) to control weeds, etc. Among the most widely accepted definitions of organic agriculture, the definition introduced by Lampkin and Padel (1994) stands out, who define organic agriculture as both a philosophy and a production system that aims to create an integrated, humane, economically sustainable agriculture, oriented towards protection of the environment, which maximizes the use of renewable resources produced on the farm itself and the management system of ecological and biological processes for the purpose of obtaining an acceptable level of the yield of plant crops, growth of animals and the level of nutrients required for human consumption, protection against diseases and pests and ensuring an appropriate level of return on investment human capital and means of production. Beauchesne and Bryant (1999) define organic agriculture as a social and technological alternative to conventional production, although a slightly more complex reality is hidden in this dichotomy. Organic agriculture is often associated with the "old" way of production, that is, it often acts as a "return to the past". However, it should be noted that there is a difference between organic farming and peasant production (Ćifrić, 2003). Ecological (organic) agriculture is not a conservative concept, and should not be understood as a requirement:

– for a return to the pre-industrial way of production in terms of technological backwardness, and,

– for the return to the peasant, traditional way of life, which includes the return to the old relations in the family, between the sexes, etc.

Ecological (organic) agriculture is a social innovation and should be understood as: giving up the dominance of the paradigm of industrial agriculture; the possibility of additional employment of labor on the family farm, settlement and society; the convenience of producing quality products on small areas; encouraging the development of "closed" production systems, with greater use of natural energy and organic processes. Ecological agriculture is a broader concept than peasant agriculture, because it presupposes (a) the appreciation of some experiences from the peasant (agricultural) economy (primarily those

concerning a balanced relationship with nature), but also (b) it includes such an application of science that guarantees its ecological character (Cifric, 2003).
Pudjak and Bokan (2011) state that ecological (organic) agriculture is a concept of agricultural production that is much more complex and whose essence is not only in the omission of agrochemicals, but in the overall economy by which it is possible to achieve this. Likewise, it is not a return to the old ways, a return to the agriculture of our grandparents. On the contrary, ecological (organic) agriculture is a part of modern agricultural production, trade and agronomic science, and is based on its latest findings and achievements. Ecological (organic) agriculture is an idea (and practice) that represents a change in thinking about food, and goes beyond the question of the nutritional composition of our meals and becomes part of our way of life. Some authors call food produced according to organic principles alternative food (Lowe et al., 2008). It creates different social structures with specific ethical and social ideas. The practices of ecological agricultural production are inseparable from social issues, ranging from restoring rural communities, avoiding the exploitation of workers, overcoming the divide between rural and urban, and producer and consumer, to the creation of alternative lifestyles of connection with others and with nature. Through efforts to uncover the complex connections between ecology and food production, the environmental movement has initiated debates about the broader sustainability of food production systems and has been a key factor in linking sustainable agricultural production with socially conscious consumption (Lowe et al., 2008).
Despite some differences in the definition of the term organic agriculture, the main goal of this production system is a sustainable system of agricultural production. The term "sustainable" is used in a broader sense, including economic, social and natural sustainability. The development of organic agriculture is, above all, related to farmers, who were pioneers in this production, and knowledge and information were distributed through informal networks in the beginning. This was followed by the establishment of organic farming organizations, sometimes encouraged by individuals interested in agricultural and rural development. Research, otherwise an important factor in agricultural development, played a minor role here (Padel, 2001).
Organic agriculture can best be defined through its goal, which is the production of health-safe, high-quality food in an ecologically sustainable way. The goal of organic agriculture is to improve the health and productivity of interdependent communities, soil life, plants, animals and people (Mirecki et al., 2011).
Organic production represents a complex way of production that must comply with strict legal norms, and this reflects the advantage of organic products, which are synonymous with protecting the health and life of people, nature and the environment. High natural potential and favorable climatic conditions make Serbia one of the countries where organic production can be successfully developed. Organic products of Serbia can be one of the factors of economic development and recognition of the Republic of Serbia. This type of production makes it possible to make a significant profit on small farms characteristic of Serbia.
The organic system of agricultural production has attracted a lot of attention over the past decades, as it seems to offer a solution to some of the problems that currently exist in the agricultural sector of industrialized countries. Organic agriculture has the potential to provide benefits in terms of environmental protection, conservation of non-renewable energy sources, improved food quality, reduction of by-product output and reorientation of agriculture towards markets where there is demand. Serbia's natural resources make it one of the countries where organic production can be developed even more intensively. Given that today the quality of food is measured by its impact on people's health, we can say that consuming organically produced food contributes to a higher quality of life, while this method of production makes a great contribution to the preservation of our environment. Growing

consumer awareness of food safety and environmental protection has contributed to an increase in organic agricultural production in recent years. Organic production should be seen as part of a sustainable agroecosystem, which supports traditional agricultural production.
The constant growth of the world's population caused an increase in the area used for agricultural production. The increased need for food has also changed the way of agricultural production. Today, it is obvious that conventional (industrial) methods of agricultural production, in addition to providing enough food and other various products, also lead to a series of negative, not only ecological, but also social and economic consequences (Kovačević et al., 2011). Rodić et al. (2008) state that agricultural land is one of those resources without sustainable use of which we cannot talk about the sustainable development of agriculture and society as a whole. The need for food, if the current trend continues, will increase almost twice by 2050, which presupposes increased land exploitation and an increase in the consumption of mineral fertilizers and plant protection products.
The agricultural sector has a relatively large importance in the economic structure of Serbia. Agrarian policy is an integral part of economic policy applied in agricultural production, and it cannot be constituted outside of economic policy and the economic system. Many issues of global economic development depend to a large extent on the development of agriculture. Serbia should use the experiences of other countries that have gone through the process of transition and joining the EU.
The importance of agriculture for the economic structure of Serbia, measured by the share of the sector in GDP and total employment, is very large. The systematic and structural reformation of the Serbian agricultural sector began after 2000. The basic direction of action of the agrarian policy was to support agricultural holdings with incentives for changing the production structure. The most significant elements of reform processes in the agricultural sector of Serbia from 2000 until today were: market liberalization, privatization of the processing industry, activation of the agricultural financial market, formation of new institutional forms at all levels.
The production of organic food is a very important source of competitiveness of our economy. The expansion of this method of production leads to the development of rural areas, contributes to more serious environmental protection and has a positive effect on people's health. Intensification of organic food production creates conditions for dynamizing the export activities of the domestic economy and improving the overall economic condition of the country. The main goal of organic agriculture is the production of high-quality food (high nutritional value) and the development of sustainable agriculture, while preserving ecosystems and maintaining and increasing soil fertility. Organic food production is becoming more and more popular than production based on conventional methods, and as such, it is becoming one of the most profitable businesses in the world. In this sense, in this paper we point out the importance of the development of organic food production, which represents a serious development opportunity for the Serbian economy.
Organic production in Serbia has exceptional opportunities and is an excellent opportunity for our country, given that the demand for these products has increased both here and in the world. There is interest in this production in Serbia, which is shown by the increase in the area and number of organic producers, as well as the growth in the export of organic products. Domestic organic products have long been present on the market of European countries, which are our main export markets, but, bearing in mind that in previous years we have opened many new markets for the export of agricultural products – such as the markets of China, Russia, Turkey, India, Egypt – organic products can become interesting for export to those countries.

Conclusions

The Republic of Serbia has great potential in organic agricultural production. The basic possibilities for the development of organic food production are: arable land, nature reserves, forest zones, great climatic diversity as a condition for the production of different plant and animal species, the offer of organically produced food on the European market is less than the demand, etc.

Organic production, as a sustainable food system, is a significant source of innovations that can help agriculture to overcome existing challenges and contribute to improving the competitiveness of producers and domestic agriculture as a whole. The Republic of Serbia recognized this potential and started advocating for the development of organic production at all levels, initiating and designing numerous initiatives aimed at creating a favorable environment that can contribute to the significant development of the organic production sector.

People are increasingly paying attention to environmental, health, social and ethical issues and are looking more than ever for fresh, less processed food from sustainable sources and are interested in those foods that can provide them with safety regarding certain risks of food products. The need for shorter supply chains has become even more pronounced during the current crisis. It should be easier for consumers to choose food from sustainable sources and systems, and all participants in the food chain should accept this as their responsibility and unique opportunity.

One of the obstacles that could be overcome in the coming period is the greater media coverage of organic products, which are still on the sidelines in the sea of aggressive advertisements of various products. Highlighting the importance of organic food can be achieved by applying the basic principles and instruments of market positioning as well as some additional instruments, such as ingredient branding, nutritional and health claims, food origin marketing, promotional and educational activities. Accordingly, the health brand can be viewed from the company's perspective in terms of conveying the importance of personal health and as a means of socially responsible behavior by emphasizing the value of public health of citizens.

A large number of citizens of our country know what these are and which organic products they are, they are informed about them in stores by means of a declaration and by asking employees who work in commercial stores. The price is the biggest obstacle why a large number of citizens do not buy organic products in the Republic of Serbia. Most of the organic products in our country are bought fresh fruits and vegetables, followed by milk and milk products. In recent years, there has been an increase in the purchase of vegetarian - vegan organic products. Also, a large number of respondents believe that organic products are sufficiently represented in stores in Serbia. In order for organic products to be sold more on the territory of the Republic of Serbia, they need to be available to target customers at an appropriate price.

In order to successfully achieve this, organizations, first of all, must communicate with their intermediaries, consumers and audiences. We believe that it is necessary to promote organic products in a proper and educational manner, so that consumers are informed about the quality of organic products, but also how and in what way organic production protects not only the health of the consumers of these products, but also the living environment in which they live and which should be left in the best possible condition for future generations.

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THE POWER OF RURAL TOURISM: INSIGHTS FROM LOCAL COMMUNITY IMPACT STUDIES

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Abstract

This paper explores the multifaceted impacts of rural tourism on local communities, synthesizing insights from various empirical studies. Rural tourism is increasingly recognized as a catalyst for economic development, cultural preservation, and social cohesion, particularly in areas with limited traditional development opportunities. Through a comprehensive literature review and analysis of case studies, this paper highlights the economic benefits of rural tourism, such as income generation and employment, which contribute to economic resilience and improved public services. Additionally, the paper examines the role of tourism in preserving local culture and heritage, fostering intercultural exchange, and reinforcing community identity. However, the social impacts of tourism are complex, with potential for both community strengthening and social tension. Environmental impacts are also discussed, noting the balance needed between conservation efforts and the risk of environmental degradation. The concept of community-based tourism (CBT) is evaluated as a model for enhancing the positive impacts of rural tourism, emphasizing the importance of local ownership, participation, and benefit-sharing. The role of policy and governance in facilitating sustainable tourism development is underscored, advocating for multi-stakeholder collaboration. This paper aims to contribute to the discourse on rural tourism by providing evidence-based recommendations for policymakers, practitioners, and community stakeholders. The findings suggest that while rural tourism has significant potential to drive sustainable development, its success largely depends on context-specific factors, effective governance, and genuine community engagement.

Keywords: *Rural tourism, community impact, community-based tourism, sustainable development, literature review.*

Introduction

Rural tourism has emerged as a significant area of interest within tourism studies, driven by the global shift towards sustainable and community-focused travel. Research indicates that rural tourism can serve as a catalyst for economic development, cultural preservation, and social cohesion in rural communities. The intricate dynamics between tourism and local communities have been explored extensively, revealing both positive and negative impacts. For instance, rural tourism can generate income and employment, fostering economic resilience in areas that may lack other development opportunities. This income generation is crucial for the sustainability of rural areas, often leading to improved infrastructure and services, which in turn can enhance the quality of life for local residents.

Economic benefits are among the most frequently cited positive impacts of rural tourism. Studies by Jaafar et al. (2013) and Liu et al. (2023) highlight how tourism can diversify rural economies traditionally dependent on agriculture. These studies suggest that the influx of tourists provides local businesses with new markets, thus promoting entrepreneurial activities. Moreover, the economic upliftment often translates into better public services and amenities, contributing to the overall development of the region. However, the extent of these benefits can vary significantly based on factors such as the level of tourism development, the local economic structure, and community involvement. In addition to economic impacts, rural tourism plays a vital role in the preservation and promotion of local culture and heritage. Researchers like Tang and Xu (2023) emphasized that tourism can help in maintaining traditional crafts, folklore, and festivals, which might otherwise decline. Cultural tourism not only preserves heritage but also provides a platform for intercultural exchange, enhancing mutual understanding and respect between visitors and locals. This cultural exchange can foster a sense of pride among residents, reinforcing their cultural identity. Social impacts of rural tourism are multifaceted and complex. While tourism can strengthen community bonds by encouraging collective participation in tourism-related activities, it can also lead to social tensions. The literature by Isaza and Salas (2024) underscored the potential for tourism to disrupt social structures, particularly if the benefits are unevenly distributed or if there is inadequate local involvement in tourism planning and management. Effective community engagement and equitable benefit-sharing mechanisms are essential to mitigate these negative social impacts and ensure that tourism development is inclusive and sustainable. Environmental impacts are another critical aspect of rural tourism, with both positive and negative outcomes documented. On one hand, tourism can promote environmental awareness and conservation efforts, as noted by Weyland et al. (2021). On the other hand, increased tourist activity can lead to environmental degradation if not properly managed. The balance between promoting tourism and preserving natural resources is a recurrent theme in the literature, emphasizing the need for sustainable tourism practices. Studies advocate for strategies such as ecotourism, which aims to minimize environmental footprints while maximizing socio-economic benefits.

Community-based tourism (CBT) has been proposed as a model to enhance the positive impacts of rural tourism. CBT emphasizes local ownership, participation, and benefit-sharing, aligning tourism development with community needs and aspirations. Research by Mora et al. (2019) suggested that CBT can empower communities, foster inclusive growth, and ensure that tourism revenues are reinvested locally. However, the success of CBT initiatives depends on factors such as community capacity, governance structures, and external support, indicating that a one-size-fits-all approach may not be effective. The role of policy and governance in shaping the outcomes of rural tourism is also highlighted in the literature. Government policies and frameworks play a crucial role in facilitating sustainable tourism development. Study by Ghaderi et al. (2018) argued that supportive policies, infrastructure investments, and capacity-building programs are essential for harnessing the potential of rural tourism. Additionally, multi-stakeholder collaboration involving governments, local communities, private sector, and NGOs is vital for integrated and sustainable tourism development. While the literature provides extensive insights into the impacts of rural tourism, there is a growing need for empirical studies that examine these impacts within specific contexts. Local community impact studies offer valuable insights into how tourism affects different communities in varied settings. These studies can reveal the nuanced ways in which tourism interacts with local socio-economic and environmental conditions, providing a more comprehensive understanding of its impacts.

The aim of this paper is to explore the multifaceted impacts of rural tourism on local communities through an empirical investigation. By synthesizing findings from various case studies, this research seeks to identify patterns and draw conclusions about the effectiveness of rural tourism as a tool for sustainable development. Ultimately, the paper aims to contribute to the ongoing discourse on rural tourism by providing evidence-based recommendations for policymakers, practitioners, and community stakeholders.

Material and methods

This study employs a systematic literature review (SLR) approach to investigate the impact of rural tourism on local communities. By synthesizing findings from various studies, this review aims to provide a comprehensive understanding of the economic, social, and environmental effects of rural tourism. The SLR method ensures a structured and replicable process, enhancing the reliability and validity of the findings.

The literature search was conducted using several academic databases, including Web of Science, Scopus, JSTOR, and Google Scholar. The following keywords and phrases were used to identify relevant studies: "rural tourism," "community impact," "economic effects," "social effects," "environmental effects," "local development," and "sustainable tourism". To ensure the quality and relevance of the literature included in this review, the following inclusion criteria were applied: peer-reviewed articles published in academic journals, studies focusing on rural tourism and its impact on local communities, publications in English to ensure accessibility and understanding, and studies published between 2000 and 2023 to capture contemporary insights and trends. On the other side, the following exclusion criteria were included: non-peer-reviewed articles, such as opinion pieces, editorials, and book reviews, studies not directly related to rural tourism or local community impacts, publications in languages other than English, and studies published before 2000 unless they provided seminal insights. Data from the selected studies were extracted using a standardized form to ensure consistency. Key information extracted included: author(s) and publication year, geographic location of the study, methodology used (qualitative, quantitative, or mixed methods), main findings related to economic, social, and environmental impacts, and recommendations for future research or policy implications. The extracted data were then synthesized using a thematic analysis approach. Themes were identified based on recurring patterns and findings across the studies. This thematic synthesis allowed for the identification of common trends, gaps, and contradictions in the literature.

To assess the quality of the included studies, the Critical Appraisal Skills Programme (CASP) checklists were used. The CASP tool evaluates studies based on several criteria, including clarity of research aims, appropriateness of methodology, rigor in data collection and analysis, and the relevance and coherence of findings. Studies were rated as high, medium, or low quality, and only those rated as high or medium were included in the final synthesis.

Results and discussion

A total of 50 studies were included in the systematic literature review after applying the inclusion and exclusion criteria. The geographic distribution, methodologies used, and main findings of these studies are summarized in Table 1.

Table 1. General information extracted from the included scientific papers

Geographic Location	Number of Studies	Methodology	Main Findings
Europe	18	Qualitative (10), Quantitative (5), Mixed Methods (3)	Economic benefits, community cohesion, environmental conservation
Asia	12	Qualitative (6), Quantitative (4), Mixed Methods (2)	Poverty alleviation, cultural preservation, resource management
North America	10	Qualitative (5), Quantitative (3), Mixed Methods (2)	Job creation, infrastructure development, environmental degradation
South America	5	Qualitative (3), Quantitative (1), Mixed Methods (1)	Economic diversification, social inequality, conservation challenges
Africa	3	Qualitative (2), Quantitative (1)	Economic empowerment, social inclusion, environmental sustainability
Oceania	2	Qualitative (1), Quantitative (1)	Community resilience, economic stability, ecological impact

The results of systematic literature review showed that rural tourism significantly contributed to local economies through job creation, increased income, and economic diversification. In Europe, several studies highlighted the role of rural tourism in enhancing local economies by creating employment opportunities and supporting local businesses. For instance, in rural Spain and Italy, tourism initiatives have led to increased income for local artisans and farmers (Maroto-Martos et al., 2020; Giaccio et al., 2018).

Table 2. Mostly defined economic impacts of rural tourism from the extracted research papers

Region	**Key Economic Benefits**	**Example Studies**
Europe	Job creation, income increase, business support	Maroto-Martos et al. (2020)
Asia	Poverty alleviation, economic diversification	Su et al. (2019)
North America	Job creation, infrastructure development	Rocket and Ramsey (2017)

According to the extracted papers, social impacts of rural tourism included community cohesion, cultural preservation, and social inclusion. Many studies, particularly from Asia and Africa, have emphasized the role of rural tourism in preserving cultural heritage and fostering social cohesion. For example, in India, rural tourism has facilitated the preservation of traditional crafts and rituals (Choudhury et al., 2018).

Table 3. Mostly defined social impacts of rural tourism from the extracted research papers

Region	**Key Social Benefits**	**Example Studies**
Asia	Cultural preservation, social cohesion	Nair and Hamzah (2015)
Africa	Social inclusion, cultural heritage protection	Haywood et al. (2020)
Europe	Community cohesion, social capital	Demirović et al. (2019)

The environmental impacts of rural tourism are diverse, ranging from positive conservation efforts to negative ecological degradation. In North America and Oceania, studies have shown both the potential for rural tourism to support environmental conservation and the risks of environmental degradation due to increased tourist activity.

Table 4. Mostly defined environmental impacts of rural tourism from the extracted research papers

Region	Key Environmental Benefits	Example Studies
North America	Environmental degradation, conservation	Raza et al. (2017)
Oceania	Conservation efforts, ecological impact	Abascal et al. (2016)
Europe	Environmental conservation	Demirović et al. (2018)

The economic benefits of rural tourism are evident across different regions, with significant positive impacts on local economies. Job creation and income generation are the most frequently reported benefits, highlighting the potential of rural tourism as a tool for economic development. However, the extent of these benefits varies based on the region's existing economic conditions and the scale of tourism activities. Social impacts are also substantial, with rural tourism playing a critical role in preserving cultural heritage and fostering social cohesion. The review indicates that community involvement and participation are key factors in achieving positive social outcomes. In regions like Asia and Africa, where traditional cultures are rich and diverse, rural tourism has been instrumental in maintaining cultural practices and enhancing community bonds. Finally, environmental impacts present a more complex picture, with both positive and negative outcomes reported. While rural tourism can promote environmental conservation by generating funds and awareness for conservation projects, it can also lead to environmental degradation if not managed sustainably. The balance between promoting tourism and preserving the environment is crucial, requiring effective policies and community involvement.

Conclusions

This study set out to explore the multifaceted impacts of rural tourism on local communities through a comprehensive empirical investigation. By synthesizing findings from a diverse range of case studies, we have identified several patterns that highlight the effectiveness of rural tourism as a tool for sustainable development. Our research indicates that rural tourism can significantly contribute to economic growth, social cohesion, and environmental conservation within local communities. Economic benefits are evident through increased income opportunities, job creation, and the stimulation of local businesses. Socially, rural tourism fosters a sense of pride and community identity while promoting cultural exchange and preserving local traditions. Environmentally, responsible tourism practices have been shown to enhance the conservation of natural resources and biodiversity. However, the literature review also revealed challenges that need to be addressed to maximize the benefits of rural tourism. These include the need for improved infrastructure, the risk of cultural commodification, and the potential environmental impact of increased tourist activity. Effective management and strategic planning are crucial to mitigating these challenges.

Based on our findings, we offer several evidence-based recommendations for policymakers, practitioners, and community stakeholders. Policymakers should prioritize sustainable tourism policies that balance economic, social, and environmental goals. Practitioners are encouraged to adopt best practices in tourism management, emphasizing community involvement and environmental stewardship. Community stakeholders should actively participate in tourism planning and development to ensure that their needs and perspectives are adequately represented.

In conclusion, rural tourism holds significant potential as a catalyst for sustainable development in local communities. By leveraging its benefits and addressing its challenges, rural tourism can create a more resilient and prosperous future for rural areas. This research contributes to the ongoing discourse on rural tourism and provides a foundation for further studies to build upon, ensuring that rural tourism continues to evolve as a viable and sustainable development strategy.

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THE INFLUENCE OF YIELD AND PRICE FROM THE CURRENT YEAR ON SOWING AREA OF POTATO IN FOLLOWING YEAR – A MULTIPLE LINEAR REGRESSION

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Abstract

The subject of this research is the dependence of yield and price on the sowing area of potato in the Republic of Serbia. The goal of the research is to observe the impact of the natural (yield) and economic(price) results of potato from the current year on the sowing area in the following year. The analyzed period is 2005-2022 and the basic tools of descriptive statistics. The average yield of potato was 16.76t/ha. The average price was 130,92euro/t. Potato yield (X_1) and price (X_2) are growing at annual rates of 1.42% and 21.93%, while potato sowing area in hectares (Y) decrease at annual rate of -4,91%. In order to establish the influence of yield and price of potato on the number of sowing hectares in the next year, a multiple linear regression model is created: $\hat{Y} = 86201{,}051 + 1555{,}001X_1 + 148{,}97X_2$. The statistical significance of the model is tested by ANOVA and the obtained results determine the statistical significance of the model ($p<0.05$). The estimated parameters of the regression model are also significant, and there is a positive relationship between the yield and price from the current year, on the number of sowing hectares of potato in the next year. The model demonstrates a positive significant impact of both independent variables on the sowing area of potato in Serbia, but the yield has a greater impact than its price.

Key words: *potato, yield, price, sowing area, Serbia.*

Introduction

Potato production in the Republic of Serbia is very important forits agriculture. In this research authors quantify the influence of production and market results in potato production, in the previous year, on the sowing area of potato in the Republic of Serbia in the next year. For this purpose multiple linear regression models are used. Novkovicet all. (2024) used a multiple linear regression model for established dependents of yield and price of sugar beet on sowing area in the next year. Since 1993. the scientific team of the Department of Agricultural Economics, Faculty of Agriculture, University of Novi Sad, Serbia developing statistical models in agriculture (Nikolic-Djoricet all., 1993). In a long period scientific team developed and published many scientific papers: Novkovicet all 2019.; Nikolic et all.2022.; Novkovicet all 2022.; 2023; 2024.

The subject of this research is the dependence of yield and price on the sowing area of potato in the Republic of Serbia.

The goal of the research is to observe the impact of the natural (yield) and economic(price) results of potato from the current year on the sowing area of potato in the following year in Serbia.

Material and Methods

The main statistical methods used in this research were descriptive statistical analysis and multiple linear regression.

Descriptive statistics consist of:

1. Average value$\overline{X}$
2. Extreme value (min, max)
3. Coefficient of variation (CV)
4. Annual rate of change % (r)

The annual change rate is calculated directly from the absolute data of the analyzed series using the following expression:

$$Y = (G - 1); G = \left(\frac{Y_n}{Y_1}\right)^{\frac{1}{n-1}}$$

Where is: r - annual rate of change, G - constant relative change of the phenomenon, Yn - absolute value of the last member of the series, Y1 - absolute value of the first member of the series and n - total number of members of the series.
For the purposes of revealing the impact of potato yield and price, on potato sowing area in the next year, a multiple linear regression model was applied. Regression analysis is a method whichis widely used to determine the relationship between the included variables. Additional analysis determines the direction and strength of the identified connections.Also, regression analysis can be defined as an assessment of the value of the dependent variable based on one or more independent variables (Mutavdžić and Đorić, 2018):

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \cdots + \beta_p X_{pi} + \varepsilon_i$$

Where Yi represents the value of the dependent variable, X_{1i}, X_{2i}, … , X_{pi}represent the values of the independent variables and $\beta_1, \beta_2,$ ….. $\beta_p$are regression parameters. Parameterβ_0 ,shows the average initial level of the dependent variable, while$\varepsilon_i$is accidental mistake. To check the statistical significance of the defined model as a whole, a regression variance analysis was performed. The data used in the analysis were taken from the website of the Statistical Office of the Republic of Serbia. The data refer to the price of potato, the yield of potato, year production of potato, well as the potato sowing area in the Republic of Serbia for the period from 2005-2022 years. The IBM STATISTICS 21 software package was used for statistical data processing.

Results and discussion

Based on the official statistical data of the Statistical Office of the Republic of Serbia (table1), a quantitative analysis of the influence of yield and price of potato on the sowing area of potato in the next year was calculated.
Price in linearregression modelwas in euro per ton. Nominal price in RSD (Republic of Serbia Dinar) per ton isexchanged in euro per ton, by official change rates for eachyear National Bank of Serbia. It wasdoneso, that data couldbecomparedon the international level.

Table 1. Production and value elements of potato production in the Republic of Serbia in the period of 2005-2022

Year	Sowing area in ha	Yield in t/ha	Year production in ton	Nominal price in RSD/t	Nominal price in EURO/t
2005	58.529	16.6	969.562	7.010	81.59
2006	58.180	16.0	930.305	13.180	167.30
2007	56.102	13.2	743.282	14.880	187.01
2008	55.993	15.1	843.545	17.040	195.19
2009	53.925	16.7	898.282	15.090	157.21
2010	52.839	16.8	887.363	23.400	220.18
2011	54.057	16.5	891.513	24.030	233.45
2012	52.035	11.1	577.966	24.930	219.57
2013	50.740	15.1	766.829	28.170	245.58
2014	51.987	11.4	592.046	24.330	200.20
2015	41.658	15.3	639.410	23.950	204.35
2016	40.105	17.8	714.350	20.240	163.99
2017	38.472	15.3	589.241	22.780	191.20
2018	28.232	17.3	487.909	32.020	270.72
2019	34.110	20.6	702.086	30.590	260.25
2020	29.676	22.4	664.891	22.730	193.32
2021	26.388	23.3	613.785	26.170	222.57
2022	24.870	21.1	523.762	56.070	477.96

Source:Statistical Office of the Republic of Serbia

Based on the official statistical data of the Statistical Office of the Republic of Serbia(table 1), a quantitative analysis of the annual production of fattening pigs, as well as the price of pigs and corn in the period from 2005 to 2022 in the Republic of Serbia, was carried out. Based on the average annual prices of fattening pigs and corn, the average annual parity was calculated.
The main elements of descriptive statistics for the production and value characteristics of potato are presented in table 2.
The total sowing potato area in the Republic of Serbia were 44.883 hectares per year, on average. They were changing from 24.870 ha in 2022, to 58.529 ha in 2005. The coefficient of variation in the observed period was 26,58%. Average yield of potato in the observed period was 16.76 t/ha. Maximum yield was 23.30 t/ha in 2021, while minimum yield was 11.10 t/ha in 2012. Total average year production of potato in the Republic of Serbia in the period 2005-22. Was 675,056 ton. Maximum potato production was almost 970 thousand tons, and in the last year of analyzed period was a minimal production, 488 thousand tons.
In the second part of the research, on base of independent variables (yield and price of potato) multiple regression model is formulated. Dependent variable was the sowing area of potato in the next year. For testing of the model, the first was tested the significance of the model by using analysis of variance (table 3).

Table 2. Descriptive statistics independent and dependent variables in the Republic of Serbia in theperiod 2005-2022

Elements	**Average**	**Minimum**	**Maximum**	**Coefficient of variation in %**	**Year change rate in %**
Sowing area (ha)	44,883.22	24.870	58.529	26.58	-4.91
Yield (t/ha)	16.76	11.10	23.30	20.13	1.42
Year production (t)	675,056	487,909	969,562	29.52	-3.56
Price (EUR/t)	130.92	81.59	477.96	74.35	21.93

Source: Author's calculation

Table 3. Analysis of variance for regression model dependency of sowing area, form yield and price of potato in the previous year

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1485948414.342	2	742974207.171	14,136	0,000
	Residual	735835099.893	14	52559649.992		
	Total	2221783514.235	16			

Source: Author's calculation

Based on analysis of variance, it can be concluded that formulated model is statistically significant. ($p<0,05$).
Evaluate parameters of the regression model are shown in table 4.

Table 4. Regression model dependency of sowing area, form yield and price of potato in the previous year

Model		Un-standardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	86201,051	9432,177		9,139	0,000
	Yield	1555,001	574,835	0,0434	2,705	0,017
	Price	148,97	41,326	0,579	3,604	0,003

Source: Author's calculation

Based on getting results from table 4 multiple regression model is:

$$\hat{Y} = 86201{,}051 + 1555{,}001X_1 + 148{,}97X_2 + \varepsilon$$

The first coefficient of regression, parameter β_1 profile independent variable, yield of potato, show a high statistically significant ($p<0,05$),while the second regression coefficient profile second independent variable, the price of potato, and it is also statistically significant

($p<0,05$),Both independent variables (yield and price) have a positive influence on dependent variable, sowing area of potato in the next year.
Increasing yield for one ton per hectare increases the sowing area of potato for 1,555 ha in the next year. Increasing the price of potato for one euro per ton, increase the sowing area for 149 hectare in the next year.

Conclusion

The results for the observed period from 2005-2022. year showed:
- the average potato sowing area in Serbia was 44,883 hectares,
- potato sowing area decreased by average annual rate of -4.91%,
- the average potato yield was 16.8 tons per hectare,
- potato yield increased by the average annual rate of 1.42%,
- the average potato year production 675.000 ton,
- potato year production decreased by average annual rate of -3.56%,
- the average price of potato was 131 euro per ton,
- potato price increased by an average year rate of 21.93%,
-the research results show a statistically significant influence of yield and price of potato in the present year, on sowing potato area in the next year,
-both independent variables (yield and price of potato) have a positive influence on potato sowing structure in thenext year,
- much higher influence on sowing area of potato in the next year has potato yield in the present year, than the price of potato,
- one ton per hectare more, increase sowing area of potato for 1555 ha in the next year.
- one euro per ton increase in the price of potato, increase sowing area for 149 hectares in the next year.

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CHALLENGES OF SETTING UP AN ORGANIC VEGETABLES COOPERATIVE IN SERBIA

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Abstract

The presentation describes action research in Strength2Food, a H2020 project to improve effectiveness of food quality schemes and public sector food procurement (including schools) and to stimulate short-food-supply-chains. The EUTA team helped 5 organic producers from Vojvodina and Belgrade to establish an organic cooperative BioLogika, with the main objective to directly supply primary schools with fresh organic vegetables. This was a financial opportunity for them, and an opportunity to contribute to the community. Main challenges were to identify genuine organic growers, and those with necessary expertise (including understanding of the procurement tendering process), certifications, commitment, and to establish a basis of trust amongst each other and us, and arrange distribution and deliveries to ensure that schools were supplied regularly and on time with a variety of organic vegetables throughout the whole school year, and, finally, avoid significant meal price increases for parents. A key factor determining the scheme’s success was convincing schools to modify food procurement tenders to provide a separate lot for “organic vegetables” to allow the cooperative to compete with other suppliers. Thus, contracts with 2 Belgrade schools were signed in 2019 and 2020, but the Covid-19 pandemic significantly reduced meal numbers and, because of the small scale of school contracts, the coop closed a year after the Strength2Food project ended. Nevertheless, we believe this model for local organic cooperatives targeting school meal vegetable (and fruit) supply has potential elsewhere, given effective management and marketing skills, and negotiation with schools, which recognise the health benefits of organic produce.

Keywords: *Short food supply chains, organic, public procurement, school meals, rural development.*

Introduction

This report discusses the findings from an action research effort in Serbia, as part of the EU H2020 project ‘Strength2Food’ (2016-2021). Strength2Food (S2F) undertook research and demonstration activities to improve the effectiveness of food quality schemes, public sector food procurement (PSFP) and to stimulate short-food-supply-chains (SFSCs). The work described here formed part of a pilot scheme of school meals initiatives in Serbia to stimulate SFSCs, focusing on years 1 and 2 in primary schools (7-8-year-olds). Anecdotal evidence indicated that meal provision in Serbia’s approximately 1200 primary schools was very varied, and that meal quality was unlikely to be good. Although at the outset of the project, the Ministry had a database on educational aspects of each primary school, it had no database of information on school meal provision. We knew that the large majority of these schools had to provide their own food through public procurements prepared and carried out independently by each school. The Serbian Law on Public Procurement required schools to accept the cheapest bid, which in practice meant that children were typically given meals using poor quality ingredients. Although the nutritional content of meals given daily to

children at kindergarten was carefully regulated, very little was known at that time of the nutritional composition of primary school meals.
Serbia has many growers of organic vegetables and fruit, though these are generally rather dispersed and few of them have considered forming organic food cooperatives - only one had been formed and supplying schools was not their priority either because of the size of the contracts and frequency of deliveries required or their business model was focused on exports. Although school directors were all in favour of improving their children's school meal quality, especially because of a widespread belief that Serbian vegetable and fruit growers use crop protection chemicals excessively, organic produce in Serbia is generally extremely expensive compared with the rest of Europe because of low market demand. Thus, schools were reluctant to commit to including organic produce in their food procurements because of fears that parents would object to an inevitable steep rise in the price charged for their children's meals.
Proponents of both the supply and demand sides of the school meal equation agreed that school children would benefit considerably from eating meals with organic ingredients, but considerable scepticism and challenges faced the EUTA team in achieving this.
Thus, the objective of the research described here was to find a "win-win" formula acceptable to both sides during the 5-year S2F project. The approach adopted was action research. Action research provides an opportunity to engage with research subjects in a collaborative project to facilitate and bring about changes which have been agreed upon by all parties, but places high ethical demand on researchers to take responsibility for the social consequences of the research (Hilsen, 2006). It aims to increase knowledge and change some aspect of the world at the same time. By its nature, action research requires extensive stakeholder engagement and collective discussions to identify the direction of and barriers to change, and requires careful thought about where we were going at each stage, weighing transformative potential against risks (e.g. *of intervening in supply-chain dynamics; of leaving schools in a worse position and even of children not being fed!).* We were mindful throughout our approach of the need to create win-wins for all.
The action research had 2 elements, the first involving a set of arrangements to understand the current situation (e.g. with schools, municipalities and ministries) with regard to PSFC contracts. The second was a set of SFSC arrangements for the co-ordination of local farmers to fulfil supply contracts to provide better quality food for local schools within local municipalities.

Materials and Methods

Our research began with an initial situation analysis in Serbia of the policy and market environment for food procurement by primary schools. Thus, at the beginning of the project a comprehensive questionnaire was sent to schools by the Ministry of Education to collect information on school meal provision, its type, scale and whether it was provided by caterers or the school's own cooks. This established a baseline of school meal provision, used to select suitable schools to approach to take part in the action research.
The subsequent action research necessitated engagement with a number of actors in various settings (central and local policymakers; school directors, administrators, catering staff; actual and potential suppliers; nutritionists, experts in public procurement).
Improving primary school meal quality required discussions with the following stakeholders:

- 104 meetings with school directors and S2F school coordinators from Belgrade, Novi Sad, Valjevo and Arilje areas of Serbia
- 2 joint meetings with Novi Sad school directors/representatives

- 9 meetings with representatives from Ministries (Education, Agriculture); 16 meetings with local government representatives in Novi Sad, Belgrade, Valjevo and Arilje
- 4 meetings with Vojvodina Chamber of Commerce, and public company ‘Market Place’
- 10 meetings with agricultural extension and advisory services
- 10 meetings with existing agricultural co-operatives and associations
- 3 meetings with large existing commercial supplier of food to schools
- 24 meetings, 10 Skype meetings and 2 presentations with/for producers from Vojvodina and around Belgrade, and the towns of Valjevo and Arilje
- Food fair discussions with organic growers in Belgrade and Novi Sad
- 7 meetings with representatives of USAID, Nordic embassies, Terras NGO working with organic producers, public procurement experts, nutritionists, organic food restaurant.
- 3 Forums, including: “Opportunities for suppling primary schools with organic vegetables”.

In addition, extensive procurement documentation on school meal tenders was downloaded from the Serbian public procurement portal and analysed according to tender criteria, number of lots, food category, ingredients, quantities, target prices, as well as accepted bidder unit prices. During school visits, weekly menus and meal normatives (where available) were collected and analysed for nutritional compositions using the Serbian EuroFir (European Food Information Resource) database. This allowed the existing nutritional quality of school meals to be established, as well as typical quantities of fresh vegetables in meals, as these would be the target foods for organic growers to bid for.

Results and Discussion

Demand-side patterns

Our initial questionnaire (completed by ca 900 schools) and school visits established baseline characteristics for school meal provision. Just over 25% of Serbia’s primary schools were found to provide meals for children using their own kitchens and cooks. The average price of the school lunch for parents was around €1.21 (range €0.33-2.28 in 2017). School directors could charge whatever amount they decided for school meals, provided the school's parents council approved it. Ministry regulations required the amount charged to parents to be sufficient only to cover meal ingredient costs (no kitchen staff or running costs could be charged to parents). In reality most schools had a poor idea of how much each meal cost, so some schools charged too much to parents, whilst others sometimes ended up subsidising the cost of meal ingredients.

Procurement of all products and services related to school meal provision had to be carried out in accordance with national public procurement law. The food procurement process was not centralized, which made schools responsible for their own procurement. In consequence, to minimise the administrative load for food procurements, documentation was usually "copy-pasted" from previous years' documentation.

Schools could vary the numbers of lots from one (a single supplier for all foods) to at least nine (given the average size of contracts, this could mean potentially nine different suppliers bidding for lots worth maybe no more than €250). Our analysis of procurement documentation showed that the most frequent number of lots used by schools for food procurement was one. This meant around 25% of all schools buying food for their own meals used general food distributors to deliver everything, again reducing the school's administrative load. Food suppliers varied from small companies, with a local catchment area to major national food suppliers.

Table 1. Value of annual procurement contracts with Serbian primary schools for 10 food suppliers for 12 months during 2016-2017

Supplier name	Supplier location	Type of supplier	Annual public procurement contract value (million RSD)	Number of public procurement contracts	Number of public procurement contracts with OŠ schools	Total OŠ school contract value (million RSD)	Total OŠ school contract value % total value	Average OŠ school annual % total contract value
Avala Merkur	Beograd	General	36.93	16	3	2.74	7.42	2.47
Big Trade doo	Novi Sad	Meats	83.86	26	3	3.72	4.44	1.48
Božilović Luxor doo	Svilajnac	General	107.46	90	13	14.03	13.10	1.00
Illi Group doo	Novi Sad	General	595.11	28	2	8.22	1.38	0.69
Ila Promet doo	Čačak	General	153.77	71	12	8.76	5.70	0.47
Komercservis-produkt	Novi Sad	General	248.17	71	15	9.70	3.91	0.26
Mlekobel doo	Novo Miloševo	Dairy	65.02	21	2	1.70	2.61	1.31
Štrand doo	Novi Sad	Meats	8.83	10	3	3.83	43.36	14.45
Univerexport doo	Novi Sad	General	162.98	79	5	15.08	9.26	1.85
ZZ PKB Povrtar	Beograd	Vegetables	206.08	35	1	0.53	0.26	0.26

Only a few schools used the services of an external agency to organise their procurements, as they had to pay for this out of their own budget. Complicated tendering procedures and lack of competence for their preparation were big issues for schools, so any suggestion to change the procurement documentation wording to improve quality criteria was met with resistance and suspicion that it would be "against the law". Most importantly, the current legal provisions defined 'lowest economic price' as the key selection criterion in procurement decision making.

Because school contracts were generally of relatively small value for many companies (only 0.25% to 2.5% the value of all annual public sector food contracts for the large food companies, Table 1), primary schools were often given a low priority when it came to scheduling delivery times, so food was occasionally delivered too late to prepare lunch, for example.

Our initial analysis showed that PSFP tended to generate sub-optimal effects for food quality and quantity (food content, freshness), and little or no attention was paid to food origin (with impacts for SFSCs and local economic development, as well as carbon footprints). Further,

- the PSFP process did not always encourage strategic planning of procurement needs
- the PSFP process did not always encourage the formation of an effective market
- inadequate specification of demand often affected supply-side outcomes (e.g. food quality)
- inadequate specification of demand often affected demand-side outcomes (e.g. excessive contract variation)
- end user dissatisfaction was usual (food preparation chefs, consumers – parents and children)

Supply-side patterns

It was also important to assess the situation from a systemic supply-side perspective; even if action was to be taken to improve the situation on the demand-side, would there would be capacity on the supply-side to respond? An assessment of supply-chain patterns identified four key possibilities for promoting SFSCs: 1) a more strategic approach from market intermediaries to use local products in the supply chain for PSFP, 2) using single local producers to provide products fitting the needs for primary school food procurement, 3) using existing co-operatives whose product range provided a good fit with needs for primary school food procurement, and in which trade with local producers is prioritised, and 4) developing new co-operatives of local producers to provide products that fit the needs for primary school food procurement.

We found no incentive for other market intermediaries to invest in any greater commitment to or strategy for developing SFSCs. We also found a general mis-match in the scale and scope of existing co-operatives to supply to our primary schools. Furthermore, we found no existing single local producers of sufficient scale and scope to engage effectively in primary school food procurement. In this situation, we concluded that the most promising alternative to serve the needs of primary school food procurement was new co-operative development - potentially one of the most challenging alternatives, and many stakeholders we consulted (especially producers themselves) were generally sceptical about the chances of overcoming those challenges.

Implementing improvements

The best option for SFSCs was to gather local organic producers into a new organisation that would meet regulatory requirements. The project team worked to do this with a group of 6 organic vegetable producers in Vojvodina who were interested in selling organic food to schools in the Novi Sad and Belgrade areas. However, these producers had 5 challenges to overcome.

Challenge 1: Length of school year

The school year lasts for around only 40 weeks of the year, so that there is no demand for around 3 months in the summer (June-August). To overcome this challenge and establish a viable business model for SFSC arrangements, an innovative scheme was proposed and discussed with all stakeholders that combines public procurement and an organic box scheme for parents. The box scheme involved selling organic vegetables directly to parents, using the school as a neighbourhood collection point. The box scheme provided the level of scale required to make the overall supply arrangements viable, notwithstanding the length of the school year.

Challenge 2: Maintaining year-round supply

Maintaining year-round supply might be difficult, especially during the April-June “hungry gap”, requiring additional consideration of the benefits and costs of solutions such as polytunnels and cold storage to extend the season and variety. We helped here as follows: we established months of the year that producers were able to supply particular produce, and in what quantities; we introduced new standardised menus in schools using the S2F Excel Meal Planner (Quarrie, 2021), which targets use of seasonal vegetables in good local supply at particular times of the year; we established a procurement framework agreement to recognise that some organic vegetables will be unavailable out of season; and some of the larger organic producers were investing in polytunnels and cold storage to extend the season and variety.

Challenge 3: Engaging with the procurement process

Small producers often have difficulties in preparing the excessive tendering documentation. Thus, we gave technical support for local producers to help them understand how to bid for tenders. Although we encountered hesitancy from schools in dealing with a new-entrant to the supply chain, we worked with schools interested in buying organic vegetables as well as experts in procurement documentation to ensure that no unnecessary eligibility criteria were included to prevent a new organic co-operative from bidding. To build trust we brokered meetings of producers and school directors, administrators and chefs, including sampling of the produce.

Challenge 4: Arrangements for distribution and deliveries

Although growers were widely dispersed around Vojvodina, the initial arrangements necessary for collecting together the produce from different local producers ready for delivery to the schools could be achieved within producers’ existing resources, and negotiation of delivery dates and times with schools allowed producers to schedule deliveries to fit in with existing customer arrangements as far as possible.

Challenge 5: Forming a new legal entity: the new co-operative

Despite initial scepticism, as they collectively worked through their issues and questions, a strong commitment developed to doing everything possible to make the scheme work, with support and advice from the S2F team on different types of agricultural co-operatives (and organic box schemes) and how they work. In response, an agreement on the establishment of a new co-operative, 'BioLogika', was signed by five producers on 5th February 2020 and officially registered by the Serbian Business Registers Agency on 11th March 2020.

Challenge 6: Size of procurement contracts and meal cost increases

There were problems of the small size of procurement contracts for the primary schools, for which we discussed the box scheme to supplement income, and using school procurement budgets and prices given by producers we showed that, despite the relatively high prices for organic vegetables in Serbia, buying fresh vegetables direct from local organic growers would increase schools' annual food budgets by only ca 5-6% (Table 2).

Table 2. Increase in schools' annual food budgets required to include organic vegetables

Location	School	Total annual contract including VAT	Conventional vegetables including VAT	Organic vegetables excluding VAT*	Annual contract increase for organic veg	% Annual contract increase for organic veg
Novi Sad	Kosta Trifković	4943780	172018	579215	407197	8.24
Novi Sad	Ivo Lola Ribar	2796781	179011	256710	77699	2.78
Novi Sad	Djordje Natošević	5596271	283140	416385	133245	2.38
Novi Sad	Djura Daničić	2194758	134257	231275	97018	4.42
Novi Sad	Petefi Šandor	6969305	349217	736265	387048	5.55
Belgrade	Drinka Pavlović	4909833	219120	379779	160659	3.27
Belgrade	Dositej Obradović	1862630	153549	284984	131435	7.06
Novi Sad	Dositej Obradović	4986957	194073	511229	317156	6.36
		* Co-operative sales too small for school contracts to incur VAT.				

Action research success: school contracts with BioLogika for organic vegetables

The first primary school in Serbia to submit procurement documents using the standard text produced by procurement legal experts for fresh organic vegetables was submitted in July, 2020. The new co-operative, BioLogika, submitted its bid correctly by the deadline, and won the contract in late August by offering the only bid. A second Belgrade school submitted procurement documents for organic vegetables soon afterwards which Biologika also won. However, by this time Covid-19 pandemic restrictions meant that some schools had shortened the working day and/or ceased to provide school meals, or changed meal numbers. The Covid-19 restrictions also prevented EUTA researchers from supporting the co-operative in setting up a box scheme for parents as well as promoting the benefits the co-operative could offer through accessible organic vegetables for children in other schools. Thus, BioLogika became a victim of the pandemic, as its subsequent lack of income led to it closing down during 2021.

Nevertheless, we feel that an important basis for change has been put in place. Establishment of a new co-operative, formed from high-quality small-scale local producers to supply primary schools through PSFP, has provided a demonstration of what can be achieved with a level of network brokerage and creative stakeholder engagement. Our action research was an example of 'vertical mutuality' (Simmons, 2020), in which policy stakeholders retain an interest and engagement, rather than simply delegating responsibility to lower levels of co-ordination.

Conclusions

This research intervention sought to overcome some of the difficulties involved in improving the quality of primary school meals in Serbia, through the construction of innovative partnership and collaborative working arrangements that align the incentives throughout the system. This has required action research interventions in (i) the policy and enabling environment, on both (ii) demand and (iii) supply sides, and (iv) in relation to contract/relationship management. The research revealed the need to develop potential new supply arrangements, through both strengthening supply chain co-operation and organising local producers to supply.

Although sceptical at the beginning, crucially, when we asked people if they would lend their support to a collective change effort, very few people ever gave us an outright 'no'. Our initiative appeared to have exceeded stakeholders’ expectations in terms of bringing high-quality, locally-grown organic produce into the school kitchen - with an additional potential positive outcome of substantially increasing the incomes of smallholder farmers. Our action research helped to:

- enhance the quality of food served to primary school children
- enhance farmer co-operation through clearly-defined, shared goals (whilst also raising farmers’ incomes and acknowledging the dignity in their labour)
- build stronger partnerships between municipalities and their communities

The fact that the ‘BioLogika’ co-operative subsequently closed should not be seen as detracting from the considerable achievements described here. On a practical level, there is almost certainly potential to build a broader network of ‘trusted’ producers in various locations, who are together capable of building appropriate supply structures to encourage all the social, economic and environmental benefits of SFSCs.

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COMMON AGRICULTURAL POLICY INCENTIVES FOR HEDGEROW MANAGEMENT: A SLOVENIAN CASE STUDY

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Abstract

Hedgerows are recognised as linear structures of woody and shrubby vegetation occurring in different agricultural ecosystems. They are crucial in preserving biocultural diversity and associated ecosystem services in agricultural areas. The general public primarily identifies the conservation and environmental values of hedgerows, which have been extensively researched with numerous studies available. When farmers manage hedgerows within their cultivated areas, they often face challenges as hedgerows can obstruct their farming activities such as reduced crop yields, increased production costs, lower profitability. In response to these challenges, the Common Agricultural Policy (CAP) has supported farmers to allocate space for hedgerows on agricultural land and enhance ecological connectivity through financial compensation schemes. In the previous CAP (2015-2020) as well in the present CAP Strategic plan (2023-2027) specific financial support for hedgerow management and preservation have been addresed. However, regardless of specific hedgerows CAP financial support, Slovenia is witnessing hedgerow degradation. The paper aim is a comparative analysis between previous and present hedgerow operations and the evaluation of financial support for specific farm. The farm is located on intensive agricultural area with land consolidation measures, but still with a fairly well-preserved network of hedgerows. The effectiveness of hedgerow operation for CAP period (2015-2020) and CAP Strategic plan (2023-2027) for considered farm showed significant decreased of financial support of considered farm. The decrase is largely related to the change of calculation from EUR/m to EUR/ha.

Keywords: *Hedgerow, CAP, agriculture area, comparative analysis.*

Introduction

The agricultural landscape is a dynamic system that is constantly evolving. Particularly in intensive agricultural ecosystems, changes occur, causing positive and negative environmental effects (Golobič *et al.*, 2015). One negative consequence is the disappearance of agricultural landscape elements, e.g., hedgerows, stone walls, riparian vegetation… (Boughey *et al.*, 2011). In Slovenia, hedgerows are the most recognized agricultural landscapes. They are included in CAP measures, known as linear strips of woody and shrubby vegetation that appear in various agricultural ecosystems: in lowlands, valleys, on hills at different altitudes, and relief forms (Burel, 1996; Boughey *et al.*, 2011). Their role in maintaining biodiversity in areas of intensive agriculture is well-established, providing numerous environmental benefits such as wildlife habitat, soil erosion control, and microclimate regulation (Merot, 1999; Montgomery *et al.*, 2020; Tromp-van Meerveld *et al.*, 2007). These functions are widely acknowledged by the public and have been extensively documented in the scientific literature (Borec *et al.*, 2023). Despite these benefits, farmers often encounter significant challenges in managing hedgerows. Notably, the presence of hedgerows can obstruct farming operations leading to reduced crop yields, elevated production costs, and diminished profitability (Montgomery *et al.*, 2020; Albrecht *et al.*, 2020; Alignier *et al.*, 2020).

The Common Agricultural Policy (CAP) has introduced measures to encourage farmers to integrate hedgerows into their farming practices, recognizing the importance of hedgerows for biodiversity and sustainable agriculture. Under Pillar II (Rural Development Programmes), voluntary schemes provide financial incentives to support farmers in adopting more sustainable land management approaches. These schemes offer compensation to mitigate the economic costs of hedgerow maintenance while promoting ecological connectivity across agricultural landscapes. In response to the decline in hedgerows and its impact on biodiversity, Agri-environment-climate (AEC) commitments are designed to maintain or restore hedgerow networks, ensuring their ecological and agricultural benefits (Turpin et al., 2015).
The CAP provides financial support for hedgerow conservation in several EU countries. For example, in France, farmers receive up to 0.40 EUR per meter for planting and maintaining hedgerows. Maintenance is provided as a "*bonus Haie*," approximately 7 EUR per hectare (Label Haie, 2023). In the UK, the Environmental Stewardship scheme provided payments of up to £13.52 per meter for hedgerow restoration (Government of the United Kingdom, 2023). In Germany, funding for hedgerows is currently available through Agri-Environment-Climate Measures (BF 8). Establishing hedgerows is supported with 12.068 EUR per hectar (1.20 EUR per meter) (BaumLand - Eine Kampagne des Fördervereins Arbeitsgemeinschaft bäuerliche Landwirtschaft, 2024).
In Slovenia, the official records include 4522 hedgerows totaling 458.5 km (Lampič and Kastelic, 2021). Slovenian farmers have received financial support for preserving hedgerows since 2014. The paper evaluates hedgerow financial support under the previous (2015–2023) and current (2023–2027) CAP frameworks.
For these propose, the specific farm was examined. The farm is situated in the Drava Plain, a region in Slovenia recognized for its high agricultural potential due to its flat relief, fertile soils, and favorable climatic conditions (KIS/eTLA, 2023). The farm cultivates 38.66 hectares of agricultural land owned and leased.

Hedgerows CAP financial support in Slovenia

In the previous programming period of the CAP, the "Preservation of Hedgerows" (KRA_MEJ) operation, part of the Rural Development Program 2014–2020, could only be implemented within Natura 2000 sites, with an annual payment of 1.60 EURO per linear meter for hedgerows at least 10 meters long and no more than 20 meters wide (Ministry of Agriculture, Forestry and Food, 2019). In the new CAP 2023–2027 period, however, the "Hedgerow Conservation" (MEJ) operation expands its scope to sites beyond Natura 2000, allowing implementation across a broader range of agricultural landscapes, with payments of 450 EURO per hectare for hedgerows covering a minimum of 25 m² (Ministry of Agriculture, Forestry and Food, 2023). A significant change is the shift from payments per linear meter to payments per hectare, reflecting a major adjustment in the funding approach. Both operations aim to preserve hedgerows, but the expansion of eligible areas and revised financial support mark important shifts in the new CAP period. The CAP measures were delayed by three years due to the COVID-19 pandemic so they just started in 2023.

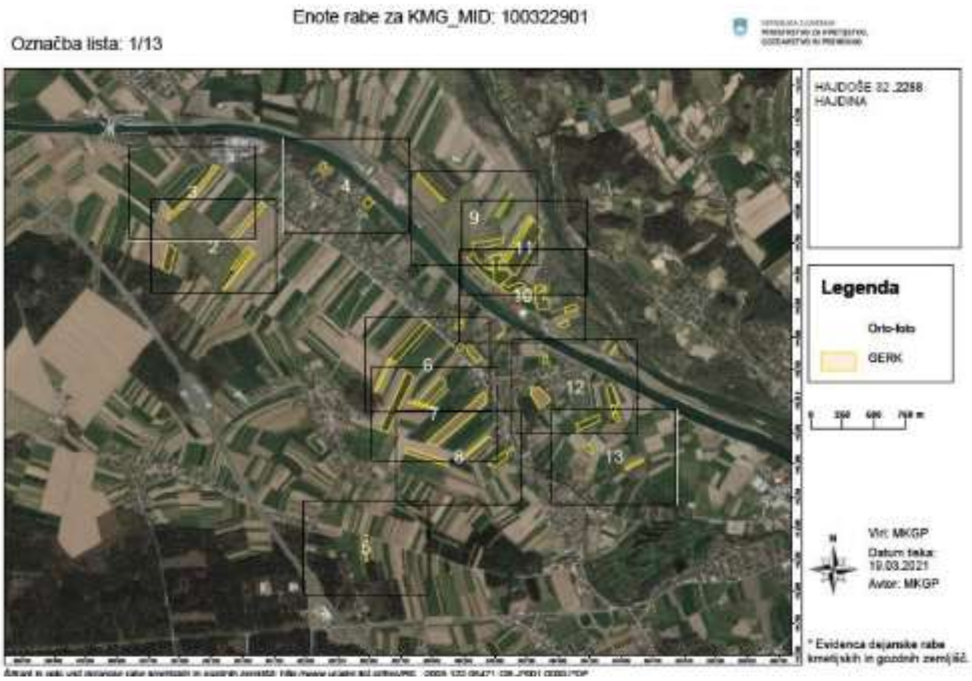
Material and Methods

Study Area

The case study farm is intended for intensive agricultural production due to the favorable climate, flat relief, and land consolidation processes (Novak et al., 2023). The soil type is characterized by Alluvial soils, Eutric, and Calcaric (Vidic et al., 2015).

A characteristic of the farms in the study area is the fragmentation of agricultural land with a diversity of crop products. The area between the agricultural land is covered by corridors mostly formed by hedgerows.
The case study farm operates with a mixed crop-livestock system, rotating crops extensively for sale and animal feed. They cultivate 38.66 hectares of agricultural land owned and leased (Figure 1). The agricultural producer has hedgerows located both within and outside the Natura 2000 protected sites.

Figure 4. Map of the case study area with the selected farmer's agricultural land (yellow).



**Source: Javni prikazovalnik grafičnih podatkov MKGP (2024)*

Hedgerows and Graphical Unit of Agricultural Land Use (GERKs) Area
The farm registration data for the year 2022 was derived from the KMRS_2022 input layer. To comply with the previous and current “Hedgerow Operation”, we used a layer of non-productive habitats, which include hedgerows, obtained from MKGP and the layer of the Natura 2000 sites (only used for the current CAP period).
For the period of the previous CAP (2015-2020), the subsidy amount of the operation was calculated only for the hedgerows located in the Natura 2000 sites with a total of 7 hedgerows on the case study area. The current CAP period (2023-2027) include subsidies for both hedgerows within and outside the Natura 2000 sites, totaling 11 hedgerows. The subsidy amount for both CAP periods was determined based on the hedgerows' length (in meters) and area (in hectares).

Results and Discussion

Table 1 presents the financial support provided for hedgerows under the previous CAP during the 2015–2020 period, based on the length of hedgerows in meters and the annual payments per meter for each year for the case study farm. It presents seven examples of hedgerows with lengths ranging from 52 meters to 300 meters, all receiving consistent yearly payments of 1.60 EUR per meter. These payments accumulate over five years, resulting in total financial supportfor each hedgerow. The overall total support for all hedgerows listed in the table is 10,588.80 EUR.

Table 9. Hedgerows CAP financial support in 2015-2022

No.	Hedgerow length (m)	Financial support (€/m)	year 2015 (€)	year 2016 (€)	year 2017 (€)	year 2018 (€)	year 2019 (€)	year 2020 (€)	Total CAP 2015-2020 period (€)
1	180	1.6	288	288	288	288	288	288	1728
2	150		240	240	240	240	240	240	1440
3	60		96	96	96	96	96	96	576
4	52		83.2	83.2	83.2	83.2	83.2	83.2	499.2
5	200		320	320	320	320	320	320	1920
6	161		257.6	257.6	257.6	257.6	257.6	257.6	1545.6
7	300		480	480	480	480	480	480	2880
Total	1103		**1764.8**	**1764.8**	**1764.8**	**1764.8**	**1764.8**	**1764.8**	**10588.8**

**Source: Author's calculation based on the CAP financial support for the period from 2015 to 2020*

Table 2 outlines the financial support for hedgerow conservation under the CAP during the 2023–2027 period. Unlike the previous period, where payments were based on length, this period expressed support based on hedgerow area (in hectares), with an annual rate of 450 EUR per hectare. The hedgerows of the case study farm, according to the CAP (2023-2027), ranged from 0.03 to 0.40 hectares both inside and outside Natura 2000 sites with consistent yearly payments over the five-year period. The increase in hedgerows is due to the inclusion of hedgerows outside of NATURA 2000 sites. The total financial support for all hedgerows over this period amounts to 4,207.5 EUR, illustrating the shift from payment per meter to payment per hectare, reflecting a broader focus on area-based environmental benefits.

Table 10. Hedgerows CAP financial support in 2023-2027

No.	Hedgerow area (ha)	Financial support (€/ha)	year 2023 (€)	year 2024 (€)	year 2025 (€)	year 2026 (€)	year 2027 (€)	Total CAP 2023-2027 period (€)
1	0.34	450	153	153	153	153	153	765
2	0.16		72	72	72	72	72	360
3	0.33		148.5	148.5	148.5	148.5	148.5	742.5
4	0.07		31.5	31.5	31.5	31.5	31.5	157.5
5	0.04		18	18	18	18	18	90
6	0.4		180	180	180	180	180	900
7	0.03		13.5	13.5	13.5	13.5	13.5	67.5
8	0.06		27	27	27	27	27	135
9	0.15		67.5	67.5	67.5	67.5	67.5	337.5
10	0.21		94.5	94.5	94.5	94.5	94.5	472.5
11	0.08		36	36	36	36	36	180
Total			**841.5**	**841.5**	**841.5**	**841.5**	**841.5**	**4207.5**

**Source: Author's calculation based on the CAP financial support for the period from 2023 to 2027*

This results section compares the two CAP periods and highlights the policy changes' potential impact on farmers and the environment. The data from Tables 1 and 2 show a clear reduction in annual financial support between the two CAP periods. Under the previous system, the total financial support was 1,764.8 EUR annually, while under the current system, it is only 841.5 EUR annually for a larger surface area of hedgerows (1.47 hectares vs. 1,103 meters). This represents a reduction of approximately 60% in total financial support between the two periods, indicating a significant shift in CAP's hedgerow funding model. While the new approach encourages the management of larger hedgerow areas, the overall reduction in payments may impact smaller farms more heavily. The Common Agricultural Policy (CAP) has shifted its approach to hedgerow conservation between the 2015–2020 and 2023–2027 periods. In the earlier period, financial support was provided based on the length of hedgerows, with payments of 1.60 EUR per linear meter annually. For example, a 180-meter hedgerow would receive 288 EUR per year, totaling 1,728 EUR over six years. This system incentivized farmers to maintain hedgerows based on their physical extent, encouraging the preservation of longer hedgerows.

The CAP's transition from length-based to area-based payments aligns with the EU's broader sustainability goals, promoting the preservation of hedgerows and their contribution to biodiversity and ecosystem services. This change supports the CAP's aim to enhance the environmental quality of agricultural landscapes while offering targeted financial incentives.

Conclusions

Hedgerows remain essential elements of agricultural landscapes, providing significant ecological services such as biodiversity support, soil protection, and climate regulation. However, the shift in the CAP funding model from a length-based to an area-based system has substantially reduced financial support for hedgerow conservation. As expected, the results of this study show that financial aid for hedgerows has decreased by over 60% in the 2023–2027 period compared to the 2015–2020 period, which may negatively impact farmers' motivation to maintain these features, especially in regions where hedgerows are smaller or fragmented.

This reduction raises concerns about the long-term sustainability of hedgerows, as the current funding system does not fully reflect their ecological value. Smaller, linear landscape features may be particularly vulnerable under this new model, despite their disproportionate benefits in supporting biodiversity and preventing soil degradation.

To ensure effective hedgerow preservation, future CAP frameworks should consider reinstating length-based payments or introducing supplementary incentives. Additionally, incorporating hedgerow management into broader environmental schemes that reward not only the size but also the ecological health of these features could help maintain their vital role in sustainable agriculture.

In conclusion, while the new CAP funding model simplifies administration, a more targeted approach is needed to safeguard the ecological and environmental benefits of hedgerows, ensuring they continue to thrive in agricultural landscapes.

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GROSS PROFIT ANALYSIS OF LEGUME-CEREALS ROTATION

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Abstract

Legumes provide high protein content for human nutrition and contribute to the mineral balance of soil in agriculture. Through this contribution, it makes them ecologically and economically profitable, particularly in rotation systems with cereals. Especially in dry conditions, they have important contributions in rotation systems with cereals, and this contribution has been proven by the calculations in this study. The study was conducted in the Konya province of Türkiye, an area with high agricultural potential. Data were obtained through face-to-face surveys with approximately 310 farmers. Gross Margin analysis was conducted by calculating the gross production value and variable costs for both cereals and legumes, using data obtained from producers practising cereal-legume rotation in both dry and irrigated conditions. It was found that beans generated the highest gross profit, while the wheat-legume-wheat rotation resulted in the highest total gross profit (214,33 $/da). Legumes were observed to have a significant share in gross profits in all rotations, with the wheat-bean rotation under irrigated conditions contributing the most at 66.49%. The study revealed the significant contributions of legumes to agricultural production. It is recommended that legumes should be included in agricultural production planning, particularly in light of the climate change, water scarcity and drought problems experienced worldwide in recent years.

Key words: *Gross Margin, Legume, Rotation System.*

Introduction

Legumes are a crucial agricultural crop worldwide due to their high protein content. They also contribute significantly to the export of agricultural products, providing financial gains for the agricultural sector (Verma et al., 2023). Legume cultivation plays a vital role in ensuring sustainability in agricultural production, both ecologically and economically, through crop rotation (Reckling et al., 2014; Stagnari et al., 2017). Legumes have the ability to fix atmospheric nitrogen, which eliminates the need for nitrogen fertilization during production (Cernay et al., 2018). Additionally, they can break weed cycles and improve soil structure (Schneider and Huyghe, 2015). The inclusion of legume crops in the rotation can increase in-house profitability by ensuring production stability (Nath et al., 2023) and providing a sustainable crop intensification model (Lal et al., 2023). Legumes are a group of products that are valued for their nutritional benefits and their ability to contribute to crop rotation. Although they are not as profitable as cereals, which are more commonly grown in agricultural enterprises, their role in crop rotation with cereals is often overlooked. According to a study by Jouan et al. (2019), alternating with legumes is more economically beneficial than alternating without them. Over the past two decades, there has been a global increase in legume production. The legume crop group comprises chickpeas, lentils, beans, peas, broad beans, and cowpeas. Table 1 provides information on the production areas and quantities of total legumes in Türkiye and worldwide. Although the cultivation area has increased by 43.5% in the last 21 years, the production amount has increased by 58%. The increase in production amount, as opposed to cultivation area, is due to improved yields resulting from advancements in the agricultural sector. Conversely, there has been a decrease in both the cultivation area and production amount of legumes in Türkiye, leading to a decrease in its global market share. The decrease in agricultural production is attributed to the rise in input costs and the shift towards alternative products that generate higher income.

Table 1. Legume Production of the World and Türkiye

Year	Harvest Area (ha)			Production (ton)		
	World	Türkiye	Share (%)	World	Türkiye	Share (%)
2000	48.180.021	1.285.489	2,67	56.775.765	1.264.100	2,23
2005	52.395.294	1.168.513	2,23	62.017.691	1.582.600	2,55
2010	59.044.517	808.740	1,37	73.247.272	1.346.010	1,84
2015	60.183.664	697.633	1,16	80.414.487	1.220.200	1,52
2020	65.724.937	883.817	1,34	91.416.493	1.453.815	1,59
2021	69.145.464	905.386	1,31	89.820.034	1.204.641	1,34

Reference: FAO, 2024.

Dry beans have the highest production (31%) among legumes worldwide. Green peas come in second place with 22%, followed by chickpeas in third place with 18%. In Türkiye, chickpeas have the largest share (40%) among legumes, followed by dry beans with 25% and lentils with 22%. According to TÜİK (2022), the degree of sufficiency in dry beans was 106,7%, 96% in chickpeas, 57% in red lentils, and 50,9% in green lentils in 2021. In times of pandemics and wars, the self-sufficiency of countries becomes increasingly crucial. To promote self-sufficiency and compete in global markets, Türkiye must increase its efforts to boost legume production. This is particularly important given the country's favourable ecological conditions. Focusing on high-demand products such as peas will also benefit the national economy.

Upon analysing the foreign trade of legumes, it is evident that the exports of dry beans have increased over the years, along with chickpeas. However, lentil exports have decreased in 2021 compared to the previous year. In terms of world legume imports, there has been an overall increase over the years. However, there was a decrease in lentil and chickpea imports in 2021 compared to the previous year. The 2021 situation regarding exports and imports may be linked to the global drought. In Türkiye, dry bean exports have significantly increased since 2020, and imports have decreased in 2021 due to a high self-sufficiency rate. A similar trend was observed in chickpeas, while both imports and exports of lentils increased in 2020 but decreased in 2021 compared to the previous year (Table 2).

Table 2. Legume Trade of the World and Türkiye

		Export Quantity (ton)		Export Value (1000 $)		Import Quantity (ton)		Import Value (1000 $)	
		World	Türkiye	World	Türkiye	World	Türkiye	World	Türkiye
Dry Bean	2005	2.728.405	2.603	1.387.707	3.183	2.478.101	37.687	1.408.725	22.296
	2010	3.698.645	1.620	3.292.567	2.551	3.106.205	37.718	2.913.104	39.761
	2015	4.172.784	4.413	3.767.346	5.437	3.423.184	32.265	3.510.089	40.537
	2020	4.547.538	96.200	4.721.722	135.152	3.978.399	114.863	4.612.013	77.469
	2021	4.974.827	117.056	4.360.706	105.210	4.269.055	69.164	4.783.601	118.095
Lentil	2005	1.403.589	118.421	631.831	71.014	1.316.448	64.281	622.629	29.162
	2010	1.993.725	194.549	1.725.675	216.605	1.884.949	210.289	1.772.961	193.831
	2015	3.575.866	219.220	2.812.938	261.441	3.314.863	313.162	2.910.343	237.461
	2020	5.085.283	406.144	2.823.858	272.138	4.987.082	629.536	3.154.518	379.943
	2021	3.780.249	289.418	3.813.776	537.106	3.971.070	536.702	4.243.964	414.639
Chickpea	2005	845.176	123.593	441.709	83.026	832.377	646	468.640	359
	2010	1.188.103	56.896	822.489	54.709	1.031.403	7.586	751.887	7.287
	2015	2.454.930	22.474	1.599.957	20.600	1.794.936	37.306	1.250.575	45.410
	2020	2.048.830	241.261	1.616.916	200.419	1.922.111	123.271	1.435.756	116.748
	2021	2.350.710	243.011	1.687.425	191.128	1.850.192	129.498	1.695.414	109.082

Reference: FAO, 2024.

Legume production is significant for the country's economy in terms of foreign trade at the macro level. At the micro level, it contributes to the agricultural income of the producer. However, global disasters such as pandemics, wars, and climate change, as well as recent increases in input prices, have had a serious negative impact on agricultural production. To ensure the sustainability of agricultural production, which is the main factor in food supply security, strategic production planning is necessary within the framework of efficient resource use. This study aims to investigate the profitability and contribution to soil sustainability of cereal-legume rotation.

Materials and Methods

Konya in Türkiye, the research area, is located between 36°41' and 39°16' north latitudes and 31°14' and 34°26' east longitudes. The average elevation of the province is 1,016 metres. Konya experiences a continental climate, with dry and hot summers and cold and snowy winters. As of 2023, the population of Konya is 2.320.241 and constitutes 2,71% of Turkey's population. It has the largest surface area (40.838 km^2) in Türkiye, with approximately 27 million decares of land, covering 65% of the total area of the province. Of the total land area, 55.15% is allocated to field crops, 30.41% to pasture, 11.50% to fallow, 1.79% to fruit and 1.16% to vegetable cultivation. Ornamental plants are cultivated in a total area of 836 decares in the province. Also, Konya Province meets 6.39% in terms of crop production value and 5.66% in terms of livestock value. While 63.79% of the cultivated agricultural land in Konya is dry, 36.21% is irrigated of Turkey in 2021 and ranks first with 6.07% in terms of total agricultural production value. Due to Konya province's arid climate, it is suitable for legume and grain production. Konya province has vast agricultural areas where sugar beet, corn, and sunflowers are commonly grown. Additionally, the province borders the Mediterranean region and is known for its fruit and vegetable cultivation. Notably, Konya produces high-quality cherries and carrots that are often exported. Konya province ranks first in the production of crops such as wheat, barley, grain corn, sugar beet, carrot, lupin, canola, tulip and cattle in Turkey.

The study's primary data was obtained through face-to-face surveys with landowners in Konya province. The survey was conducted in 31 districts, with at least 10 surveys completed in each district. Data was collected from a total of 900 parcels for the agricultural production period of 2021.

In order to reach the aim of this study, the gross profits of legumes and cereals, which are considered the most appropriate crops for rotation, will be calculated, and their contribution to crop alternation will be determined. Gross margin is calculated by subtracting the changing costs from the gross value of production, as described by Açıl and Demirci (1984). The gross value of production is calculated by multiplying the product yield by the product price. The variable costs of production include soil preparation, plant protection, irrigation, fertilization, harvesting, and interest in revolving capital. To calculate variable costs, we considered the production inputs (such as production materials and labour) used at each stage, based on the market price at the time of the research. We included revolving fund interest in the variable costs by taking half of the current interest rate (18%) at the time of the research.

Results and Discussion

Upon analysing the demographic situation of the enterprises examined in this research, it was found that 14.54% of the total population in Konya province is under the age of 15, while 31.22% constitutes the active population working in agriculture between the ages of 15-49. The rate of individuals aged 50 years and over is 32%. It is evident that the younger population has shifted away from agricultural activities due to their desire for education and urban employment. The study found that 41.57% of enterprise owners and their families had completed primary school, 24.56% had completed high school, 18.11% had completed university, and 13.97% had completed secondary school. The low level of education in rural areas of Konya province may be attributed to seasonal migration. The population of the districts close to the city centre, especially, have houses there. As a result, they spend six months of the year in rural areas and the other six months in urban areas, allowing the young population to continue their education.

The research area, Konya province, has a wide range of products due to its large lands. However, mostly cereals and legumes are produced due to its arid climate structure. Table 3 presents Variable Costs, Gross Production Values, and Gross Margin for wheat, barley, lentil, chickpea, and beans, which are commonly grown in the research area, separately for irrigated and dry farming practices.

Upon analysis of table 3, it is evident that products obtained from irrigated agriculture generate higher gross margins. Beans yield the highest gross margin (142.51 $/da), followed by wheat (71.82 $/da) and chickpea (58.90 $/da). In terms of variable costs, beans ranked first at 95.62 $/da, followed by chickpea at 67.26 $/da and wheat at 57.02 $/da. The table indicates that the higher variable costs of chickpea compared to wheat are due to fertilization. Upon analysing the cost items on a product basis, it becomes apparent that soil preparation costs are roughly equivalent across all crops. Sowing costs are highest for chickpeas under dry conditions, while fertilization costs are highest for chickpeas under irrigated conditions. Pesticide, irrigation, and harvesting costs are highest for beans.

Table 3. Gross Margin and Variable Costs of Cereals and Legume ($/da)

	Wheat Irrigated	**Wheat Dry**	**Barley Irrigated**	**Barley Dry**	**Lentil Dry**	**Chickpea Irrigated**	**Chickpea Dry**	**Dry Bean Irrigated**
Soil Preparation	5.35	4.81	5.84	4.51	4.92	4.35	4.03	5.41
Sowing	8.93	8.00	7.71	6.67	19.63	10.88	21.79	14.42
Fertilization	17.01	12.17	13.95	11.34	14.32	21.44	7.65	20.07
Pesticide	1.66	1.10	1.36	1.15	1.30	1.17	1.19	5.14
Irrigating	13.81	0.00	12.11	0.00	0.00	10.15	0.00	21.50
Harvest	5.63	4.55	4.83	4.17	4.12	13.73	5.38	19.88
Revolving Fund Interest	4.68	2.76	4.12	2.51	3.98	5.55	3.60	7.80
Variable Costs	57.02	33.44	49.31	30.39	48.26	67.26	43.64	95.62
Yield (kg/da)	527.86	314.00	513.33	310.19	180.00	237.50	152.14	274.00
Price	0.22	0.21	0.18	0.17	0.51	0.55	0.52	0.87
Byproduct Revenue	7.61	4.59	7.12	4.29	0.00	0.00	0.00	0.00
GPV	128.83	70.96	97.75	57.40	91.65	126.17	77.88	238.13
Gross Margin	71.82	37.52	48.43	27.01	43.39	58.90	34.24	142.51

* 1$ = 8,57 Turkish Liras in research time * 1 da = 1000 m^2

The variation of variable costs, gross value of production and gross margins of the products can be seen more clearly in figure 1.

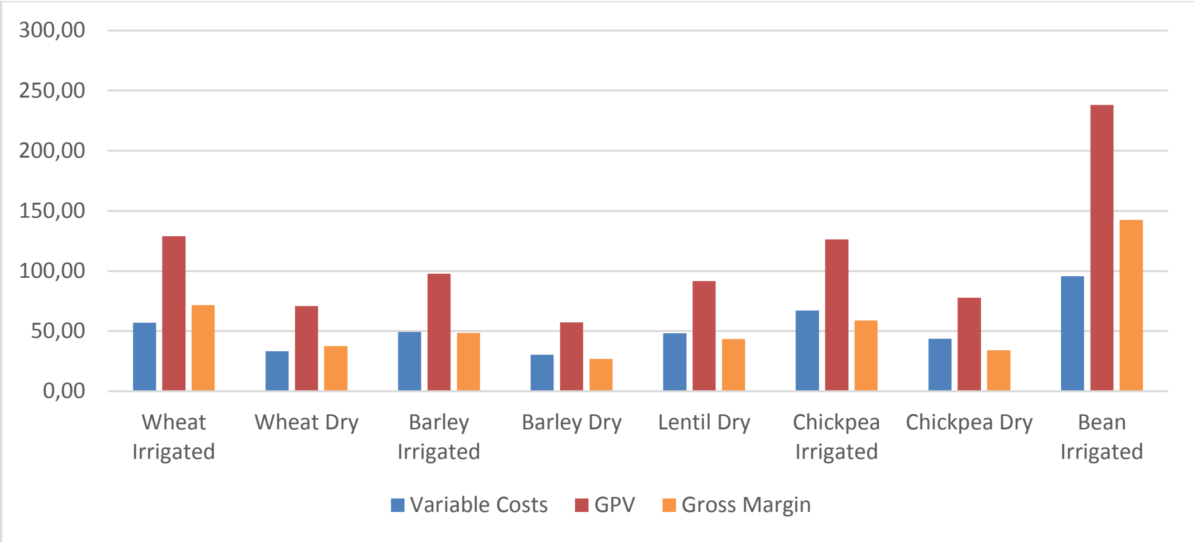

Figure 1. Variable Costs, GPV, Gross Margin According to Products

Cereal cultivation is practised in both irrigated and dry environments, while legume alternations are typically carried out in dry farming. Among cereal-legume rotations, the Wheat-Poach-Barley rotation yielded the lowest gross margin (98.77 \$/da), whereas the Wheat-Beans-Wheat rotation yielded the highest total gross margin (286.15 \$/da).

Table 4. Gross Margins of Legume-Cereal Rotations

Rotation	**Total Gross Margin (\$/da)**	**Share of Legume (%)**
Chickpea-Wheat-Chickpea-Barley	133,01	25,74
Wheat-Chickpea- Barley	98,77	34,67
Wheat-Chickpea (irrigated)	130,72	45,06
Wheat-Lentil-Barley-Lentil	151,31	28,68
Wheat-Lentil-Barley	107,92	40,21
Wheat-Dry Bean-Wheat (irrigated)	286,15	49,80
Wheat-Dry Bean (irrigated)	214,33	66,49

The text presents information on common rotation systems involving cereals and legumes in the research area, including their total gross profits and the shares of legumes in total gross margins. It is observed that lentils are rotated only under dry conditions, chickpeas are rotated under both wet and dry conditions, and beans are rotated only under wet conditions. Among the rotations with cereals, beans have the highest share in the gross margins, accounting for 66,49% in the wheat-beans double rotation. In a wheat-chickpea rotation applied under irrigated conditions, chickpea accounts for 45,06% (Table 4). The study found that legumes are preferred in rotations due to their nitrogen content and ability to balance the nitrogen content in the soil, which is deficient from cereal cultivation, as well as their significant contribution to gross profit. Therefore, it is recommended to plan for increased legume production.

Conclusions

The fundamental objective of an agricultural enterprise is to maximize profit through efficient resource utilization and production planning. The aim of this study was to determine the profitability obtained through the alternation of cereals and legumes, which are compatible with each other in terms of soil fertility and economic profitability, particularly in dry conditions, and the contribution of legumes to this profitability.

The results showed that the highest gross margin was obtained from dry beans, with $142,50/da in the investigated enterprises. Dry beans were rotated with wheat, resulting in a total gross margin of $286,15/da in triple rotation and $214,33/da in double rotation. Beans, a legume, contributed approximately 50% or more to this rotation. Chickpea and lentil also contributed similarly. It has been determined that legumes contribute not only nitrogen to the soil, but also have economic benefits. Therefore, it is important to utilize legumes in dry conditions for agricultural production, especially considering the impact of climate change. Agricultural policies should take into account the importance of legumes in supporting and planning production.

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COST BENEFIT ANALYSIS OF BULB ONION PRODUCTION USING MANUAL HARVESTING SYSTEM IN THE SOUTHEAST REGION, USA

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Abstract

Onions (*Allium cepa*) are a staple agricultural commodity in the United States of America and around the world with India and China being the 1st and 2nd largest producing countries. Onions were first brought to the United States by early European settlers and their cultivation spread across the nation. Onions can be segregated into three marketable categories, i.e., the "green" onions, which consist of varieties pulled while the tops are still green, with a small bulb such as scallions and two other categories that are harvested primarily for their bulb. Our research focuses on the two bulb categories, irrespective of the cultivar, with the aim of analyzing its horticultural production practices to determine the profitability margins of the industry using a sensitivity risk-rated enterprise budget economic model. Primary and secondary data were used to gather pertinent information needed for the analysis at the University of Georgia, Southeast District Vidalia Onion & Vegetable Research Center in Lyons, GA, USA. Average yields of 600 boxes (40 lbs., ~18.14Kg) and a price of $22 per box were used for the study. The study depicted an expected net return of $4,887/acre with a 99% chance of profit 50% of the time. The profitability margins vary significantly with changes in price and yields respectively. A break-even analysis revealed total cost of $13.85 per box and a yield of 306 lbs. (~138.80 Kg) per acre and that bulb onion was profitable in 2024 despite the increase in input prices in the past two years.

Keywords: *Bulb onion, Variable costs, profitability margins, United States.*

Introduction

Onions (*Allium cepa*) are a staple agricultural commodity in the United States of America. They were first brought to the country by early European settlers and their cultivation spread across the nation (Boyhan, 2001). There are three marketable categories of onion, i.e., the spring or "green" onions, which consist of varieties pulled while the tops are still green, with a small bulb such as scallions, while the remaining two categories are "dry bulbs" that are harvested primarily for their bulb (AMRC, 2021). Bulb onion is cultivated for the spring/summer fresh market, which are typically milder with a shorter shelf life and in the fall/winter, which tends to be more pungent with a longer shelf life (NOA, 2017). Dry bulb varieties are split between: (a) short-day varieties which require 10 to 13 hours of daylight, (b). intermediate varieties which need between 13 and 14 hours of daylight, and (c). long day varieties which require >14 hours (Boyhan, 2001). Short and intermediate varieties are planted in the southern and western portions of the USA in fall/winter while the long-day varieties are planted in the northern regions of the USA during the spring (AMRC, 2021).
Onion is not only an important crop to the state of Georgia, but also regionally and nationally, as it ranks third behind lettuce and tomatoes. Onion crops were worth $1.6 billion in 2022

with a six-year average of $1.2 billion (USDA NASS, 2020; 2023). On the other hand, onions were the second most consumed fresh vegetable in 2022 with per capita consumption of 19.18 lbs., and a six-year average of 21.13 lbs. (USDA ERS, 2023). According to the USDA Report (2023), onion planted acreages dropped by 2.5% over the last three years and the utilized production equally decreased by 11.9% in the same time frame (USDA NASS, 2023). As of 2022, the state of Georgia (USA) ranks fourth in terms of both acreage and value of utilized production after California, Washington, and Oregon respectively. These states are responsible for over 70% of both areas planted and value (USDA NASS, 2023). Still in 2022, Georgia (USA) obtained the highest price of $62.40 per cwt, followed by Texas with $52 and New Mexico with $46.50 respectively (USDA NASS, 2023). These prices further increased in 2023 with Georgia and New Mexico experiencing 40% and 60% respectively while Texas recorded 80% at the same time. On average, the price per cwt has increased by 38% in the USA from 2021 to 2022 and 93% percent from 2020 (USDA NASS, 2023).

Despite the ever-increasing demand for onions, planted acreages have been trending downward for the past few years. This is due to the increased cost of inputs in the form of fertilizers, pesticides, and fuel (USDA ERS, 2023). According to USDA ERS Report (2023), "average annual nitrogen prices increased by 67 percent, pesticide prices increased by 39 percent, and diesel prices increased by 54 percent." This increase affected all vegetable producers in the United States who paid on average 14% more for production inputs in 2022 compared to 2021 (USDA ERS, 2023). The same report forecasted that input prices could increase slightly or drop for the 2023 growing season. Exports of onions for 2022 stand at 633 million pounds which is a 22% decrease from 2019 whereas imports stand at 1.46 billion pounds, a 22% increase from 2019. Almost 105 billion pounds of onion are produced annually worldwide with an average per capita consumption of 14 pounds while the United States consumes 19 lbs. The major world producing countries are India, China, the United States, Egypt, and Turkey (FAOSTAT, 2024).

This study strictly focuses on the productivity and profitability of bulb onion using manual harvesting techniques in the southeast region of the United States. It is the first step in a larger study where mechanical harvesting technologies will be introduced, and a comparison of both systems will be conducted to determine which of the two production systems offer pareto optimum profitability to the growers.

Material and Methods

Our study focusing on the cost-benefit of bulb onion production using manual harvesting is the first step towards a larger USDA/NIFA Awarded Grant entitled, "Ensuring Future Economic Viability of US Short-day Onion Production Through Mechanical Harvesting" as we plan to compare the two harvesting technologies to determine the better option in terms of profitability to the growers in the region. Primary data was collected from the University of Georgia, Southeast District Vidalia Onion & Vegetable Research Center, Lyons, GA, USA and from growers. Secondary data was obtained from various organizations including the University of Georgia Extension Onion Production Guide and recommendations and other scientific publications such as the Food and Agriculture Organization of the United Nations (FAO), the United States Department of Agriculture (USDA), the National Onion Association (NOA), and the Agricultural Marketing Resource Center (AMRC) respectively (Boyhan et al., 2021; FAOSTAT, 2024; NOA, 2017; AMRC, 2021).

The yield of 600 boxes per acre was the average obtained from the growers and the price of $22/40 lbs. box was obtained from USDA/ERS. We adopted five case scenarios where the "optimistic" and "best" yield and price were 10% and 20% increase from the "Expected"

while the "pessimistic and "worst" cases were 10% and 20% decrease respectively (Brigham, 1982; Fonsah and Hudgins, 2007; Fonsah et al., 2018; Fonsah et al., 2007; Fonsah et al., 2012; Fonsah, Kunwar, Diaz-Perez, 2023).
We calculated the pre-harvested variable costs (P-HVC) by taking into consideration inputs such as plants and setting, fertilizers, insecticides, fungicides, and herbicides applications respectively to their standard use and labelled usage rates. We included 9% interest on operation costs. The harvesting and marketing costs (H&MC) included manual and general harvesting labor, grading, labelled mesh bags, cartons, and commission fees. Fixed cost (FC) calculations included machinery, irrigation and overhead and management (Fonsah et al., 2008; Kunwar and Fonsah, 2022; Fonsah et al., 2020).

Results and Discussion

Agricultural practices, farm size, yields and prices vary from farmer to farmer. In this study, the different yields from best to worst per acre is the average obtained from growers in the onion producing areas (Table 1). The price of $22/40 lb. box was a summary average bulb onion prices from USDA report (2024). The "optimistic" and "best" prices were a 10% and 20% increase assumption made by the authors following financial management model (Fonsah and Chidebelu, 2012; Brigham, 1982; Gallo, 2012).

Table 1. Estimated Yields and Prices of Bulb Onion Production in the Southeast Region of the United States, 2024.

Description	Best	Optimistic	Expected	Pessimistic	Worst
Yield (40 lb. boxes)	800	700	600	500	400
Price per box ($)	26.40	24.20	22.00	19.80	17.60

The pre-harvesting variable costs (P-HVC) of $3,537.75 per acre was obtained by determining the quantity and price of the inputs most growers in the region use in their agricultural practices. A classic example are plants and setting, different fertilizers, insecticides, fungicides, herbicides, fuel, lubricants and maintenance and labor (Table 2). The combined cost of plants and setting was $1,236 equivalent to 34.94% of the total P-HVC. Other expensive inputs were Nitrogen ($108), insecticides ($152.32), fungicides ($418.70), machinery fuel, lubrication, and maintenance ($246.09), labor and crop insurance. The "Yours" column in Table 2 and subsequent tables is for the grower to input their own actual cost for comparation and guidance purposes (Table 2).

Table 2. Estimation of Total Pre-Harvesting Variable Cost of Producing Bulb Onion in the Southeast Region of the United States, 2024

Pre-Harvest Variable Costs	**Unit**	**Quantity**	**Price**	**Total/Ac**	**Yours**
Plants and setting	Thou	92.00	8.00	736.00	
Set Plants	Acre	1.00	500.00	500.00	
Lime, applied	Ton	0.33	47.20	15.58	
Nitrogen	Pounds	120.00	0.90	108.00	
Phosphorous	Pounds	120.00	0.65	78.00	
Potassium	Pounds	120.00	0.50	60.00	
Insecticide	Acre	4.00	38.08	152.32	
Fungicide	Acre	10.00	41.87	418.70	
Herbicide	Acre	2.00	15.50	31.00	

Machinery (fuel, lubrication & maintenance)	Acre	1.00	246.09	246.09	
Labor	Acre	35.00	14.68	513.80	
Crop Insurance	Acre	1.00	400.00	400.00	
Irrigation	Appl	1.00	125.93	125.93	
Interest on Oper. Cap.	$	3385.41	0.09	152.34	
Pre-Harvest Variable Costs (P-HVC)	Acre	1.00		**3,537.75**	
Total Harvest and Marketing (TM&HC)				**3,463.40**	
Total Variable Costs (TVC)				**7,001.15**	

Source: Author's compilation, 2024.

The harvesting and marketing quantity of 570 boxes was the assumed 5% field loss during harvesting from the expected 600 boxes. Manual labor was 25.93% of total H&MC while grading labor was $900.60, equivalent to 26% of total H&MC. Although some studies have shown that agriculture workers earn 40% less than their nonagricultural counterpart, and that the migrant H-2A program is too expensive, the current administration still went ahead and increased the Adverse Effect Wage Rate (AEWR) to $14.68/hour, which was adopted in our estimation. Migrant Farm Worker wage is a serious US Congressional policy topic of debate as growers and farming associations argue that wages have increased exponentially increasing cost of production (Costa, 2023; Fonsah and Kunwar, 2024). For instance, the combined labor cost is equivalent to 31% of TVC. The TVC which is the summation of P-HVC + TH&MC was $7,001.15 per acre (Table 2).

Estimated Machinery Cost Analysis

Total machinery cost items included tractor, plow, disk, subsoiler, and transplanter. All costs of equipment were considered new, but only 33% of the cost of the equipment and amortization was considered simply because growers in this region of the US cultivated at least three different crops per year using the same implements (Fonsah et al., 2007; 2008). Our calculations also considered the salvage value of the equipment, lifespan, depreciation, interest, taxes, and insurance. The total machinery cost which is part of the fixed cost (FC) was $597.74 which was calculated based on 40 acres farm and divided by 40 to obtain the per acre cost.

Irrigation Cost Analysis

Our research adopted sprinkler irrigation even though most of the larger growers in the region prefer central pivot irrigation system. The total investment cost on irrigation materials was $53,790. This included sprinkler system, power unit, an 8 inch well, pump, and filters. The calculations were based on 40 acres (~16 Ha) which is the average onion farm size in the region, and we considered the lifespan of the equipment, depreciation, interest, taxes, and insurance. Although the total annual irrigation cost on 40 acres (~16 Ha) was $7,320, the cost per acre was reduced to $183.01 while the operation cost was $125.93. Irrigation has two different costs, i.e., the installation cost which is FC and the operation cost which is a P-HVC.

Total Cost (TC)Analysis

The total cost (TC) of production of $8,312.57 is the summation of P-HVC + TH&MC+TFC. TFC including machinery, irrigation, overhead and management. Overhead and management cost was 15% of TP-HVC.

Break-Even Analysis (BE)

The break-even analysis is usually used to "determine the number of units or amount of revenue that is needed to cover one's business's total cost". For instance, the BE TC per box of $13.85 means that, at this price, the grower is not making any profit nor is he/she is losing money. However, if the TC is above $13.85 per box, then the grower is making money. On the other hand, if the total yields are lower than the BE yields of 306 lbs., then the grower is losing money (Fonsah, Kunwar and Diaz-Perez, 2023)

Sensitivity Risk-Rated Returns

The best, optimistic, expected, pessimistic and worst yield and price values in Table 1 were used to calculate the sensitivity risk-rated return (Table 3). We assumed a 10% and 20% increase and decrease from the expected price of $22 per box of bulb onion (Fonsah and Chidebelu, 2012). Our findings showed that with a yield of 600 boxes (40 lbs., or ~ 18kg) and $22 per box, a grower's expected net profit would be $4,887 per acre of cultivating bulb onion in the Southeast region of the US. The study also reveals that occasionally (7% of the time), a grower could obtain $8,025, all things being equal while $1,750 per acre (0.40 Ha) could be obtained in the worst-case scenario, 7% of the time (Table 3).

Table 3. Sensitivity Risk-Rated Returns of Producing Bulb Onion in the Southeast Region, USA over Total Cost, 2024.

Description	**Best**	**Optimistic**		**Expected**	**Pessimistic**		**Worst**
Returns ($)[1]	8,025	6,979	5,933	**4,887**	3,842	2,796	1,750
Chances (%)[2]	7%	16%	31%	50%			
Chances (%)[3]				50%	31%	16%	7%
Chances for Profit =		**99%**	Base Budgeted Net Revenue ($) =				**$4,887**

[1]Net return level (Top row)
[2]The chance to obtain this level or more (Middle row)
[3] The chance to obtain this level or more (Bottom row)

Conclusion

Onions (*Allium cepa*) are a staple vegetable in the United States of America and around the world with India and China being the 1st and 2nd largest producing countries (FAOSTAT, 2024). Worldwide, approximately 105 billion pounds (~47.63 million MT) of onion are produced annually with an average per capita consumption of 14 pounds (~6.4 kg) while the US consumes 19 pounds (8.6 kg) (FAOSTAT, 2024). The objective of this study was to investigate the productivity and profitability of bulb onion in the southeast region of the United States using manual harvesting techniques and determine its financial lucrativeness, if any.

The results depicted that the Pre-harvest variable cost (P-HVC), which was the summation of the most important generally used inputs such as plants and setting, a combination of fertilizers, pest and disease control measures, crop insurance and labor was $3,537.75 per acre (0.40 Ha). The harvesting and marketing cost (H&MC) was $3,463.40 and included manual harvesting labor, general labor, grading, and cartons. The total variable cost (TVC) was $7,001.15 which was obtained by adding the P-HVC to H&MC. Investing $8,312.57 per acre (0.40 Ha) of any vegetable enterprise is quite risky especially given their perishable nature.

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CONSIDERATIONS CONCERNING THE EVOLUTION OF AGRITOURISM GUESTHOUSES IN ROMANIA

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Abstract

Romania has huge agritourism potential, thanks to its varied landscapes, rich natural resources and unique traditions that are still preserved in many rural areas. The development of the authentic tourism sector could bring substantial economic and social benefits, both to rural communities and to the tourism industry as a whole. The paper highlights the evolution of agritourism guesthouses in the period 2018-2022, in Romania, and a series of indicators are presented and analyzed, such as: the accommodation capacity of agritourism guesthouses and the number of agritourism guesthouses at the national level, through the development regions and counties, and also the utilization index of the tourist accommodation capacity of agritourism guesthouses at the national and regional level. Data for these indicators were obtained from the National Institute of Statistics. This methodology offers a structured approach for understanding the evolution of agritourism guesthouses in Romania over the specified time period.

Keywords: *agritourism guesthouses, accommodation capacity, authentic rural tourism, Romania.*

Introduction

According to data published by the World Tourism Organization, tourism occupies the third position in the export of services and goods globally, registering a higher growth in recent years compared to the international trade.

At present, tourism is an industry on the rise, as more than a billion people travel abroad annually to spend their holidays, to have fun, to visit new places etc. The first three reasons for choosing a tourist destination are: visiting cultural vestiges, spending time in a natural setting and the gastronomic culture (Totcum.com, 2017; Privitera *et al.*, 2018).

In many countries in Europe, tourism actively contributes to economic development. Here, a number of types of tourism are practiced, thus offering tourists a wide range of attractions and services.

The development of tourism in Europe and beyond was possible due to the existence of tourism infrastructure. It is made up of a variety of interconnected elements that allow tourists to arrive, stay and benefit from the tourist attraction of their destination. The tourist infrastructure includes the following: basic services; the road system; transportation; gastronomy; reception units comprising hotels, villas, bungalows, tourist pensions, agritourist pensions etc. (Ilieş *et al.*, 2017; Pop *et al.*, 2017); services for sports and recreational activities; services for cultural activities; other services; security services and store networks (Ciornei, 2013; Ionel, 2019; Ștefan (Matei), 2017; Simoni, 2017).

In the economies of the European states, rural entrepreneurship plays a particularly important role in terms of promoting tourism both internally and externally (Sima *et al.*, 2015).

It was noted that Romania is one of the countries with a tourist vocation, having numerous areas with an abundant flora and fauna in unique species in Europe, but also a series of tourist

attractions, which contribute to boosting tourism (Călina *et al.*, 2017; Cimpoca *et al.*, 2024; Herman *et al.*, 2020; Yilmaz *et al.*, 2018).
Several types of tourism are practiced in our country, as it has a variety of landforms, historical vestiges, folk art, traditions, special culinary dishes etc. Rural tourism has been identified by the Romanian authorities as a key area of development, which can address social and cultural inequalities (Ilieş *et al*., 2017; Pop *et al*., 2017).
In Romania, agritourism is an industry that stands out for its accelerated growth, with an average growth rate related to tourist arrivals four times higher compared to the general average. However, there are notable regional differences in the flow of attracted tourists (Tenie and Fîntîneru, 2020).
Agritourism fully enhances the resources of rural areas and offers the possibility of preserving biodiversity and capitalizing on resources in a sustainable and rational way, in a world where there is an increasing emphasis on reducing the effects due to pollution (Băieș, 2022; Ciornei, 2013; Hontuș, 2016; Ștefănică *et al.*, 2021).
At the same time, agritourism contributes to rural development (El Bilali *et al.*, 2014), by capitalizing on the anthropic, natural and cultural potential for tourism purposes, by creating new jobs for rural inhabitants, but also by promoting Romania abroad (Călina *et al.*, 2018). Agritourism, along with other forms of tourism, can contribute to both economic growth and income growth (Bujdoso *et al.*, 2015; Mack *et al.*, 2018; Pocol *et al.*, 2017).
In order to develop tourism and agritourism at national level in the medium and long term, it is necessary, on the one hand, to develop the tourism infrastructure, and on the other hand, this to be carried out in the area of the National Parks, having a positive impact on the economy and on the social and cultural life (Călina *et al.*, 2017).

Materials and methods

In this article, the authors used statistical data from the National Institute of Statistics (INS, 2024). At the same time, other professional sources and articles listed in the bibliography were also checked.
Data related to Romania, its development regions and counties, for the period 2018-2022, were extracted and there were analyzed the follow indicators: the accommodation capacity and the number of agritourism guesthouses at the national level, at the development regions and counties level, and also the utilization index of the tourist accommodation capacity of agritourism guesthouses at the national and regional level.

Results and discussion

In 2022, agritourism guesthouses accounted for 38.20% of the total existing accommodation units in Romania (INS, 2024). During the period under analysis, both at national and regional level, with 2 exceptions (South-Muntenia Region and Bucharest-Ilfov Region), the number of agritourism guesthouses increased by 23.50% – Table 1. The most obvious increase was in the South-East Region, 57.26%. The accommodation capacity also increased, except in the Bucharest-Ilfov Region, where the decrease was by 67.74%. On the other hand, in the South-West Oltenia Region the highest increase was recorded, of 38.22%.
It should be noted that, after the Covid-19 Pandemic, we have witnessed a revival of tourism and agritourism, both nationally and internationally (Gherdan *et al.*, 2020). This health crisis has led to significant changes in the behavior and habits of tourists (Crețu *et al.*, 2021; Morosan-Danila and Bordeianu, 2020). According to some authors, it has led to an increase in the number of overnight stays in agritourism guesthouses, which shows the tourists' preference for these accommodation units (Popescu *et al*., 2021).

Table 1. Number and Accommodation capacity of Agritourism guesthouses in Romania and by Regions of Development, 2018-2022

Specification	2018		2019		2020		2021		2022		2022/2018 %	
	Number of Agritourism guesthouses	Accommodation capacity	Number of Agritourism guesthouses	Accommodation capacity	Number of Agritourism guesthouses	Accommodation capacity	Number of Agritourism guesthouses	Accommodation capacity	Number of Agritourism guesthouses	Accommodation capacity	Number of Agritourism guesthouses	Accommodation capacity
Romania Total	2,821	48,574	2,800	49,053	3,022	52,389	3,460	55,778	3,484	56,850	123.50	117.04
North-West Region	520	9,405	538	9,543	616	10,918	750	12,317	806	12,977	149.81	137.98
Center Region	979	16,043	892	15,466	967	16,526	989	17,191	1,019	16,964	114.24	105.74
North-East Region	458	7,632	458	7,623	475	7,907	488	7,930	489	8,086	106.77	105.95
South-East Region	234	4,534	234	4,708	237	4,592	461	5,460	368	5,752	157.26	126.86
South-Muntenia Region	244	4,253	256	4,435	249	4,450	256	4,501	247	4,394	96.48	103.32
Bucharest-Ilfov Region	2	62	2	62	1	20	1	20	1	20	50.00	32.26
South-West Oltenia Region	185	3,006	209	3,330	238	3,720	254	3,934	269	4,155	128.71	138.22
West Region	199	3,639	211	3,886	239	4,256	261	4,425	285	4,502	135.07	123.72

Source: own calculation after INS, 2024

In recent years, agritourism has become more and more preferred, both by Romanian and foreign tourists, who are looking to relax away from the hustle and bustle of the big cities. In Romania, agritourism has always been practiced, but on a much smaller scale than it is today. The Romanian countryside largely preserves the old customs, the elements of craftsmanship and a series of ethnography elements contributing to the development and promotion of agritourism (Gonța, 2020).

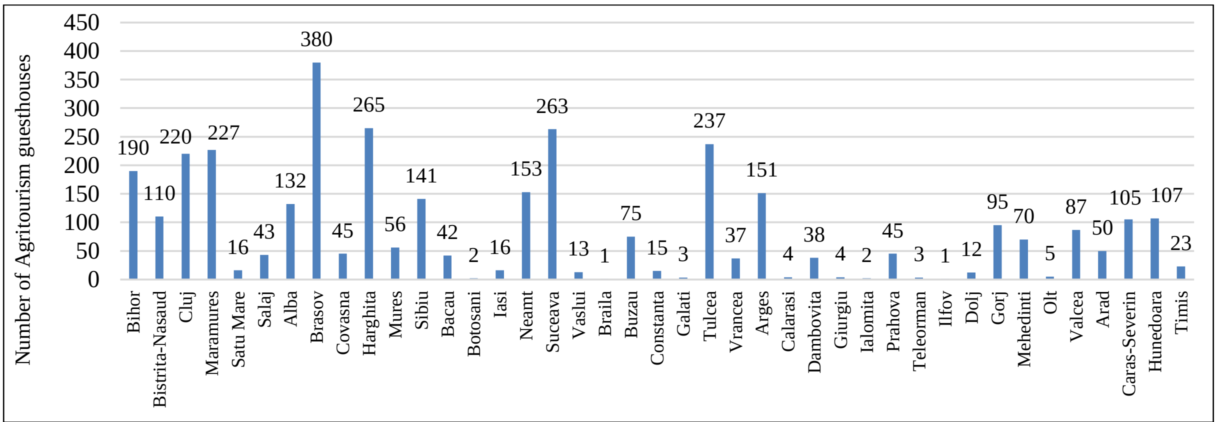

Figure 1. Number of Agritourism guesthouses in Romania's Counties, in 2022
Source: own interpretation after INS, 2024

Figure 1 shows the Number of Agritourism guesthouses existing in Romania's Counties, in 2022. In Brașov County (well known for its natural and anthropogenic resources) the highest number was found, 380. At the opposite pole were the counties of Brăila and Ilfov, located near large cities (Firățoiu *et al.*, 2024), with one agritourism guesthouse each.

The existing accommodation capacity in agritourism guesthouses in 2022 reflects the fact that Brasov County had the most accommodation places (6,688), leading the way in the top of the counties – Figure 2. The counties of Ilfov (20 places) and Brăila (10 places) were on the last places. It should be noted that during the analyzed period, the counties of Satu Mare, Brașov, Mureș, Bacău, Teleorman, Ilfov, Dolj and Timiș registered decreases in the number of accommodation places in agritourism pensions.

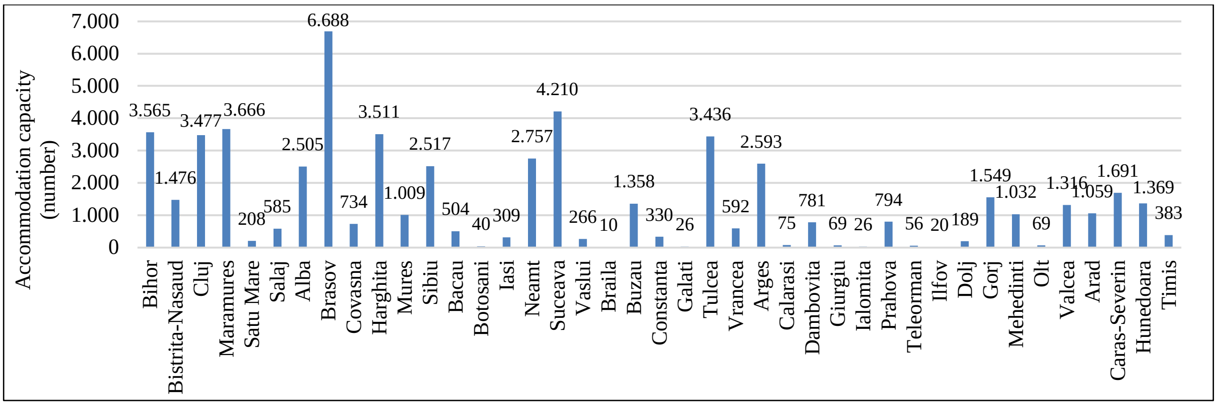

Figure 2. Accommodation capacity of Agritourism guesthouses (number) in Romania’s Counties, in 2022

Source: own interpretation after INS, 2024

Although at the country level there was an increase of 7.69%, the net utilization index of accommodation capacity recorded decreases in the period 2018-2022 in the North-West Region (12.44%), South-East (24.75%) and West (6.72%) – Table 2. The largest increase was for the Bucharest - Ilfov Region, 1,486.89%.

Table 2. Index of net utilization of tourist accommodation capacity in operation, in Romania and by Regions of Development, 2018-2022

(percentage)

Specification	June 2018	June 2019	June 2020	June 2021	June 2022	2022/2018 %
Romania	18.2	22.2	12.4	17.2	19.6	107.69
North-West Region	20.9	24.4	9	15.8	18.3	87.56
Center Region	16.4	21.9	12.2	17.3	21.4	130.49
North-East Region	18.8	21.7	12.1	17.1	21.3	113.3
South-East Region	20.6	22.5	15	16.7	15.5	75.24
South-Muntenia Region	15.1	18.4	10.1	15.4	18.2	120.53
Bucharest - Ilfov Region	6.1	30.1	3	64.7	90.7	1,486.89
South-West Oltenia Region	21.1	24.5	17.5	21.8	21.5	100
West Region	16.6	19.3	14.1	17.3	15.5	93.37

Source: INS, 2024

Conclusion

Due to its geographical position, Romania has favorable conditions for the development of agritourism and tourism in general. Tourists who prefer less populated places, closer to nature, found in agritourism an alternative to traditional tourism.

In Romania, in 2022, agritourism guesthouses held 38.20% of the total number of accommodation units. Their number varied from one county to another. Thus, Brasov county, known for its natural and human resources, had the largest number of agro-tourism guesthouses (380), with the largest accommodation capacity (6,688). On the other hand, in the territories of Brăila and Ilfov counties, there was only one agro-tourism guesthouse.

In the future, rural entrepreneurs will have to diversify their businesses in the agritourism sector as well, because changing preferences will lead to more and more requests from both Romanian and foreign tourists.

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FORESTRY AND AGRO-FORESTRY

STUDY OF THE CORK QUALITY FROM *QUERCUS SUBER* L. IN NORTH-WEST ALGERIA

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Abstract

This work is a contribution to a study of the quality of cork from *Quercus suber* L. to determine a possibility of variation depending on environmental factors. The study is carried out on cork bark from three cork oak forests in northwestern Algeria (Zarieffet, Tagdempt and Ami-Moussa) subjected to the same management for 14-year harvest cycles. The quality indices obtained for each provenance show the effect and the "brushwood status" on cork quality. Indeed, poor quality indices < 6.5 result from high competition with undergrowth and scrubland, which explains a cork porosity > 6%, a density > 300 kg/m3, low productivity (< 6.5 kg/m2), physiological and pathogenic defects (earthy cork, lignified cork, blown cork and insect galleries), and a dieback index of 2.70. In stands maintained by silvicultural care and in good health, quality indices seem better > 8.5. This is a corky growth > 2.5 mm/year, a productivity > 7.5 kg/m^2, a low porosity < 3%, a satisfactory density (195 kg/m^3), dotted with minor growth defects (cork back, cork belly, split cork) and a healthy state (1.25). The results also show the role of good forest management in improving the productive and qualitative performances of stands with economic and ecological value, such as cork oak. It appears more clearly that in the absence of intervention by the forest manager, the cork oak forest declines and only produces poor quality cork with a low economic yield.

Keywords: *Cork oak, northwest Algeria, scrub, quality index.*

Introduction

Cork is a natural material of cellular structure with an interesting set of properties, i.e., low density, very low permeability to liquids, large compressibility, low conductivity, chemical stability, and durability (Pereira, 2007). All these properties give cork very wide applications. Heated ground cork can be manufactured to form tiles and carpets used in floor covering due to their pleasant elastic feel and sound absorbing properties (Toribio et *al*., 2005). Currently, cork is the second most important marketable non-wood forest product in the western Mediterranean, and the world cork market exports represent near US$2 billion annually (Mendes and Graça, 2009).
Cork is the bark of the cork oak (*Quercus suber* L.) which is concentrated mainly in the Mediterranean region. Cork forests cover area of 2.5 million hectares (Aronson et *al*., 2009). These areas have the weather conditions necessary for its growth: dry summers and mild winters (Johnson, 2009).
The cork oak has a remarkable capacity to create cork tissue from its inner bark. This tissue, formed specifically by the phellogen of the cork oak (the tissue responsible for the formation of new cells), derives its name from the Latin suber (cork) (Graça and Pereira, 2004). One of the most important characteristics of cork is the presence of lenticular channels, called cork porosity and they are usually quantified by a porosity coefficient, calculated as the proportion of pores in the total area. The cork porosity ranges from below 2% to over 15% (Pereira et *al*.

1996). Through its formation process, changes occur in the cork due to environmental perturbations that affect its growth, colour, texture, density, and its porosity (Dehane and Chorana, 2021). Among the factors disrupting the quality of cork, we quote the dieback of trees. It is a phenomenon which began to take on relatively significant dimensions from the 80s-90s in the Mediterranean (De Sampaio, 2013) and from the 2000s in the north-west region of Algeria (Dehane et *al*., 2013).The symptoms of forest decline are easily observed: loss of leaves or needles, change in color of leaves which turn yellow and orange, tree mortality. Softwoods were the first affected, but hardwoods such as oaks are also reached, whether young or old. (Costa et *al*., 2010).
According to the standards of the Instituto de Promoción del Corcho (IPROCOR, 2006) in Merida, Spain, cork planks are classified commercially by visual observation, in nine extended quality classes (first to ninth) related to two criterions. The first criterion of quality is related cork thickness expressed in number of lines (one line corresponds to 2.25 mm). The second criterion is allied with the presence of porosity, density and defects, and the impact that they may have on the classification process. The recent application of digital technology has made it possible to implement several systems for the detection and the quantification of thickness, porosity, and quality, such as image analysis and use of the Coveless CQ05™ (Dehane and Chorana, 2021). In Algeria, the cork oak occupies potentially 450,000 ha (Dehane, 2012). The history and the economic fluctuation of Algerian cork sector have been reported (Dehane et *al*., 2014). The current situation of cork oak woodlands can be regarded as unstable. The cork forests of the northwest of Algeria are abandoned without management. Various problems have arisen: tree decay, the recurrence of fires, difficult natural regeneration, and a reduction in the quality of the cork. The objective of this work is to detect the causes of the decline in quality through experimental plots installed in these degraded cork forests.

Materials and methods

Three provenances of *Quercus suber* situated in north-west Algeria were selected from a 400-km- long gradient, ranging from the sub-humid to the semi-arid area (Zarieffet (ZA), Tagdempt (TA) and Ami-Moussa (AM). The dominant substrate in this area is of two types: the Late Jurassic sandstone, consisting mostly of alluvium. The area has a mild Mediterranean climate with cool humid winters and warm-dry summers: sub-humid for Zarieffet forest and semi-arid for others. The rainfall averages recorded (for the period 1992–2021) vary between 541.90 mm (Zarieffet), 356.85 mm (Tagdempt) and 298.59 mm (Ami-Moussa). The mean annual temperature is about 14.5°C (ZA), 15.25°C (TA) and 18,09°C(AM) (National Bureau of Meteorology, 2022).

In each cork oak forest, two experimental plots of 4 ha each were protected over a period of 10 years. The first plot (cleaned plot) is made up of productive cork oak stands with all possible silvicultural work and the cultural care. The second plot (brushy plot) is abandoned without management to the undergrowth (*Arbutus unedo, Phillyrea angustifolia, Pistacia leniscus, Erica arborea, Daphne gnidium, Chamaerops humilis, Ampelodesma mauritanica, Genista tricuspidata, Juniperus oxycedrus, Tetraclinis articulata, Calicotome spinosa, Cistus monspeliensis, Olea europaea var. sylvestris, Quercus coccifera)*, and presence of holm cork (*Quercus ilex* L.) the zean oak (*Quercus faginea* Lam.) and the aleppo pine (*Pinus halepensis* Mill.) In every plot, 50 sample trees were chosen randomly. A square of 20×20 cm (called 'cala') was chalked on the trunk of each sample tree at 1.30 m height, and then probed instantly (by Coveless CQ05™) for an ulterior validation of the cork quality. Subsequently, the sample of cork was extracted carefully without hurting the mother cork. Each sample was labelled according to its origin and the nature of the plot (Cleaned plot: ZA, TA and AM); (Brushy plot : ZA, TA and AM).

In the laboratory, the samples were put in boiling water for 1hour, without undergoing any chemical or industrial treatment. Then, they were stabilized at room temperature (20°C) till constant weight to remove the water netted during the boiling. Prior to commencing the annual increment measurements, each sample underwent light sanding of its cross-sections followed by compressed air cleaning. For the analysis of annual cork growth, each cross-section was divided into three equal distance parts represented by three vertical lines on which the growth rings are marked by a thin pen. The scanning of the cross-sections allowed us to measure the thickness of the growth rings by image analysis, converting the resolution of the scanner to mm by the ImageJ software. The cork anomalies were identified according to the methodology developed by Dehane (2012). To determine the volumetric density (kg.m^{-3}) and productivity (kg.m^{-2}), each sample was weighed on a precision scale (to the nearest 0.01 g). Porosity was measured by scanned image analysis as previously described by Pereira et *al*. (1996).

Following to the standards of Iprocor (2006), the Coveless CQ05™ validates digitally the commercial classification of quality in nine extended quality classes (first to ninth): Q_1 (19 up 6th up), Q_2 (15–19 5th up), Q_3 (15–19 6th), Q_4 (13–15 5th up), Q_5 (13–15 6th), Q_6 (11–13 5th up), Q_7 (11–13 6th), Q_8 (11down 4th up), Q_9 (raw cork waste). Each of the nine quality classes has a market value according to its use in the cork processing industry (stoppers, discs, paper and trituration). According to IPROCOR, a quality index (IQ) is assigned for each class: (Q_2=19.5; Q_4=19; Q_6=12.75; Q_8=12) (represent high quality (A)). For medium quality (B) ($Q_{1=}$11; $Q_{3=}$7;
$Q_{5=}$6.5; $Q_{7=}$5). On the other hand, cork of low quality (C) (raw cork waste) does not exceed 1.5 (Q_9). Monitoring the health of trees (Ten years of observation) is part of the cork quality monitoring network in Oranie (northwest-Algeria), installed by Dehane (2012). Health index (ID) was calculated, in each cork forest, by applying the formula of Sechi et *al*. (2005): ID = Σ (C × F)/N

Where:

C = value of trees of defoliation class (0-1-2), Class 0 (defoliation < 25%, healthy trees), Class 1 (defoliation 25-60%, weak trees), Class 2 (defoliation > 60%, decadent trees).

F = frequency of the same class, and N = total number of trees examined.

In order to study in more detail the influence of the state of the scrubland on the quality indices and associated variables, a univariate analysis of variance (ANOVA) was carried out for all the variables studied. All statistical tests were carried out using IBM SPSS Statistics 21 software.

Results and discussion

Cork quality indexes

Within the same forest, the distribution of average quality index values varied from one plot to another (Table.1).

Table 1. Relative frequencies of occurrence for the three classes of quality index (QI)

	Quality indices classes	Quality classes number	Relative frequency	QI
Cleaned plot (ZA, TA and AM)	A (19.5, 19, 12.75, 12)	2, 4, 6 and 8	0.72	15.60
	B (11, 7, 6.5, 5)	1, 3, 5 and 7	0.13	8.72
	C (1.5)	9	0.15	1.50
	Total		150	8.60
Brushy Plot (ZA, TA and AM)	A (19.5, 19, 12.75; 12)	2, 4,6 and 8	0.2	7.5
	B (11, 7, 6.5, 5)	1, 3,5 and 7	0,22	6.65
	C (1.5)	9	0,58	1.50
	Total		150	5.21

By grouping the quality classes under their indices according to IPROCOR standards, we have observed a high percentage of stopper cork bark (72%) in cleaned plot compared to the percentage of brushy plot (overgrown by the maquis and undergrowth) (20%). The average values of the quality indices register respectively 8.60 (IQ- Cleaned plot) and 5.21 (IQ-Brushy plot).

Cork quality indexes/ cork features

The results obtained for each measured variable are shown in the table 2.

Table 2. Mean for each measured variable according the state of undergrowth

	QI	N	Thickness (mm)	Growth (mm.year-1)	Porosity (%)	Productivity (kg/m²)	Density (kg/m³)	ID
Cleaned plot	Low (C)	22	26.47(2.90)	2.17(0.41)	4.28(2.23)	6.3(0.85)	258.18(61.69)	1.94(0.73)
	Medium(B)	20	24.14(3.97)	2.46(0.11)	4.51(1.36)	6.25(0.00)	250.12(10.50)	1.66(0.09)
	High (A)	108	28.57(4.62)	2.65(0.28)	2.97(1.15)	7.52(065)	194.89(0.00)	1.25(0.06)
	Total	150	28.02(4.50)	2.57(0.34)	3.25(1.48)	7.27(0.84)	207.59(38.45)	1.37(0.37)
Brushy plot	Low (C)	88	24.20(3.11)	1.99(0.23)	6.47(2.51)	5.72(0.56)	302.16(58.31)	2.72(0.32)
	Medium(B)	33	25.42(3.07)	2.47(0.22)	4.24(1.49)	6.33(0.20)	242.50(16.46)	1.87(0.33)
	High (A)	29	25.56(2.11)	1.98(0.81)	5.10(1.50)	5.23(2.84)	213.41(47.65)	1.86(1.08)
	Total	150	24.79(3.01)	2.12(0.45)	5.60(2.31)	5.81(1.31)	269.53(60.36)	2.33(0.68)

In parentheses (standard deviation)

The variables measured show significant differences depending on the scrub condition and quality, varying between 28.57mm (Thickness, cleaned plot, A) and 24.79 mm (Thickness, brushy plot, A). The annual increases go from 2.65 mm/year (Cleaned plot, A) to 1.99 mm/year (Brushy plot, C). It is the same for porosity, in the maintained and cleaned plots, the coefficient of porosity is low (2.97%) (Cleaned plot, A) whereas it is strong in those covered with brush (6.47%) (Brushy plot, C). Productivity and density also go in this direction: 7.52 kg/m^2; 194.84 kg /m^3 (Cleaned plot, A) and 5.72 kg /m^2; 302.16 kg/m^3 (Brushy plot, C). The health status of brushed trees is not satisfactory (ID = 2.72) compared to those cleared of undergrowth (ID = 1.25).The analysis of variance for all the variables has showed an influence of brushwood state on the quality indices ($p < 0.05$; $p < 0.001$) (Table 3).

Table 3. Results summary of test between subject's effects of the variables studied (ANOVA)

Variable		Brushwood status	Quality index	Brushwood status* Quality index
Growth	F	20,64	13,97	13,77
	p	0,000	0,000	0,000
Thickness	F	4,44	6,22	3,26
	p	0,036	0,02	0,04
Porosity	F	18,97	11,06	4,73
	p	0,000	0,000	0,000
Density	F	6,90	70,15	4,14
	p	0,000	0,000	0,000
Productivity	F	27,29	2,57	20,24
	p	0,000	0,078	0,000
Health status	F	48,73	60,75	3,71
	p	0,000	0,000	0,026

$p < 0.001\%$; $p < 0.01\%$; $p < 0.05\%$.

Cork oak is known to be very feverish in competition with other forest species. According to Starkey and Steven (1989), the bushy plants accompanying the cork oak includes evergreen species fleeing calcareous soils frequently succeed in crowding out the cork oak. Some authors claim that the cork oak is more sensitive than the holm oak and the pubescent oak to the negative effects of competition for environmental resources, especially in relation to light, water resources and mineral elements (Gentilesca et *al.*, 2017).
The progression of brushwood in the studied areas has increased the risks of dieback and fires.
The fierce competition from Zean oak and Aleppo pine for water and mineral elements is affecting the physiological state of the cork oak, particularly the growth of cork (earlycotk and latecork). The result is stunted, weakened trees in search of photosynthesis and light. In this situation, dieback affects (state of health) the proper functioning of the cambium and phellogen, causing an increase in porosity. This is a form of adaptation in trees dominated by stress, with the production of large pores favouring good gas exchange with the outside environment despite the loss of foliage at the crown.
It follows then, a rising in the density of the cork tissue, acting on the physical and mechanical characteristics of the cork as the basis of the quality (flexibility and elasticity). According to Pereira (2007), the general tendency is towards higher density values (>300kg/m^3) in corks with more and larger lenticular channels. The lenticular channels contain a filling material of loosely bound cells of different chemical composition and are occasionally lined by heavy lignified cells (Anjos et *al.*, 2008). Indeed, porosity and density are the two main parameters regulating the quality of cork: good cork will have few and small pores superimposed on great flexibility.
The effect of overgrowth is also felt in the physiological response of the trees through the appearance of anomalies other than porosity and excess density. Eleven types of defect were detected by descriptive analysis of the frequencies of cork anomalies (Figure 1).

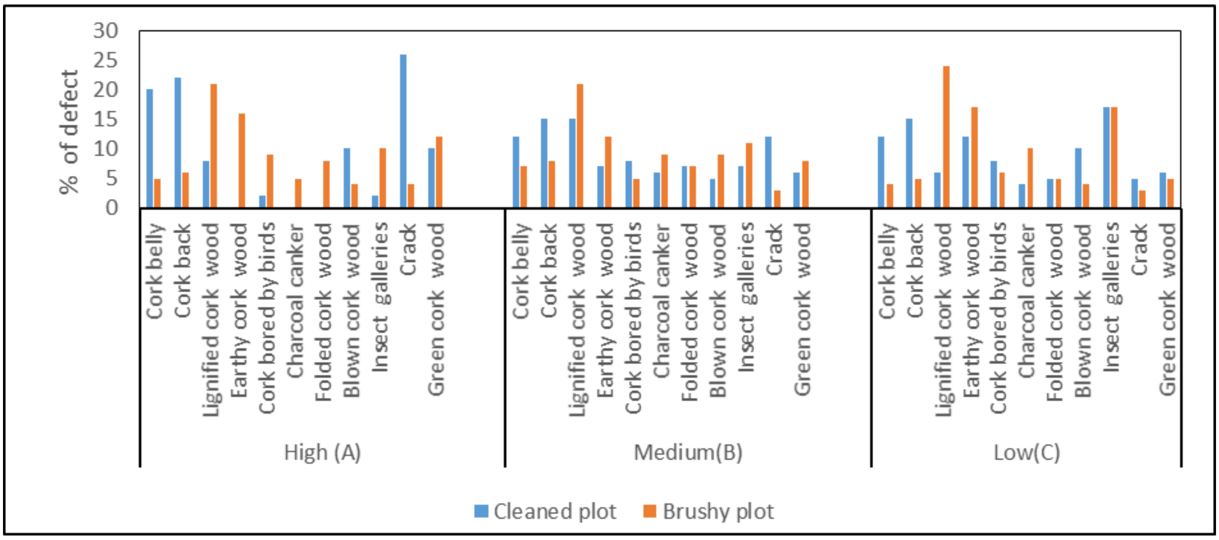

Figure 1. Distribution of cork defects according to the quality indices and the undergrowth

From the figure 1 it becomes clear that the two states of the brushwood produce an amalgam of defects in the cork tissue. In the maintained and cleaned plots, the cork of well-growing trees is strewn with minor anomalies (cork back (A, 22%), crack (A, 26%), green corkwood (A, 10%), blown corkwood(A,10%) and cork belly (A, 20%)) which do not have a significant impact on the quality of the cork. On the other hand, the excess of the undergrowth, and the competition of the Zean oak and the Aleppo pine disadvantage the trees by affecting the phellogen activity by replacing the corky tissue suber by a pulverulent and lignified tissue, considerably affecting the quality of the cork. These are the appearance of intrinsic defects (earthy corkwood (C, 17%), lignified corkwood (C, 24%) and folder corkwood (C, 5%)) and also extrinsic anomalies (insects galleries (C, 17%) and charcoal canker) (C, 10%) linked to phytopathogenic agents such as insect attacks (*Platypus cylindrus, Crematogaster scutellaris* and *Coroebus undatus*) and pathogenic fungi (*Biscogniauxia mediterranea* and *Phytophthora Cinnamomi*).

Conclusion

The marked decline in cork production in quantity and quality in the western region of Algeria is mainly due to poor management: lack of appropriate forestry interventions against scrubland, resination, tree aging and the almost total failure of natural regeneration. This study has shown through two different examples of management that the abandonment of cork oak stands induces the proliferation of undergrowth and scrubland, promoting health problems (dieback characterized by the inability of trees to reconstitute their foliage and produce good quality cork). The overall homogenization of quality indices to the technological variables of cork has supported the role of human responsibility in accentuating the low scores obtained in scrubland plots. Excess undergrowth deprives the cork oak of water resources and mineral elements, significantly affecting the activity of the cortical cambium, which will lead to a poor response of the phellogen by producing a hard and thin cork dotted with various anomalies.

Those responsible for management in the three forests must imperatively opt for moderate and channeled undergrowth after each harvest. It is known that the cork oak left to its own devices without silvicultural treatment and management is doomed to disappear from its natural area.

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INFLUENCE OF *JUNIPERUS SABINA*, ALGINITE AND BIOREGULATORS ON SEED GERMINATION KINETICS OF ROZNAVA ORIGIN BEECH

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Abstract

The purpose of this research was to study the effect of *Juniperus sabina* extract, a natural substance of mineral origin Alginite and bioregulators genistifolioside and Moldstim on the germination kinetics of beech seeds of Roznava (Slovakia) origin. The gibberellic acid served as standard plant growth regulator. Seed viability, determined by two express tests, averaged 78.9%. The highest rate index (2.60-2.66) of seed germination was observed in the variants with Alginite, *J. sabina* and genistifolioside (in the concentrations 0.001%), the lowest in the control (water) – 2.38. Moldstim showed a low germination rate index (2.42), however, the mean germination time was only 50.90 days, which was significantly lower than in other variants (by 1.79-6.85 days). The highest index of mean daily germination (1.33) was in variants with *J. sabina* and Alginite, the lowest in the control – 0.98. Considering that the germination time of a large batch (1000 and more) of beech seeds is rather long and amounts to 90-140 days, stimulation of daily seed germination from 0.98 up to 1.33 (*J. sabina* and Alginite) and 1.25 seeds (genistifolioside) leads to a significant reduction in the total period of seed germination by 24-36 days. Statistical analysis of data on the germination of beech seeds during 126 days of stratification showed that Alginite and *J. sabina* exhibited similar activity and increased the total germination of seeds significantly in comparison with other bioregulators ($LSD_{0.05}$ = 1.88, p<0.001). Genistifolioside stimulated seeds germination at the level of gibberellic acid that was significantly higher than Moldstim and control.

Keywords: *Fagus sylvatica, Juniperus sabina, Alginite, bioregulators, germination.*

Introduction

Fagus sylvatica seeds are deeply dormant seeds (Kolařova *et al*., 2010). The duration of stratification for beech seeds according to various authors (Gugala, 2002; Soltani, 2003; Bezděčková and Matějka, 2015) ranges from 60 to 180 days. Most authors agree that in order to successfully interrupt the dormant period, beech seeds need cold stratification (+ 3 - 4 ºC) at a moisture content of 28-30% for at least 12 weeks, which can last up to 17-20 weeks. However, prolonged cold stratification can also lead to a loss of seed viability. Therefore, the problem of reducing the dormancy period of beech seeds is one of the most important tasks at the present stage, which scientists from many European countries are trying to solve (Staszak *et al.,* 2019).

Numerous modern scientific researches had shown that the addition of ethephon, ethylene, abscisic or gibberellic acids was effective in breaking beech seed dormancy (Soltani, 2003; Calvo *et al.*, 2004, Kolařova *et al.,* 2010; Staszak *et al*., 2019). Our previous studies showed

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the positive effect of some natural growth regulators on the germination of beech seeds of various origins (Elisovetcaia et al, 2022, 2023). At the same time, beech seeds of different origins and years of collection have varying degrees of dormancy.
Therefore, the purpose of this research was to study the effect of plant extract from *Juniperus sabina*, natural mineral Alginite and bioregulators genistifolioside and Moldstim on the kinetics of germination of *Fagus sylvatica* seeds originating from Roznava (Slovakia).

Materials and methods

The experiments were carried out in the laboratory Natural Bioregulators, in solarium of the Institute of Genetics, Physiology and Plant Protection, Moldova State University and in the Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources during 2022-2023.
The study used *Fagus sylvatica* seeds from 3 populations collected in the fall of 2022 in the area of Roznava (Rožňava) – Nižná Slaná, Rožňava District, Košice Region, Republic of Slovakia. The beech seeds were collected at an altitude of 560-580 m above sea level.
Seed moisture was measured using the RADWAG moisture analyser by gradual drying with a halogen lamp at a temperature of 160 °C to a constant weight (if the change in weight does not exceed 1 mg in 10 sec) (Elisovetcaia *et al.,* 2023). For each determination, 3 grams of seeds were used.
The viability of seeds was determined by two test using the 2.3.5-triphenyltetrazolium chloride (TTC) solution (Kerkez *et al.,* 2018; França-Neto and Krzyzanowski, 2019) and hydrogen peroxide (HP) solution (Sharma and Sibi, 2020). Root length (in cm) of germinated seeds in the HP test was measured.
The seed germination test was carried out in accordance with the recommendations of the International Seed Testing Association (ISTA, 2006). Each variant consisted of 200 seeds on 4 replications. Before the germination by stratification, the beech seeds were soaked for 22-24 hours at room temperature in aqueous solutions (0.001%) of *Juniperus sabina* plant extract, natural mineral Alginite, and bioregulators genistifolioside from *Linaria genistifolia* (L.) Mill) and Moldstim from *Capsicum annuum* L. (Mascenco *et al.*, 2015; Borovskaia *et al.*, 2020). Distilled water was used as a control, and 0.001% solution of gibberellic acid was used as a standard.
The following observations: total germination percentage (TGP), mean daily germination (MDG), mean germination time (MDT), germination rate index (GRI) were calculated by the standard formulas. On the 70th day of stratification, the length of the seed roots was measured.
Statistical analysis. The results were processed with the use of Statgraphics Plus 5.0 software based on general statistical procedures, including one-way ANOVA and Multiple Range Tests (Tukey Honestly Significant Difference). Pearson's correlation analysis was conducted using the CORR procedure.

Results and discussion

The TTC and HP tests for the assessment of the viability of beech seeds in laboratory conditions showed a strong positive correlation, the Pearson coefficient was greater than 0.9998, which allows their equivalent use. The highest viability (76.0% TTC test, 81.8% HP test) by both methods was demonstrated by beech seeds collected in location no. 1, Roznava. Statistical analysis of the data showed that the viability of seeds from Roznava locations number 2 and 3 was insignificantly lower ($LSD_{0.05}$=5.2 for HP, and 6.6 for TTC test) compared to seeds from location no.1. The weight of 100 seeds ranged from 28,60±1,31 to 29,62±0,84 g with a humidity of 12,64±0,44-13,71±0,49%. Thus, based on the data obtained on seed viability, as well as according to quantitative assessment, seeds from plot no. 1 of Roznava with the highest germination rate 76.0-81.8% and the heaviest weight of 100 seeds equal to 29.62 g with a humidity of 13.71% were selected for experiments to increase germination and resistance under the influence of bioregulators (*Juniperus sabina*, Alginite, Moldstim, genistifolioside, gibberellin).

Seed germination during stratification is shown in Figure 1. The total seed germination (TGP) by the 126th day of stratification was achieved in variants with aqueous solutions of *J. sabina* extract and Alginite – 68.0% each (Fig. 1).

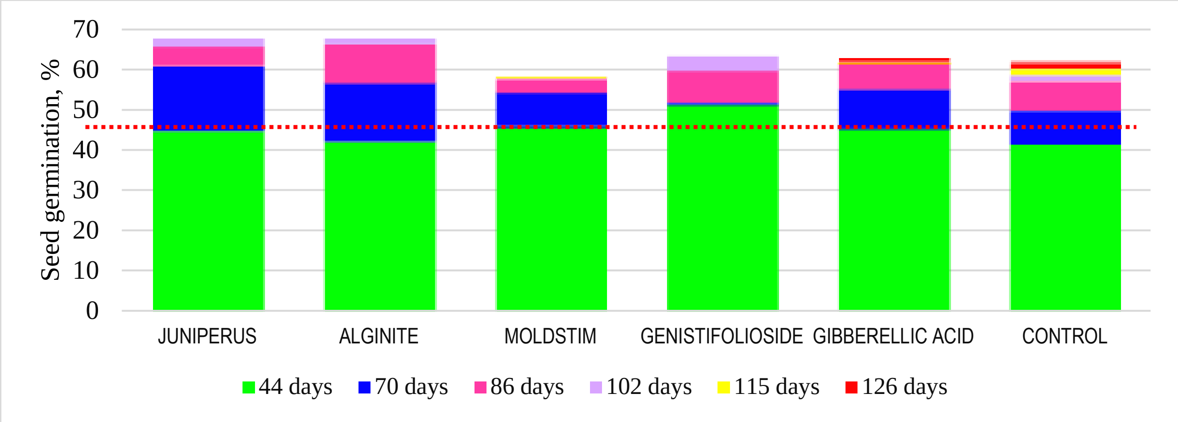

Figure 1. The influence of *J. sabina* plant extract, natural mineral Alginite, and bioregulators genistifolioside and Moldstim on the total germination of beech seeds of Roznava origin during stratification.

Germination indices of Roznava origin seeds are presented in Table 1. The highest rate index (GRI) of seed germination was observed in the variants with Alginite, *J. sabina* and genistifolioside (in the concentrations 0.001%) – 2.60-2.66, the lowest in the control (water) – 2.38 (Table 1). The germination of beech seeds in the Moldstim on the 44th day of stratification was ahead of variants with *J. sabina*, Alginite and gibberellic acid (Fig. 1). After the 86 days of stratification, seed germination in the Moldstim variant was slightly behind, but still significantly exceeded the control variant and reached 58.0%. Over the remaining 40 days of stratification (from 86 to 126 days), germination in the Moldstim variant increased by only 1.5%. The obtained data indicate that despite the low germination rate index (2.42), the mean germination time (MGT) in the Moldstim was only 50.90 days, which was significantly faster than in other variants (by 1.79-6.85 days). Consequently, treatment of seeds of Roznava origin with Moldstim significantly stimulated germination during stratification, as a result, the majority of viable seeds in this variant managed to germinate in a shorter period of time. The genistifolioside variant also characterized by low MGT (52.69 days). Variants *J. sabina* and gibberellin showed the mean germination time of 3.09-4.01 days higher than that of Moldstim and 1.30-2.22 days higher than that of genistifolioside. The longest time for seed germination was observed in the control, MGT was 57.75 (Table 1).

The highest index of mean daily germination (1.33) was in variants with *J. sabina* and Alginite, the lowest in the control – 0.98 (Table 1). Pre-treatment of beech seeds with these preparations led to a significant increase in germination (by 3.07-5.3%) of seeds in comparison with the variants of Moldstim and gibberellic acid. The lowest germination rate was in the control – 10.5% lower than that of *J. sabina* and 8.5% lower than that of Alginite (Table 1). Considering that the germination time of a large batch (1000 and more) of beech seeds is rather long and amounts to 90-140 days (Bonner and Leak, 2008), stimulation of daily seed germination from 0.98 (control) and 1.0 (gibberellic acid) up to 1.33 (in *J. sabina* and Alginite) and 1.25 seeds (genistifolioside) leads to a significant reduction in the total period of seed germination by 24-36 days (in comparison with gibberellic acid and control). This significant reduction in germination time is very important for planting.

Table 1. Germination indices of beech seeds of Roznava origin during stratification under the influence of *J. sabina*, Alginite and bioregulators.

Variants	Germination indices				Distribution by groups at p<0.05) in comparison with	
	MDG, seeds/day	MGT, day	GRI, seeds/day	TGP, %	Control	Gibberellic acid
Juniperus sabina	1.33	54.91	2.66	68.00	I	I
Alginite	1.33	56.69	2.60	68.00	I	I
Moldstim	1.02	50.90	2.42	59.50	II	III
Genistifolioside	1.25	52.69	2.61	63.50	I	II
Gibberellic acid	1.00	53.99	2.52	63.00	I	
Control	0.98	57.75	2.38	58.50		III
$LSD_{0.05}$				1.88, p<0.05=2.48E^{-08}		

Statistical analysis of data on the germination of beech seeds of Roznava origin on the 126th day of stratification showed that *J. sabina* and Alginite exhibit the same activity and increase the total germination of seeds significantly in comparison with all variants. Genistifolioside stimulated seeds germination at the level of gibberellic acid that was significantly higher than Moldstim and control. The Moldstim treatment variant is at the control level and is significantly inferior to other variants (Table 1). The main number of seeds in the variants with *J. sabina* and Alginite (57.0 and 61.0%, respectively) germinated in 70 days, while in the control and gibberellin a similar (57.5-61.5%) number of seeds germinated in 86 days (Fig. 1). As a result, the time required for germination of the main proportion of viable seeds in the variants with *J. sabina* and Alginite was 1.23 times less than in the control and gibberellin (Table 1). Thus, according to the obtained data, both *J. sabina* and Alginite had a significant impact on germination rate of beech seeds of Roznava origin and the total seeds germination percentage. Genistifolioside showed growth stimulation activities at the level of gibberellic acid, and Moldstim, despite the fact that it lagged behind in some indicators, still showed the lowest MGT (Table 1).

The data obtained are in good agreement with earlier studies on the germination of beech seeds 2019 year of collection originating from Nitra beech stand (Tribeč Mountains, Western Carpathians) in the Slovak Republic (Elisovetcaia *et al.*, 2022). The best results of increase in daily germination (up to 18.5% in comparison with control) were obtained under influences of Moldstim and genistifolioside at concentration of 0.001% (Elisovetcaia *et al*., 2022).

Data analysis showed that the longest roots of germinated seeds were observed (at $p \leq 0.001$) in the variant with genistifolioside. They were 6.77-13.16 mm longer than in other variants (Table 2, Fig. 2). The length of roots in the variant with Moldstim for a significance level of $\alpha = 0.05$ was comparable to the variants with *J. sabina* and gibberellic acid and significantly higher than in the control. For significance levels $\alpha = 0.01$ and $\alpha = 0.001$, the length of roots in the variant with Moldstim was also comparable to the control. The length of the roots of germinated seeds in the treatments with *J. sabina* and gibberellic acid was comparable to the control and Moldstim and was significantly inferior to genistifolioside at all levels of significance. The length of the roots in the variant with Alginite was at the control level at all significance levels – $\alpha = 0.05$; $\alpha = 0.01$ and $\alpha = 0.001$ and are significantly lower than other variants only at the significance level $\alpha = 0.05$. For significance levels $\alpha = 0.01$ and $\alpha = 0.001$, the length of the roots in the variant with Alginite was also comparable to the variants treated with *J. sabina* and gibberellic acid (Table 2).

Table 2. Distribution of the roots length of seeds germinated during stratification

Variants	Length of roots, mm			±	Distribution of variants into groups depending on the confidence levels									
					α = 0.05 +/- limits 3.03-3.24				α = 0.01 +/- limits 4.07-4.27			α = 0.001 +/- limits 5.12-5.46		
	average	min	max		1	2	3	4	1	2	3	1	2	3
Alginite 0.001%	22.90	7.00	47.00	8.17	X				X			X		
Control	25.74	8.00	48.00	9.35	X	X			X	X		X	X	
Gibberellic acid 0.001%	26.29	7.00	52.00	10.70		X	X		X	X		X	X	
Juniperus sabina 0,001%	26.40	9.00	48.00	9.49		X	X		X	X		X	X	
Moldstim 0.001%	28.95	3.00	53.00	11.29			X			X			X	
Genistifolioside 0.001%	36.12	10.00	70.00	12.44				X			X			X

Note: 3-4 – homogeneous groups without significant differences (Multiple Range Tests - Method: 95,0; 99.0 and 99.9 percent Tukey HSD).

a b c d

Figure 2. The roots length of germinated beech seeds in the different variants: Alginite (a, b); control (c) gibberellic acid (d)

Conclusion

The treatment beech seeds of Roznava (Slovakia) origin with plant extract from *Juniperus sabina* and natural mineral Alginite significantly increased seed germination during stratification in comparison with control and gibberellic acid, and with natural bioregulators – genistifolioside and Moldstim. The total germination of seeds was 5.0-9.5% higher than that of the control and gibberellic acid; and the mean daily germination was accordingly 33.0-35.7% more. The bioregulator genistifolioside stimulated seed germination at the level of gibberellic acid, and significantly higher than the level of Moldstim and control. The germination index reached 2.61 and was at the level of the *Juniperus sabina* (2.66) and Alginite (2.60) variants and significantly higher than the control (2.38) and gibberellic acid (2.52). Treatment with the bioregulator Moldstim contributed to a significant reduction in the time of seed germination compared to other variants, including control and gibberellic acid.

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CULTURE OF THE SILVER FIR (*ABIES ALBA* MILL.) IN ORAVIȚA VALLEY, ROMANIA

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Abstract

Silver fir is the second most widespread conifer species after the Norway spruce and occupies an area of about 0.3 mil ha, which means 4.25 % of the Romanian forests. The silver fir occurs in Oravita Valley, sub-region Cerna–Semenic, South-West of Romania, but the origin of the tree species is unclear. As located outside of the native rage, on a eutrophic and skeletal rendzina, rich in bases, with 800-1000 mm precipitation, silver fir has favorable conditions for development in Oravita Valley. According to Forest management plans of Working Circle, silver fir was introduced to Oravita Valley around 1920, at the time of Steel Works and Domains of Reșița (UDR) management. Square areas of 0.25 ha, planted with silver fir in 30-40-year-old European beech stands, constituted natural tree species distribution points. However, the dates registered in old unit management descriptions showed that silver fir already exists. In this context, the research aimed to highlight the dynamics of tree species in terms of the percent of participation in stand composition and biometric characteristics. Dates about the total and effective area (area multiplied by density of silver fir species), the participation percentage in the stand's composition, the age, regeneration way, DBH and the volume were analyzed. The analysis of the data from the existing Forest management plans showed a decrease in the proportion of silver fir from 30% (in 1955) to 8% (in 2008). Regarding the biometric characteristics, the mean diameter and volume are similar or even higher than the national average values (according to the national yield tables).

Keywords: *silver fir, biometric characteristics, stand speciescomposition, percent of participation in mixed forest.*

Introduction

One of the most valuable conifer trees in Europe, silver fir (*Abies alba* Mill) (Dobrowolska et al. 2017, Dinca et al. 2022) it is also one of the most threatened conifer species (*Ugarković et al. 2021*). Silvir fir is the tallest tree species of Europe (more than 60 m height), very shade tolerant, and forms a dense canopy (Ellenberg and Leuschner 2010). The species are important for valuable wood production and for their protective role of European forests (Simberloff 1998.). It is sensitive to ungulate browsing and fire (Tinner et al. 2000) and can be affect by insects, fungi, late frost and industrial emissions (Ellenberg 2009, Kozakova et al. 2011). Silver fir is distributed in Central, Southern and Eastern Europe, on the mountain area (200-2000 a.s.l.), with 700-1800 mm yearly precipitations (*Mauri et al. 2016*). Mixed fir-European beech represented an important part of European Mountain forest (Boncina et al. 2002) but in the last decades the decline of abundance of silver fir in European forest was significant (Ficko et al. 2010). In Romania, the area of silver fir is about 0.3 mil ha, that means 4.255% from Romanian forest area. Silver fir is found in Romanian Carpathians Mountain where tree species is mixed with Norway sprouce (Eastern Romanian Mountains) and European beech (Southern Romanian Carpathians). In The South-West of Romaniais are found mixed broadleaves with fir or mixed forest with beech and fir. Silver fir tree stands

distribution is the result of the great diversity of climatic, geological, relief and edaphic conditions (Barbu I and Barbu C 2005). Silver fir grows in sub-Mediteranean climate in the south west of the country and continental climate in the north area (Teodosiu et al. 2019).
In Oravita Valley, the silver fir was artificial introduced under Steel Works and Domains of Resita company administration. It is located at the lowest Romanian altitude, 250-300 m. In order to introduce silver fir in 30-40 years old beech stands, squares of 0.25 ha were planted. It seems to be the cores of silver fir natural spread. This kind of plantation were made on Marila, the area where silver fir seems to come from (Management Plan of Working Unit V, Oravita Valley, 2008).In the last decades silver fir was naturally regenerated in mixed stands, especially with European beech.
The research aimed to highlight the dynamics of tree species in terms of the percent of participation in stand composition and biometric characteristics.

Materials and methods

Working Unit V Valea Oraviţei with an area of 3,658.0 ha is located on the South slopes of Banat Mountain, in the hilly region of Semenic in Romania.The lithological substrate consists of calcareous rocks on which well-structured soils have formed, with medium-deep depths, with a balanced hydrological regime and physico-chemical qualities favorable to the development of forest vegetation. The slopes are between 16-30 degrees, the medium elevation between 601-800m.The climate, according to Köppen's classification system, is Cfbx (temperate oceanic climate) with a mean annual temperature of 7^0C, a maximum absolute temperature of 39°C, and a minimum absolute temperature of –21.5 °C (Management Plan of Working Unit V Oravita Valley, 2008).
Working Unit V Oravita Valley Plans from 1955, 1967, 1977, 1988 and 2008 represent the study material. Dates about the total and effective area (area multiplied by density of silver fir species), the participation percentage in the stand's composition, the age, regeneration way, DBH and the volume were registered. The evolution of the silver fir area and the dynamic of DBH and volume value according to age and yield class were analyzed

Results and discussions

Dates from Working Unit Plans showed a reduction of area with silver fir starting with 1955 (Table 1).

Table 1. The dynamic of area with silver fir in Working Unit V Oravita Valley

Working Unit Plan from	The Working Unit area (covered by forest)	The area of sub –compartment with silver fir	The effective area with silver fir
1955	3564.3	772.7	229.8
1968	3435.3	730.3	220.7
1977	3432.9	711.4	180.6
1988	4136.7	691.8	151.3
1998	3900.7	460.9	49.2
2008	3658.8	447.8	36.1

The silver fir mixed stands area decrease from 772.7 ha in 1955 to 447.8 ha in 2008. The decrease is even more accelerated according with effective area occupied by silver fir, from 229.8 hain 1955 to 36.1 ha in 2008. There are a lot of sub-compartments where silver fir just disappear or represent a small percent in stand composition. If silver fir area occupied 30% from mixed stands in 1955, in 2008 the tree species occupied only 8%.

There are changes in the participation percentage in the stand's composition. In 1955 it should be find stands with 80% of silver fir in stands composition, in 2008 there were not more than 20% (Table 2).
The same trend was recorded in Europe, a change in mixed silver fir-European beech stands composition, a reduction of silver fir percent of participation and an increase of European beech (Vrška et al. 2009).

Table 2. The dynamic of silver fir stand composition in Working Unit V Oravita Valley

Stand composition	Silver fir area in the year					
	1955	1967	1977	1988	1998	2008
1SF 9HS, SS	12.06	11.63	11.04	12.13	25.89	32.67
2SF 8HS	27.56	4.13	17.55	19.10	7.86	3.43
3DF 7HS	22.99	34.45	34.44	37.26	9.38	
4SF 6HS	41.11	6.66	23.94	18.24	0.53	
5SF 5 HS, SS	68.05	21.59	33.53	63.31		
6SF 4HS	35.42	99.71	52.77	0.25	5.07	
7SF 3HS	21.22	10.21	5.99		0.50	
8SF 2HS	1.39	32.29	1.28	1.02		
Total	229.80	220.67	180.54	151.31	49.23	36.10

SF -silver fir; HS – hardwood species; SS – softwood species.

The analysis of the areas occupied by silver fir, by age class, in the period 1955 to 2008 showed an unbalanced stands structure in the past (Table 3) with large areas of silver fir in the old class age.
According to existing data in Management Plans, many factors occurred over time: strong wind (1943 and 1974), high intensity of pests, maybe physiological age of silver fir located outside of the natural area, but especially the human factor.
The highest reduction in the silver fir area happened in 1990 due to a lack of correlation between forest exploitation and silver fir regeneration. By introducing silver fir in the optimum climate of European beech, high competition between these tree species seedlings occurred, so promoting silver fir release cutting is necessary.
The massive silver fir exploitation and probably the lower attention given to silver fir regeneration led to the reduction of the areas occupied by this species.

Table 3. The evolution of the areas occupied by silver fir, by age classes

Age class	Silver fir area in the year					
	1955	1967	1977	1988	1998	2008
1-20	2.67	1.07	1.06	5.94	11.95	5.5
21-40	7	1.60	0.68	1.16	6.23	0.2
41-60	50.9	56.93	6.44	0.96	4.02	11.3
61-80	169.23	8.04	15.40	4.99	2	1.4
81-100		153.03	144.80	113.65	11.67	10.2
>100			12.16	24.61	13.36	7.6
Total	229.8	220.67	180.54	151.31	49.23	36.1

Not only the area but dendrometric characteristics are also important. The dynamic of the maxim and minimum value of silver fir diameter at breast height (DBH) showed an increase of this value according to age and a decrease according to yield class. The DBH values are close to the average national value for many stands. There are stands where the values are higher. For example, a 90 years old silver fir from sub-compartment 41C, the II-nd yield class with a DBH of 50 cm (average national diameter is 36.1cm), or 120 years old silver fir, II-nd yield class with a DBH of 56 cm (43,6 cm national average value). High values higher than the national average were also found in the IVth - Vth yield class stands.
The same design is applied to the volume stands. Most of the silver fir registered value of volume according to national average value or even higher as the silver is from sub-compartment 28A, yield class 3, DBH 40 cm (average national value 34 cm) and volume 685 mc (average national value 637mc).

Table 1. Silver fir diameter at breat height on yield and age class

Yield class	Age class	1955			1967			1977			1988			1998			2008		
		Value of DBH (cm)																	
		min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table
I	21-40	10	10	12.3															
	41-60	30	30	29.3															
	61-80	30	40	33.6															
II	1-20																		
	21-40							14	14	12.3									
	41-60	26	26	25.6	20	20	21.8	26	26	25.6									
	61-80	26	40	29.5-33.0				36	38	33.0									
	81-100				50	50	36.1	38	38	36.1	40	44	36.1-39.0						
	101-120										56	56	43.6						
	>120													56	56	43.6			
III	1-20	7	7	5.0				3.0	3.0	2.1	6	10	3.5-5.0	1	4	2.1	8	16	3.5-5
	21-40	16	16	25.4	8	14	8.7-13.1				16	16	6.6	10	20	6.6-13.1	20	20	10.9
	41-60				18	28	17.5-21.8	20	22	15.3-19.7	20	20	19.7	24	26	17.5	24	38	15.3-21.8
	61-80				26	32	23.7-28.5	24	30	23.7-25.4	26	36	25.4-28.5	34	34	21.8	28	28	27.0
	81-100				22	44	30.0-34.0	34	44	31.4-34.0	33	44	31.4-34.0	32	42	28.5	32	42	31.4-34
	101-120							40	44	36.3	40	46	34.0-34.3	32	44	30.0-31.0	36	42	38.3
	>120																46	56	-
IV	1-20							6	6	2.8									
	21-40										14	28	5.3-6.8						
	41-60	20	22	18.4	22	22	16.2	14	14	12.3	24	24	18.1						
	61-80				26	26	24.2	24	24	19.8	20	20	19.8						
	81-100				14	38	26.7	34	44	26.7-29	28	36	25.5-29.9	28	28	25.5			
	101-120										28	42	30.0-31.0	38	38	31.0	38	38	32.7
V	1-20										8	8	2.3						
	21-40																		
	41-60							22	22	10.9									
	61-80							24	24	18.5									

Table 2. Silver fir stand volume on yield and age class

Yield class	Age class	1955			1967			1977			1988			1998			2008		
		Value of volume (cm ha^{-1})																	
		min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table	min	max	Yield table
I	21-40	443	443	231										378	378	306			
	41-60	471	591	648															
	61-80	557	725	735															
II	21-40							192	192	181									
	41-60	385	385	532	667	667	532	478	478	532									
	61-80	327	726	610-675				611	681	532-675									
	81-100				606	606	728	614	614	728	283	683	728-771						
	101-120										594	594	838						
	>120													788	788	-			
III	1-20							33	33	20	44	94	20-66	4	12	20	67	150	44-66
	21-40	150	150	228	144	193	134-228				110	110	97	150	322	97-228	267	267	179
	41-60				240	487	329-419	267	325	278-377	414	414	377	388	450	329-419	356	650	278-419
	61-80				100	579	456-550	450	512	456-490	488	613	490-550	544	544	550	450	450	521
	81-100				125	613	576-637	408	683	598-637	541	682	598-637	543	613	576-598	543	688	598-637
	101-120							648	717	655-672	650	683	637-672	400	720	672	317	643	697
	>120																		
IV	1-20							30	30	30									
	21-40										170	381	67-94						
	41-60	120	175	279-314	325	325	279	171	171	200	400	400	314						
	61-80				413	413	431	313	388	347	475	571	405						
	81-100				131	588	473	450	546	473-508	438	448	453-508	479	479	453			
	101-120										513	675	524-538	583	583	538	580	580	559
V	1-20										71	71	16						
	21-40																		
	41-60							215	215	160									
	61-80							325	325	295									

Conclusion

From 1955 to 2008, the silver fir area decreased from 30-40% to less than 8%.
The silver fir area was dramatically reduced due to strong wind, high intensity of pests, maybe the physiological age of silver fir located outside of the natural area, and human activity.
The dynamic of DBH and volume showed an increase of these values according to age and a decrease according to yield class. The values of DBH and volume are close to national average values or even higher for many stands.
As located outside of the native range, on a eutrophic and skeletal rendzina, rich in bases, with 800-1000 mm precipitation, silver fir has favourable conditions for development in Oravita Valley.
An effective way to preserve the silver fir population is their natural regeneration, using long-term and small-scale regeneration methods.
Release cuttings are necessary to promote silver fir due to high competition between silver fir and European beech seedlings, species located in their optimum climate.

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THE POTENTIAL OF LAND FOR THE IMPLEMENTATION OF FOREST CLIMATE PROJECTS IN RUSSIA

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Abstract

The article discusses the implementation of forest climate projects in Russia. The use of forest lands and agricultural lands can lead to both an increase and a decrease in the total stock of biomass of ecosystems and, accordingly, be accompanied by a net absorption or net CO_2 emission into the atmosphere. The potential of areas for carrying out project activities aimed at absorbing greenhouse gases on forest and agricultural lands has been determined. The potential of forest lands suitable for the implementation of forest climate projects for reforestation and afforestation is estimated at 1068.4 thousand hectares. Such forest climate projects involve the creation of special plantations from various fast-growing tree species, based on gentle soil cultivation technologies. The most suitable regions for the implementation of forest climate projects for reforestation and afforestation are the regions of the Central, Southern, North Caucasus and Volga Federal Districts of Russia, that have a drier climate that is less suitable for growing forests. Forest climate projects have significant potential for improved agricultural land management (agroforestry). For the implementation of climate change agroforestry projects, the most suitable areas are areas of unused arable land that are subject to overgrowth by trees and shrubs, as well as land plots that were previously used for arable land.

Keywords: *forest climate projects, agroforestry, land, forestry economics, Russia.*

Introduction

According to the data of the Global Forest Resources Assessment for 2020 (conducted by the Food and Agriculture Organization of the United Nations (FAO)), the Russian Federation owned 20% of the world's forests (815 million hectares).
According to various researchers (Emrinelson et al., 2024; Canham et al., 2024; Wang et al., 2024; Lei et al., 2024; Zhang, 2023; Stepanova et al., 2023) the key function of forests as ecosystems is carbon absorption. Forests remain the main absorber of greenhouse gases according to international reporting data.
In the context of the set goal of achieving carbon neutrality of the economy of the Russian Federation by 2060, the question arises about what measures the state can take in forestry to reduce emissions and increase carbon absorption, including national and regional projects on forests conservation (Panyavina, 2022), as well as identify cases in which representatives of the business community may participate in order to achieve carbon neutrality. One of the measures to achieve carbon neutrality, therefore, are voluntary forest climate projects aimed at reproducing the natural capital of forests (Zhang et al., 2023; Lobovikov et al., 2023; Coffield et al., 2022), namely – reforestation (Morkovina et al., 2023) and afforestation (Dadile et al., 2023), as well as agroforestry (Taillandier et al., 2023). The peculiarity of such projects is the long period of their implementation – 15 years or more.
Each of the listed types of forest climate projects is focused on preparing forest areas for appropriate activities, directly planting forest plantations and caring for such plantations (Andres et al., 2022).

Currently, many climate projects aimed at carbon capture are being implemented in various countries (Osmundsen et al., 2012). Researchers are considering the possibilities of implementing climate projects by comparing the environmental and economic effects and the costs of their implementation (Schubert, 2021). A number of studies indicate that the costs of sequestration of greenhouse gases vary significantly both between individual projects and within countries (Burniaux, 2009). Individual countries use various tools to support climate projects (subsidies, preferential tariffs, etc.) (Capros, 2013). The ongoing discussions on carbon prices and emissions charges also highlight the importance of implementing voluntary climate projects. At the same time, the implementation of voluntary climate projects is primarily limited by the potential of suitable areas. For Russia, which has significant potential for lands of various categories, it is important to establish the most suitable sites for the implementation of forest climate projects.

This is important for the formation of measures aimed at carbon sequestration in forestry and agriculture, and for attracting businesses to implement voluntary climate projects.

The purpose of this study is to determine the potential of lands for the implementation of forest climate projects at the regional level of Russia, due to their significant differences in geographical, climatic and economic aspects.

Material and Methods

In the course of this study, materials from official statistical reports of sectoral public administration bodies in the Russian Federation, including the Federal Forestry Agency, information from public administration bodies of the subjects of the Russian Federation, data from the Unified State Registry of Real Estate, the National Report of the Russian Federation on the Inventory of the Anthropogenic Emissions and Sinks of Greenhouse Gases Not Controlled by the Montreal Protocol were used.

In this work, the methods of desk research, comparative analysis, formalization in the form of modeling of forest climate projects, and investment analysis are used.

In the course of a desk study, the state forest registry were analyzed in order to determine the potential of land resources for the implementation of forest climate projects according to the criteria of expediency of carrying out forestry activities, as well as according to the criterion of economic accessibility of territories.

Results and Discussion

Reforestation projects involve the restoration of vegetation cover by planting, sowing or natural restoration of woody vegetation with human help, including the entire cycle of reforestation on forest lands. Project activities can be implemented in the form of creating carbon-depositing plantations, forest resource plantations, as well as mixed multi-species plantations using a wide range of additional measures to increase the safety of forest crops at early stages and increase the level of greenhouse gas absorption not only by the phytomass pool, but also by the soil.

Afforestation projects involve the creation of forest plantations on non-forest lands and agricultural lands. Agroforestry projects involve the deliberate creation of forest plantations in combination with crops or livestock to generate benefits and services.

Agroforestry is a dynamic, environmentally sound natural resource management system that, through the integration of trees on farms into the agricultural landscape, diversifies and supports production to increase social, economic and environmental benefits for land users at all levels. Project activities can be implemented in the form of creation and reconstruction of

anti-erosion and protective forest plantations, as well as agroforestry systems (alley and strip farming systems) on agricultural lands.
The diversity of the natural conditions of the territory of the Russian Federation determines the regional specifics of land use, forestry and agriculture, and as a result, the specifics of the implementation of climate projects.
For forest climate reforestation projects, it is possible to use the lands of the reforestation fund represented by non-overgrown burns, wastelands and clearings (Table 1).

Table 1. The potential of reforestation project activities in the forest fund of the federal districts of the Russian Federation as of 01.01.2022

Federal District	The area of forest lands intended for reforestation (reforestation fund), thousand hectares			
	non-overgrown burns	dead forest plantations	wastelands and clearings	total
Central	6.7	34.8	33.0	74.5
Southern	17.8	7.7	55.5	81.0
North Caucasian	0.7	1.1	6.4	8.2
Volga	22.1	27.2	70.7	120.0
Ural	603.0	108.0	117.2	828.2
Siberian	2,650.9	741.9	322.2	3,715.0
North Western	50.3	61.0	11.1	122.4
Far Eastern	21,935.4	297.1	1,954.2	24,186.7
Total	25,286.9	1,278.8	2,570.3	29,136.0

*Source: Author’s elaboration based on the state forest registry

The potential of reforestation project activities on forest fund lands is estimated at 29,136 thousand hectares, including 25,286.9 thousand hectares in non-overgrown burns, 1,278.8 thousand hectares of dead forest plantations area.
At the same time, reforestation should be carried out with forest species that ensure high productivity of future plantations in combination with significant the ability to absorb greenhouse gases.
Afforestation, as well as reforestation, leads to an increase in the level of carbon sequestration. Afforestation projects on the lands of the forest fund can be implemented in areas where, for various reasons, the forest has not previously grown. In this regard, prospects for the implementation of afforestation projects have areas of non-forest lands that are inconvenient for use (swamps and sands). In practice, each of the plots should be examined in detail for the possibility of afforestation, however, for orientation, it is necessary to assess the existing potential in the context of these categories of non-forest lands of the forest fund. When selecting areas for reforestation, it is necessary (according to remote sensing / field survey data) to identify areas unsuitable for tree planting: saline or difficult to access, take into account the location, proximity to watering points, soil blackening, etc.
Taking into account the above, when analyzing the lands of the forest fund for the implementation of forest climate reforestation and afforestation projects, taking into account the criteria for the expediency of forestry activities, economic accessibility of territories, the following potential areas for such projects were identified (Table 3).

Table 2. Potential of project activities on forest fund lands in the federal districts of the country

Federal District	Areas, thousand hectares intended for forest climate projects	
	on reforestation	on afforestation
Central	8.0	196.1
Southern	22.7	282.4
North Caucasian	7.3	89.6
Volga	92.8	366.5
Total	130.8	934.6

*Source: Author’s elaboration based on the state forest registry

Climate projects implemented in the regions of the Central Federal District, Southern Federal District, North Caucasus Federal District, and the Volga Federal District will have significant potential, due to the peculiarities of forest cultivation and the creation of forest crops in the southern regions of the country. A warmer climate promotes accelerated growth of tree species, and with sufficient humidity in these regional systems, highly productive plantations can be formed, providing a high level of GHG absorption. The total area for reforestation in the Central, the Southern, the North Caucasian and the Volga Federal Districts is 130.8 thousand hectares. In total, the potential of the areas for the implementation of forest climate reforestation and afforestation projects amounted to 1065.4 thousand hectares.

Climate projects on agroforestry are a promising model for sequestration of greenhouse gases. During the period from 2010 to 2020, the area of agricultural land in the Russian Federation decreased from 400 million hectares to 382.4 million hectares, that is, by 17.6 million hectares, or 4.6%. In 2023, the area of agricultural land amounted to 379678.4 thousand hectares, including lands occupied by perennial plantations amounted to 1925.2 thousand hectares. The area of unused plots of agricultural land accounted for 11.9% of the total area of agricultural land in the country, including unused agricultural land – 32.08 million hectares (16.2%), the area of unused arable land - 18.65 million hectares (16.05%) (Figure 1).

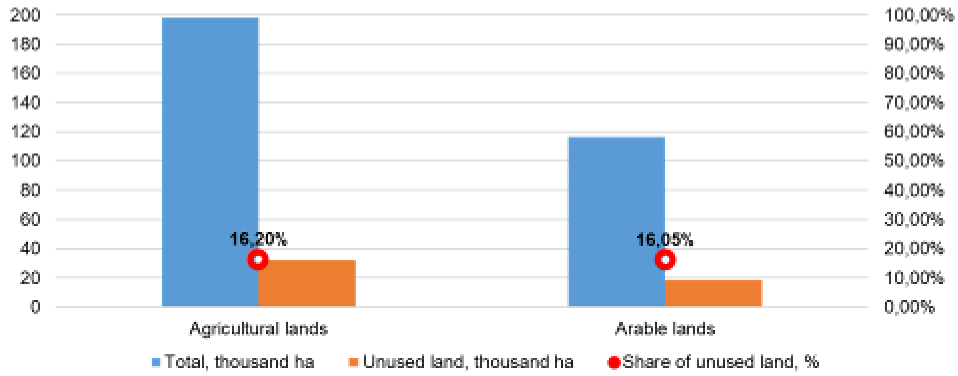

Figure 1 - Unused agricultural land and arable land in comparison with their total areas

*Source: Author’s elaboration based on the public administration bodies data and the Unified State Registry of Real Estate

The return of unused land to agricultural circulation has a number of problems from biological to economic, associated with a steady change in the qualitative characteristics of agricultural land over a long period of time. The total area of arable land suitable for agricultural use as of 01.01.2022 was 7.6 million hectares, i.e. 40.6% of all unused arable land in the country. The remaining areas of unused arable land (slightly more than 50%) are lands overgrown with woody and shrubby vegetation and subject to other negative processes.

The most significant proportion of unused arable land overgrown with woody and shrubby vegetation was noted in the Volga, Siberian and Central Federal Districts – 11%, 8% and 12% of the total arable land area in the Russian Federation, respectively. The share of the total area of unused arable land in the Russian Federation is minimal in the North Western (3.0%), Far Eastern (2.3%), Ural (2.8%), Southern (1.4%) and North Caucasian (0.2%) federal districts (Figure 2).

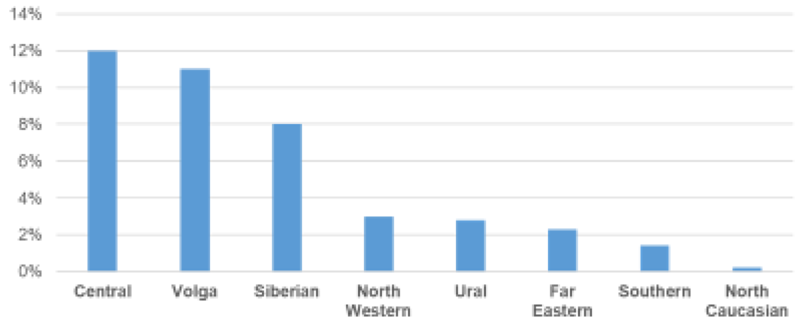

Figure 2 – The share of unused arable land overgrown with woody and shrubby vegetation in the total area of unused arable land by federal districts in the Russian Federation as of 01.01.2022, %

*Source: Author’s elaboration based on the public administration bodies data

The potential of agricultural land for the implementation of climate projects is significant, only the area of arable land unsuitable for return to agricultural circulation is estimated at between 4 and 4.8 million hectares. If the costs of bringing arable land into circulation significantly exceed the potential benefits from their use, it is advisable to abandon the introduction of plots into agricultural circulation and consider the possibility of transforming arable land into other categories of land.

Conclusions

In the course of this study, it was found that the potential of land resources for the implementation of forest climate reforestation projects will amount to only 934.6 thousand hectares, for afforestation – 130.8 thousand hectares, for agroforestry - 4-4.8 million hectares. Projects on reforestation, afforestation and agroforestry in the southern regions of Russia, and namely, in the Volga, North Caucasian, Central and Southern Federal districts, have significant potential for the implementation as climate projects on carbon sequestration. A warmer climate promotes accelerated growth of tree species, and if there is sufficient humidity in these regions, highly productive plantations can be formed, providing a significant level of greenhouse gas absorption. To assess the suitability and expediency of entering agricultural land plots for the implementation of climate projects on agroforestry, a number of factors should be taken into account, reflecting the qualitative characteristics of soils and their potential fertility, transport accessibility and economic feasibility.

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CHARACTERIZATION OF ANTAGONISM OF FUNGI *EPICOCCUM NIGRUM* AND *DIAPORTHE ERES*

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Abstract

Fungus *Diaporthe eres* represents a pathogen that occurs on a large number of hosts in forestry. In order to create strategies for reduction of damages and use of ecologically justified measures of protection of this insufficiently studied complex of species, *in vitro* research of antagonistic activity of *Epicoccum nigrum* was carried out. The study of antagonism of *Epicoccum nigrum* and *Diaporthe eres* included dual cultures method. The measurement of diameter of cultures, determining of the appearance of the mycelium and the way of cultures' reaction was carried out after 14 days. The results showed that the growth of *Diaporthe eres* mycelia in cultures with *Epicoccum nigrum* was statistically significantly smaller compared to control cultures. The cultures of *Diaporthe eres* were inhibited, whereby there was no overgrowth of mycelia of *Diaporthe eres* with mycelia of *Epiccocum nigrum*, which indicates that the antagonism was primarily reflected in the prevention of growth. The growth of *Epicoccum nigrum* was also statistically significantly smaller compared to control cultures, as well as *Diaporthe eres,* which indicates that the antagonism was not absolutely pronounced, and that for more pronounced inhibition of *Diaporthe eres* a larger quantity of mycelia is needed. Part of the cultures of *Epicoccum nigrum* showed forming of red pigment during reaction with the cultures of *Diaporthe eres*, indicating that there could be further research of its part in the antagonism process. On the other hand, clear forming of the pigment of *Diaporthe eres* was not recorded in dual cultures compared to control cultures. The obtained results enable the development of practical application of *Epicoccum nigrum*.

Keywords: *biological protection, dual cultures, interaction, antagonism.*

Introduction

Genus *Diaporthe* represents species of fungi that appear in different ecological conditions in forestry, as plant pathogens and saprophytes (Gomes *et al*., 2013; Horst, 2013). The taxonomic situation of this genus is very complex and the need for defining species is necessary due to creating adequate strategies of introduction of plants and phytosanitary protection measures (Crous and Groenewald, 2005; Wingfield *et al*., 2012). Especially since a certain number of members of this genus and related imperfect forms of fungi can occur on various hosts, or have wide geographical distribution. (Udayanga *et al*., 2011; Gomes *et al*., 2013). A complex of species *Diaporthe eres* is of great importance and present on many different hosts and also morphologically variable (Castlebury *et al*., 2002; Udayanga *et al*., 2014).
Genus *Epiccocum* consists of a large number of species, out of which *E. nigrum* (*E. purpurascens*), *E. layuense*, *E. dendrobii*, *E. mezzettii* and *E. minitans* represent the biocontrol agents of a large number of plant pathogens, whereby the species *Epicoccum nigrum* especially stands out (Taguiam *et al*., 2021). The mechanism of action of antagonism of *Epicoccum nigrum* to pathogenic organisms is necessary to research due to the need of creation of strategies for wide application of this species. The base for understanding the

antagonism between *Epicoccum nigrum* and pathogenic fungi are certainly the processes of growth of their mycelia. These researches are especially significant when studying the possibilities of protection against different complexes of species in order to obtain the data as detailed as possible on their reaction to antagonistic agents. The obtained results will enable at the same time better knowledge on antagonistic characteristics of certain strains of the species *Epicoccum nigrum* and reactions of certain members of the complex of species *Diaporthe eres* to their application.

In accordance with the above-mentioned, the objective of this research was to examine the influence of the used strain of *Epicoccum nigrum* to the development of the cultures of *Diaporthe eres*. The tested null hypotheses were the following: i) The used strain of *Epicoccum nigrum* cannot have effect on growth arrest of *Diaporthe eres*; ii) The mode of antagonism of the used strain of *Epicoccum nigrum* to *Diaporthe eres* cannot be characterized.

Materials and Methods

The experiment of in vitro effects of *Epicoccum nigrum* on *Diaprthe eres* in dual cultures was placed on 3% malt extract agar nutrient medium (MEA; 30 g/l malt Biolab, Hungary; 20 g/l agar Torlak, Serbia), on the temperature of 25ºC in December 2023. The used isolates of *Diaporthe eres* and *Epicoccum nigrum* were taken from mycological collection of the Institute of Forestry in Belgrade and the Faculty of Forestry of the University of Belgrade.

The total of 15 dual cultures of *Epicoccum nigrum* and *Diaporthe eres* were set up. The control group for *Epicoccum nigrum* and *Diaporthe eres* contained 5 cultures of each fungus. Parts of mycelia 5x5 mm of *Diaporthe eres* and *Epicoccum nigrum* were placed by sterilised scalpel in petri dishes on the same distance of 2.5 cm from the edges of petri dishes. One part of mycelia of *Diaporthe eres* and *Epicoccum nigrum* were placed per each petri dish. The control groups contained pure cultures of *Epicoccum nigrum* and *Diaporthe eres*. The experiment was completed after 14 days when all the cultures showed evident growth.

The measurement of the diameter of cultures was carried out in two transverse directions. In antagonistic cultures measurement of the growth was carried out on the places of the largest growth of mycelia. In control cultures, the measurement of the diameter was carried out from the centre of petri dish.

The testing of the normality of the arrangement and homogeneity of variance of culture dimensions was performed by Kolmogorov-Smirnov test, with Lilliefors correction and Levene's test. Kruskal Wallis test with Dunn's post hoc test was used for comparing the dimensions of cultures of *Diaporthe eres* exposed to antagonism of *Epicoccum nigrum* and control cultures. The descriptive statistics was used for presenting the dimensions of cultures in the experiment. All statistical analyses were carried out using the software package SPSS 27 (IBM Corp.) and Microsoft Office Excel 2021 (Microsoft Corp.).

Results and Discussion

Kruskal Wallis test showed statistically significant difference in the diameter of the cultures ($H = 34.899$; $p < 0.001$, Figure 1). Also, different pairs of cultures showed statistically significant difference in the development (Figure 1). The cultures of *Diaporthe eres* exposed to the effect of *Epicoccum nigrum* were significantly smaller compared to their control group (Figure 1, Figure 2). On the other hand, antagonistic cultures of *Epicoccum nigrum* had the smallest dimensions, as a consequence of preventing the growth of mycelia of *Diaporthe eres* (Figure 1, Figure 2).

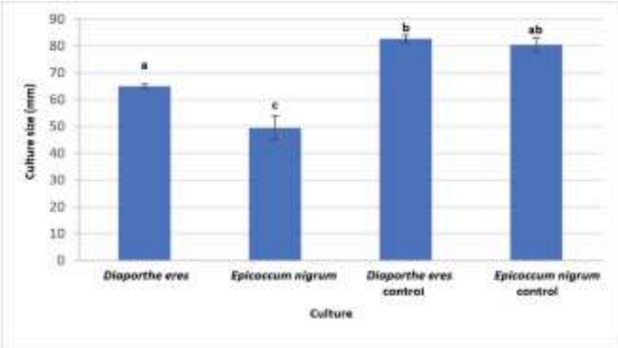

Figure 1 Dimensions of cultures of *Diaporthe eres* and *Epicoccum nigrum*
Note: Statistically significant differences were marked by different letters ($p < 0.05$)

All cultures had a distinctive appearance, in accordance with the characteristics of their isolates (Figure 2). Dual cultures of the tested fungi did not show forming of specific reaction zones between mycelia of *Diaporthe eres* and *Epicoccum nigrum* (Figure 2). However, certain colonies of *Epicoccum nigrum* in dual cultures showed pronounced secretion of red pigments which is possibly associated with metabolic enzymes, i.e. antagonistic activity (Figure 2). The secretion of black pigment in *Diaporthe eres* was recorded in all cultures, including inoculum places, so it is not considered the exclusive product of antagonism (Figure 2). In dual cultures overgrowth of mycelia of *Diaporthe eres* with mycelia of *Epicoccum nigrum* was not recorded, but the antagonism was reflected in occupation of space for growth (Figure 2).

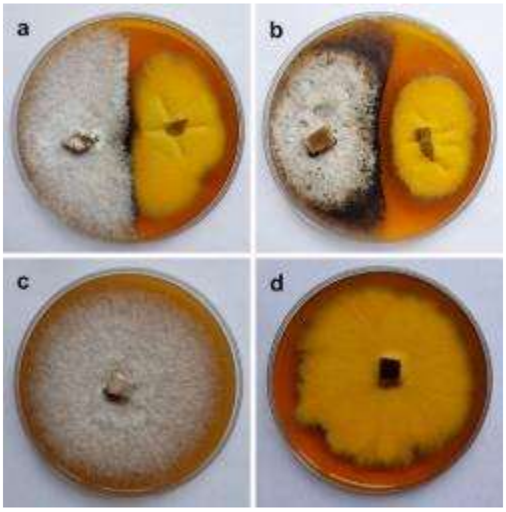

Figure 2 Pure cultures of *Diaporthe eres* and *Epicoccum nigrum*: a-b – antagonism (*Diaporthe eres* on the left, *Epicoccum nigrum* on the right), c – *Diaporthe eres* control, d – *Epicoccum nigrum* control

This study showed the way of growth of *Diaporthe eres* in case when it is exposed to interaction with *Epicoccum nigrum*. The obtained results enable development and improvement of strategies of application of *Epicoccum nigrum* as an agent of biological protection against *Diaporthe eres*. That is, the first null hypothesis was rejected and alternative hypothesis was accepted that the used strain of *Epicoccum nigrum* can have an effect on growth arrest of *Diaporthe eres*. Also, the results of this study are in line with the previous research that showed inhibitory potential of *Epicoccum nigrum* towards *Diaporthe* spp. (Abramczyk *et al*., 2020).

The performed experiment showed that blocking of the growth of *Diaporthe eres* was primary mechanism of antagonism of *Epicoccum nigrum*. In the cultures of *Diaporthe eres* exposed to *Epococcum nigrum* overgrowth of mycelia was not recorded as in the antagonism towards *Rhizoctonia solani* (Lahli and Hijri, 2010), nor degradation of mycelia as in antagonism towards *Phytophthora infestans* (Li *et al*., 2013). In this way, second null hypothesis was rejected and alternative hypothesis was accepted that the way of antagonism of the used strain of *Epicoccum nigrum* to *Diaporthe eres* can be characterised.
However, the occurrence of red pigment was noted which was present to a lesser extent in pure malt extract agar cultures, indicating the potential role of metabolites as factors that participate in antagonism or affects the inhibition of development independently. There are different methods of stimulation of natural pigments of *Epicoccum nigrum*, using potato dextrose agar and liquid nutrient media (Mapari *et al*., 2008; Kaur *et al*., 2019). Above all, forming of pigments of *Epicoccum nigrum* while interacting with *Diaporthe eres*, indicates bioecological characteristics of *Epicoccum nigrum* that can be significant for selection of antagonistic strains. The used isolates of *Diaporthe eres* and *Epicoccum nigrum* had the same growth rate, which gives a possibility of estimate of antagonistic potential of *Epicoccum nigrum*. It is noticeable that during the inhibition of *Diaporthe eres*, the colony of *Epicoccum nigrum* is also significantly smaller. This indicates that the antagonism of the tested strain of *Epicoccum nigrum* is not too pronounced and that a larger quantity or number of applications in necessary for more successful application against this pathogen. Similar examples are found while studying the antagonism of *Epicoccum nigrum* against *Botrytis cinerea*, where certain strains of *Epicoccum nigrum* did not succeed to limit the growth of *Botrytis cinerea* (Ogórek and Plaskowska, 2011), while other strains showed satisfactory effect (Christova and Slavov, 2021).
Finally, the need of studying the interaction of *Epicoccum nigrum* with certain fungi, is the first step in defining the way of reaction of plant host to *Epicoccum nigrum* aimed at successful protection from the said pathogen. From the obtained results, we conclude that the antagonism of *Epicoccum nigrum* towards *Diaporthe eres* is significantly reflected in this possibility to colonise the plant and reduce the space for growth of this pathogen. The experiences from agriculture showed that it stimulates the growth and biomass, as well as that it was re-isolated most frequently from the surface of the plant (Favaro *et al*., 2012; Ogórek *et al*., 2020). However, it is necessary to take in consideration that certain strains of *Epicoccum nigrum* are highly pathogenic towards certain woody species in forestry, which requires a detailed knowledge of pathogenicity of this fungus and to some extent limitations in application. In any case, in the future, it is necessary to develop methods by which *Epicoccum nigrum* would be more applied in the field and its positive effects would be clearer on different species of trees and shrubs, especially the ones that relate to antagonism towards *Diaporthe eres*.

Conclusion

Based on the obtained results from in vitro study on the effect of *Epicoccum nigrum* to the growth of *Diaprthe eres*, the conclusions that arise from them can be presented in the following way:

- There was statistically significant inhibition of growth of mycelia of *Diaporthe eres* in cultures that were exposed to *Epicoccum nigrum* compared to pure cultures of *Diaporthe eres*.
- Mycelia of *Epicoccum nigrum* showed red pigment in contact with mycelia of *Diaporthe eres*. In this way, it is possible that during antagonism metabolites and mycotoxins are excreted that affect the development of pathogen. In any case, the occurrence of red pigment is noted in antagonistic strains of *Epicoccum nigrum*, i.e. efficient in inhibition of

Diaporthe eres, and to a certain extent it can serve as a starting point for selection of these strains of fungi and it is necessary to be further studied.

• Antagonistic colonies of *Epiccocum nigrum* had smaller dimensions compared to the colonies of *Diaporthe eres*, which indicates that antagonism was not very pronounced, i.e. that in the case of more pathogenic strains of complex of species *Diaporthe eres* it is necessary to use larger quantity of *Epicoccum nigrum* or larger number of treatments.

• Antagonism of *Epicoccum nigrum* in relation to *Diaporthe eres* was reflected in preventing of the growth of mycelia, whereby there was no overgrowth or disintegration of mycelia. This phenomenon leads to the conclusion that the antagonism of *Epicoccum nigrum* is characterised by occupying the space for growth of *Diaporthe eres*. Therefore, strategies are necessary which will enable better development of *Epicoccum nigrum* as a positive reaction of the host plant aimed at creating conditions for application of antagonism.

The obtained results enable development of the method for practical application of *Epicoccum nigrum* in protection of woody species in forestry from the complex of species *Diaporthe eres*.

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TAXONOMY AND PHYTOGEOGRAPHY ANALYSIS OF MEDICINAL PLANTS WITHIN MANAGEMENT UNIT „GOČ-SELIŠTE"

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Abstract

The analysis of taxonomy and phytogeography features of medicinal plants at the area of MU „Goč-Selište", within the protective forests of Vrnjačka Banja, was conducted during growing season of 2023. The studied area is very significant due to the numerous functions of these forests: water protective, ecological, economics, social and recreational. There was established, based on detailed monitoring, presence of total 73 medicinal taxa. As for taxonomy, total of 34 families was recorded and the greatest number of representatives included Asteraceae (13.70 %), Lamiaceae (12.33 %) and Rosaceae (12.33 %). In terms of floral elements, there was total of 17 found and among them the most abundant were eurasian (31.51 %), submideuropean (19.18 %) and european (15.07 %). Among life forms hemicryptophytes (42.47 %) and phanerophytes (24.66 %) were the most dominant, while the other five life forms included only 1/3 of the total number. Based on the obtained results related to taxonomy and phytogeography, we can deduce there are no significant differences if compare with forests located in the other ecological conditions in Republic of Serbia. Over 70 % of recorded medicinal plants were herbaceous. The recommendation is to conduct monitoring within a longer period of time (at least three consecutive growing seasons) and to pay a special attention to some species which natural regeneration is weaker. In that way, a significant focus should be devoted to preservation and protection of these endangered species.

Key words: *MU „Goč-Selište", taxonomy, floral elements, life forms, medicinal plants.*

Introduction

The history of the medicinal plants use is closely related to the history and development of human society. The oldest written documents come from China, 3000 years BC when more than a hundred medicinal plants were known, and among them some are still used nowdays (Tucakov, 2014). Medicinal plants were also studied by Greek and Roman scientists (Jokanović, 2021). During the Middle Century, knowledge about medicinal plants was acquired only in monasteries, and it was only at the end of the 18th and the beginning of the 19th century (Jovanović, 2016). Medicinal plants are used in official or folk medicine to treat diseases or to preserve people's health. Today, about 10.000 plant species are used and great attention is paid to them precisely in order to find better medicines that would be less harmful to the human body (Došić, 2016). The importance of medicinal plants in modern medicine is increasing. Achievements in these investigations contribute to this, as well as interest in their use as natural raw material in the production of different medicinal and other preparations. Phytotherapy is developed based on medicinal plants using and it should be noted that herbal treatment has its significant place in medicine. On the other hand, herbal medicines and herbal

treatment methods are useful in some cases, especially when it is about minor and chronic diseases, where they are able to replace many official medicines (Inđić, 2020).
The locality investigated in this paper belongs to the group of protected areas, and one of the ways to conduct the conservation of nature and natural values is the implementation of permanent monitoring of plant species, which is preceded by their identification and spatial positioning (Šijačić-Nikolić et al., 2024). The studied area is characterized by the presence of species from the category of rare (when the number of individuals is small), relict (they had a large distribution in the past, and today’s range is significantly reduced), endemic (their distribution is limited to a clearly defined geographical area), endangered (big probability to disappear from this area in the near future) and those at risk (there is a danger that their population size will decrease greatly).
This study presents the results of the research related to the distribution and representation of medicinal plants in MU „Goč-Selište“ which belongs to the protective forests of Vrnjačka Banja municipality in Serbia. Special attention is paid to the phytogeographical and taxonomical features of the medicinal taxa in this area. As for herbal plants in this part of Serbia, some detailed study has not been conducted for a very long time.
The aim of the paper is to determine medicinal plants at the studied area and, based on that, to carry out phytogeographical and taxonomical research. One of the aims is also to establish if some species are very endangered according to unreasonable use by humans which leads to reducing of their potential for natural regeneration.

Material and Methods

Medicinal plants at the area of MU „Goč-Selište“, within protective forests of Vrnjačka Banja municipality, were investigated during growing season 2023. Many references were used in order to determine these herbal taxa (Kojić & Vilotić, 2006; Igić et al., 2010; Tucakov, 2014; Vilotić, 2018, 2021; Jokanović, 2021). The endangered species were discovered on the base of their reduced populations according to human activity which caused its weaker natural regeneration. Life forms classification was carried out due to Raunkier system (1934) which was lated extended by Mueller-Dombois et. Ellenberg (1974), while Stevanović (1992) developed the same system for Serbian taxa. Floral elements and taxonomy features of studied plants were identified according to a lot of references (Gajić, 1980; Sarić, 1989; Ranđelović et al., 2002; Tucakov, 2014; Vilotić, 2018).

Results and Discussion

In this study were overall 73 herbal plants which belong to 34 families determined (Table 1). Families with the most representatives are: Asteraceae (13.70 %), Lamiaceae (12.33 %) and Rosaceae (12.33 %). There are many papers (Jovanović, 2016; Đošić, 2016; Inđić, 2020; Jokanović et al., 2022; Jokanović et al., 2023) whose results of taxonomy analysis almost completely coincide with our results. In terms of life forms, the most dominant are hemicryptophytes (42.47 %) and phanerophytes (24.66 %), while all the other groups have much less representatives (Table 1). The obtained results in some other papers related to life forms, which were conducted in southeastern Serbia (Đošić, 2016; Jovanović, 2016), central Serbia (Inđić, 2020; Jokanović et al., 2023) or northern Serbia – Vojvodina (Jokanović et al., 2022) also showed a significant dominance of hemicryptophytes and phanerophytes. Among floral elements (Table 1), the most abundant were: Eurasian (31.51 %), Submideuropean (19.18 %) and European (15.07 %). As for aforementioned papers, in the area of mountain Kukavica (Jovanović, 2016) and Besna Kobila (Đošić, 2016) the most dominant floral elements were Eurasian and Mideuropean, respectively. In the area of Nature Monument

„Šuma Košutnjak" (Inđić, 2020; Jokanović et al., 2023) and lowland forests of Gornji Srem (Jokanović et al., 2022), the most present are Eurasian and Submideuropean, which is very similar to results obtained in our paper.

Table 1: Display of taxonomy and phytogeography plant characteristics (P - phanerophytes; H - hemicryptophytes; G - geophytes; T/H - therophytes/hamephytes; Ch - hamephytes; T - therophytes; S - scandetophytes; H/T - hamephytes/therophytes)

Latin name	**Family**	**Life form**	**Floral element**
Abies alba (Mill.)	Pinaceae	P	Submideuropean
Achillea milefolium (L.)	Asteraceae	H	European
Agrimonia eupatoria (L.)	Rosaceae	H	European
Agropyron repens (L.) Beauv	Poaceae	G	Cosmopolit
Allium ursinum (L.)	Alliaceae	G	Boreal
Arctium lappa (L.)	Asteraceae	T/H	European
Artemisia vulgaris (L.)	Asteraceae	H	Circumpolar
Asarum europaeum (L.)	Aristolochiaceae	Ch	Eurasian
Asperula odorata (L.)	Rubiaceae	Ch	Subpontic-submeditteranean
Atropa belladonna (L.)	Solanaceae	H	Eurasian
Bellis perennis (L.)	Asteraceae	H	Submideuropean
Betula pendula (Roth.)	Betulaceae	P	Pontic
Calendula officinalis (L.)	Asteraceae	H	Eurasian
Capsela bursa-pastoris (L.) Med	Brassicaceae	T	Cosmopolit
Centaurium umbellatum (Gilib)	Gentianaceae	T	Boreal
Chelidonium majus (L.)	Papaveraceae	H	European
Cichorium intybus (L.)	Asteraceae	H	Cosmopolit
Cornus mas (L.)	Cornaceae	P	Pontic-submediterranean
Cornus sanguinea (L.)	Cornaceae	P	Submideuropean
Crataegus monogyna (Jacq)	Rosaceae	P	Submideuropean
Equisetum arvense (L.)	Equisataceae	G	Holarctic
Equisetum palustre (L.)	Equisataceae	G	Holarctic
Erica carnea (L.)	Ericaceae	Ch	Eurasian
Fagus moesiaca (K. Maly.)	Fagaceae	P	Submideuropean
Fragaria vesca (L.)	Rosaceae	H	European
Galium verum (L.)	Rubiaceae	H	European
Geranium pussilum (L.)	Geraniaceae	H	Submideuropean
Geranium robertianum (L.)	Geraniaceae	T	Circumpolar
Geum urbanum (L.)	Rosaceae	H	European
Glechoma hirsuta (Waldst. et Kit)	Lamiaceae	H	Pontic-east-submeditteranean
Hedera helix (L.)	Araliaceae	S	Subatlantic-submediterranean
Helichrysum arenarium (L.)	Asteraceae	H	Eurasian
Helleborus odorus (L.) W.K.	Ranunculaceae	G	Submideuropean
Hypericum hirsutum (L.)	Hypericaceae	H	Cosmopolit
Hypericum perforatum (L.)	Hypericaceae	H	Subeuropean
Juniperus communis (L.)	Cupressaceae	P	Eurasian

Lamium maculatum (L.)	Lamiaceae	H	Eurasian
Lamium purpureum (L.)	Lamiaceae	T	Eurasian
Lavandula officinalis (L.)	Lamiaceae	H	Mediterranean-submediterranean
Malus sylvestris (Mill.)	Rosaceae	P	Eurasian
Malva sylvestris (L.)	Malvaceae	H	Subeuropean
Matricaria chamomilla (L.)	Asteraceae	T/H	European
Mellisa officinalis (L.)	Lamiaceae	H	Meridional-submeridional
Mentha longifolia (L.) Huds	Lamiaceae	H	Submideuropean
Pinus nigra (Arn.)	Pinaceae	P	Eurasian
Pinus sylvestris (L.)	Pinaceae	P	Eurasian
Plantago lanceolata (L.)	Plantaginaceae	H	European
Plantago major (L.)	Plantaginaceae	H/T	European
Plantago media (L.)	Plantaginaceae	H	European
Primula acaulis (L.)	Primulaceae	H	Eurasian
Prunus avium (L.)	Rosaceae	P	European-westAsian
Pulmonaria officinalis (L.)	Boraginaceae	H	Submideuropean
Ranunculus repens (L.)	Ranunculaceae	H	Pontic-submediterranean
Robinia pseudoacacia (L.)	Fabaceae	P	Adventive
Rosa canina (L.)	Rosaceae	P	Eurasian
Rousmarinus officinalis (L.)	Lamiaceae	H	Eurasian
Rubus hirtus (L.)	Rosaceae	P	Submideuropean
Rubus idaeus (L.)	Rosaceae	P	Submideuropean
Salix caprea (L.)	Salicaceae	P	Eurasian
Salvia officinalis (L.)	Lamiaceae	H	Eurasian
Sambucus nigra (L.)	Adoxaceae	P	Submideuropean
Symphytum officinale (L.)	Boraginaceae	T/H	Submediterranean-pontic
Taraxacum officinale (Web.)	Asteraceae	H	Eurasian
Thymus serpyllum (L.)	Lamiaceae	Ch	Submideuropean
Tilia platyphyllos (Scop.)	Tiliaceae	P	Submideuropean
Trifolium arvense (L.)	Fabaceae	T	Eurasian
Trifolium campestre (Schreb.)	Fabaceae	T	Eurasian
Tussilago farfara (L.)	Asteraceae	G	Eurasian
Urtica dioica (L.)	Urticaceae	T/H	Eurasian
Vaccinium myrtillus (L.)	Vacciniaceae	Ch	Boreal
Verbascum phlomoides (L.)	Scrophulariaceae	H	Continental-mediterranean
Viola alba (L.)	Violaceae	H	Submediterranean
Viola tricolor (L.)	Violaceae	T/H	Eurasian

Conclusions

Total of 73 medicinal plants were determined in MU „Goč-Selište“ within protective forests of Vrnjačka Banja municipality. As for families participation, the most dominant is Asteraceae (13.70 %), while Rosaceae and Lamiaceae came to the second place with proportion of 12.33 %. Among floral elements the greatest number of representatives was recorded by: Eurasian (31.51 %), Submideuropean (19.18 %), and European (15.07 %), while two life forms had the most abundant presence: hemicryptophytes (42.47 %) and phanerophytes (24.66 %). It should be noted that investigated area includes mixed forests consisted of silver fir and beech that belong to the associations of the highest quality from ecological and economics point of view. One of the greatest problem related to the investigated area is the endangerment of some wood species that are at risk (*Pinus sylvestris, P.nigra* and *Prunus avium*) or rare and endangered (*Betula pendula, Sambucus nigra, Malus sylvestris*). Among herbaceous herbal taxa, the difficulty of natural regeneration was recorded only in *Primula acaulis* and *Lavandula officinalis*. Taking into account the trend of climate changes, as well as the uncontrolled collecting of medicinal plants by visitors and excursionists, it is needed to preserve the existing gene pool and carry out monitoring in the upcoming years in order to make a decision in what direction to implement silviculture and management measures in the future. The knowledge of herbs used for medical purposes will allow us to competently organize protective measures in these natural areas.

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VARIABILITY OF VESSELS AND WOOD RAYS DENSITY BY CONTAINER SEEDLINGS OF DIFFERENT SPECIES FROM QUERCUS GENERA

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Abstract

The anatomical traits of one-year old container seedlings of three species from *Quercus* genera – *Quercus robur, Quercus petraea* and *Quercus frainetto* were analyzed. Seedlings were planted in the same type of container. The scope of the paper was to establish density of vessels and wood rays per 1 mm^2. These two anatomical elements play a very significant role – vessels are conductive elements responsible for water and minerals moving from the root to the upper parts of the tree, while wood rays are in charge of nutrients storage. The obtained results showed that the greatest density of both anatomical elements was recorded by *Q. petraea* (110.07 vessels, and 34.07 wood rays per mm^2) which means that this species had the narrowest vessels and wood rays. On the other hand, in *Q. robur* we found presence of 102.1 vessels, and 24.93 wood rays per mm^2, while *Q. frainetto* had the lowest vessels density (97.03), but came to the second place in terms of wood rays density (33.07). As for the possibility of using of investigated oaks container seedlings for reforestation purposes, *Q. petraea* would be more suitable for arid sites, according to the greatest density of vessels and wood rays, which should be related to the lowest width of these elements. Bearing in mind this fact, we can deduce that narrower vessels are more efficient for conducting, while smaller wood rays can store enough nutrients for a short time. However, in order to obtain reliable conclusions, in addition to analyzed parameters, it is needed to investigate some morphological (height and root collar diameter) and anatomical (vessels width, wood rays width and height) traits.

Key words: *Q.petraea, Q.robur, Q.frainetto, vessels density, wood rays density.*

Introduction

For decades, nursery production has been faced with a choice – container or bare-root seedlings. Container seedlings have many advantages compared to these produced in conventional manner: higher survival rate; lower physiological shock of seedlings during replanting; extended period of planting in spring, and earlier onset of planting in autumn, as well; afforestation of degraded sites; mechanical tools can be used during production and planting which reduce the overall costs; human activity is less needed while planting is performing (Matić et al., 1996; Ocvirek 1997; Orešković et al., 2006; Popović et al., 2014). On the other hand, the main disadvantage of container seedlings is related to irregular shape and deformation of the root system which causes many difficulties during outplanting process (Stilinović, 1991; Ivetić, 2013, 2021). The volume of the container greatly affects the growth and development of the plant, as well as its later performance after transplanting (Ivetić 2013, 2021). When we talk about forest seedlings, smaller containers are more desirable according to lower price, easy handling, and greater plants density per unit area and species with small

seed are often produced in this container type (Ivetić, 2013; Popović et al., 2014). However, larger containers are more suitable for species with big seed and strong root system (Ocvirek, 1997; Topić et al., 2006). Grossnickle (2012) claim that the most significant morphological feature that affects significantly development and growth of seedlings is root collar diameter. In terms of wood rays and tracheids dimensions, Jokanović et al. (2024 a) found these traits depend a lot on the species and container volume, as well. Wood rays are in charge of conducting nutrients through the plant and play a very significant role by hardwoods, particularly in winter when the leaves have fallen (Vilotić, 2019). As a result, all nutrients by these species in this time are stored in wood rays. Conductive elements (Jokanović et al., 2024 a) enable transport of water and mineral substances from the root to the aboveground parts of the tree. The same authors found that a smaller diameter of conductive elements is usually found in arid habitats in order to transport the small amounts of available water as efficiently as possible through the plant.
The aim of the paper is to analyse two anatomical traits (wood rays density and vessels density) in three different oak species (*Q.robur, Q.petraea, Q.frainetto*) produced in the same container type. The obtained results could indicate which of these species would be the most suitable for afforestation of difficult sites, taking into account that in order to make such conclusions it is necessary to investigate some additional anatomical and also morphological traits of the seedlings.

Material and Methods

Bosnaplast 180 container, made of plastic, was used for cultivation of investigated oak species. Seeds were collected from Gornji Srem (*Q.robur*), Fruška Gora (*Q.petraea*) and Lipovica (*Q.frainetto*). Container seedlings were produced in the nursery situated in Sremska Mitrovica that belongs to the PE „Vojvodinašume". At the end of the growing season 2022, containers were filled manually during sowing. Plants were regularly watered, especially during hot summer. According to the producer instruction, Fitogal Galenika, Serbia, foliar fertilization was performed twice a week during June and July. Systemic fungicide Previcur was used in order to protect young individuals from pests attack. Onset of *Q.robur* seed germination was in the middle of April and lasted until the end of May, while for the other two species it started a few weeks later. At the end of the growing seaason, 30 saplings per species were lifted and transported to the laboratory in order to carry out necessary anatomical analysis. 30 wood rays and 30 vessels per unit area were measured for each species (Figure 1).

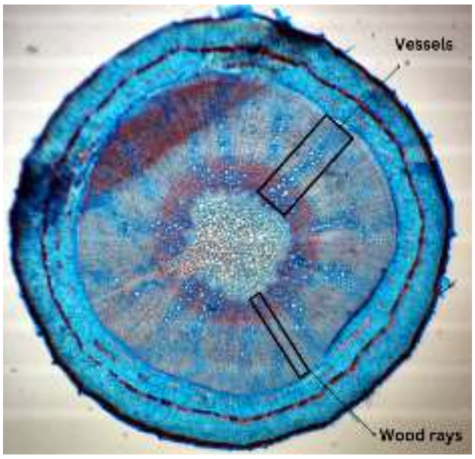

Figure 1. Display of analyzed anatomical traits

Anatomical analysis was conducted on permanent anatomical preparations made on transversal samples which were obtained by previous softening of the whole material, firstly kept in boiling water, and then in the mixture of water, glycerol and ethanol in the same volume proportions. The samples were cut using sliding microtome „Reichert“ in transversal segments 20-25 microns thick. The vessels are circular in shape and are scattered within the growth ring, unlike the wood rays that extend radially from the pith to the bark. All measurements were taken using a Boeco microscope that contains specialized software with calibration to recalculate the obtained dimensions of the investigated elements.
Regarding the measurement of the density of vessels and wood rays, 30 measuring fields were randomly selected and on each one the amount of vessels and wood rays was counted separately. After that the area of each measuring field was calculated and the number of studied anatomical traits was converted to area of 1 mm^2 using a simple proportion. It should be noted that only completely formed vessels and wood rays were taken into consideration.
The numerical data was processed using descriptive statistics. There were determined mean value, minimum, maximum, and for each mean value, standard deviation and coefficient of variation were calculated, as well.

Results and Discussion

Based on the obtained results for vessels density (Table 1) we can deduce that the greatest number of these conductive elements per unit area was recorded in *Q.petraea*, then *Q.robur* came to the second place, and finally *Q.frainetto*. As for standard deviation and coefficient of variation, there was no big difference between investigated species. A low degree of variability (0-10 %) was detected for all analyzed oaks.

Table 1. Descriptive statistics of vessels density per unit area

	Q.robur	***Q.petraea***	***Q.frainetto***
Mean value	102.1	110.07	97.03
Minimum	82	95	85
Maximum	115	123	110
Standard deviation	7.95	7.86	6.04
Coefficient of variation (%)	7.79	7.14	6.22

Number of wood rays per unit area (Table 2) is again the highest in *Q.petraea*, while the lowest values were established in *Q.robur*. In terms of this anatomical element, a very similar standard deviation was recorded in *Q.robur* and *Q.petraea*, while this value was two times less in *Q.frainetto*. As for the coefficient of variation, a very diverse trend was found – a low degree of variability was recorded in *Q.frainetto* (0-10 %), then moderate in *Q.petraea* (10-20 %) and strong in *Q.robur* (over 20 %).

Table 2. Descriptive statistics of wood rays density per unit area

	Q.robur	***Q.petraea***	***Q.frainetto***
Mean value	24.93	34.07	33.07
Minimum	16	22	27
Maximum	40	45	38
Standard deviation	6.61	6.75	3.30
Coefficient of variation (%)	26.51	19.81	9.98

The highest number of vessels and wood rays per unit area in *Q.petraea* (Table 1 and 2) can be associated with the very small width of these elements, which implies that saplings of these species could be used for afforestation of dry sites, taking into account that the small width of the conductive elements means a greater efficiency of conducting. In addition to that, a greater number of wood rays allows large amounts of nutrients to be stored, which is especially important for hardwoods when the growing season stops, because their leaves have fallen and all nutrients are accumulated in wood rays. However, it is needed to be said when recommending planting material for afforestation, it is necessary to consider the dimensions of the morphological and anatomical traits of the seedlings, as well as the shape and development characteristics of the root system in order to make the right decision (Orešković et al., 2006; Kolevska et al., 2020; Mijatović et al., 2022; Jokanović et al., 2024 a).
Jokanović et al. (2024 a) found that container volume affected very much the dimensions of the anatomical traits in Scots pine and black pine seedlings – by larger containers, both wood rays and tracheid width had significantly higher mean values. Martin et al. (2010) investigated how different ecological conditions affected wood anatomical traits by Scots pine and established that trees grew in the driest regions had a very large tracheid lumens, while cell walls were thick, and the number of ray tracheids was very high which enabled a great storing capacity in the sapwood. However, Esteban et al. (2012) found that black pine trees in arid environment had short tracheids which can be associated with their poor growth, while at higher altitudes these plants tended to form very large resin ducts as a physiological and physical barrier. In our paper, two anatomical traits of one-year-old seedlings of three oak species that were grown in the same habitat conditions (one type of container) were analyzed, and some differences, mainly caused by the bioecological features of the investigated species, were established. Jokanović et al. (2024 b) investigated the influence of pollutants on the morpho-anatomical traits of four willow clones and found that the vessel diameter and wood rays height were higher in the control compared to the contaminated site. Significant differences were also detected between individual clones in the same site conditions, especially when it comes to vessel diameter, which can be linked to the genetic characteristics of these clones.

Conclusions

In the paper was investigated the variability of two anatomical parameters (vessels density and wood rays density) in three different *Quercus* species (*Q.robur*, *Q.petraea*, *Q.frainetto*) cultivated in the same type of container. Based on the obtained results, the highest values of both traits were established in *Q.petraea* seedlings. As for the number of vessels, *Q.robur* came to the second place unlike wood rays density. The analysis of the dispersion elements of the probability distribution shows there are no major differences in the values of standard deviation and the coefficient of variation between the investigated species for vessel density. However, in the case of wood rays density, the value of the standard deviation is two times less in *Q.frainetto* compared to the other oaks, and the coefficient of variation differs significantly between the species.

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THE CONDITION OF THE TREE CROWNS ON ICP LEVEL I PLOTS IN AP VOJVODINA, SERBIA

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Abstract

Forests are threatened by numerous biotic and abiotic damaging factors. Continuous monitoring of forest conditions is of great importance for detecting changes in forest ecosystems and determining the cause and relationships between the resulting changes and factors that affect forests. In the network of ICP monitoring plots in Serbia, 13 Level I plots are located on the territory of Autonomous Province of Vojvodina and managed by the Institute of Lowland Forestry and Environment where monitoring of crown condition is performed continuously. This paper presents the analysis of the results of crown conditions' assessment on plots and the impact of some of harmful factors on crown condition of most common tree species such as: sessile oak, pedunculate oak, silver lime, Euroamerican poplar, narrow-leaved ash and Austrian pine. Assessment of tree crown condition was performed annually during July and August in the period 2017- 2023 according to the methodology prescribed by the International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests. Results showed that the highest degree of tree crown defoliation was identified on pedunculate oak, narrow-leaved ash and Austrian pine trees. The results of forest condition monitoring on sample plots indicated high effect of drought, storm, and harmful biotic factors on the tree conditions. The significant damage of the *Quercus robur* L. foliage mass was caused by the oak lace bug (*Corythucha arcuata* Say) and early season defoliators. Trees of *Fraxinus angustifolia* Vahl were damaged by *Stereonychus fraxini* De Geer. while trees of *Pinus nigra* Arn. were affected by the infestation of *Diplodia pinea* Dasmaz.

Keywords: *monitoring, crown condition, defoliation, drought, insects.*

Introduction

Continuous monitoring of forest ecosystems is of great importance for determination of changes in forests and for the detection of causal relationships between harmful factors and forest conditions. In Europe, there was an intensive decline in forest ecosystems in the 80s of the 20th century. Air pollution was then identified as the primary cause of this decline. As a result of that The International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests was established in December 1984 (Nevenić et al., 2005). At present, 42 European countries as well as the United States of America and Canada are participating in the programme, which includes assessments according to harmonized and standardized methods following this ICP Forests Manual (Schwärzel et al., 2022). The condition of forests on the territory of Europe has been monitored at about 6,000 plots at Level I (Nevenić et al., 2011). Today, the aim of this international program is to monitor the impact of air pollution on forests and to monitor changes in forest conditions related to the effects of other harmful factors. The parameters used for continuous monitoring of forest health are the intensity of defoliation and the intensity of tree damage caused by the action of a many of different harmful factors. In the Republic of Serbia network of ICP Level I with

130 monitoring plots was reconstructed in the period 2003-2004. Since the establishment of the monitoring plots, the condition of the tree crowns has been regularly monitored every year and the collected results have been analyzed, which has already been published in earlier papers (Drekić et al., 2007; 2013; 2016). The paper presents the results of the assessment of tree crown condition on 13 plots of the Level I in the Autonomous Province of Vojvodina in Serbia during the period 2017-2023.

Material and methods

The monitoring was performed from 2017 to 2023 on Level I plots in AP Vojvodina. In each plot, 24 selected trees were evaluated. All rating trees were numbered. Dominant, codominant and subdominant trees were selected for assessment. The assessment of the condition of the tree crowns was performed every year during July and August. The assessment was performed according to the methodology prescribed by the International Co-operative Program on the Assessment and Monitoring of Air Pollution Effects on Forests (Eichhorn et al., 2016). After evaluation of defoliation, the trees were classified into the classes which are listed in Table 1.

Table 1. Defoliation clasess

Defoliation clasess	Percentage of defoliation
Not defoliated	0 – 10%
Slightly defoliated	>10 – 25%
Moderately defoliated	>25 – 60%
Severely defoliated	>60 – <100%
Dead trees	100%

Impact of biotic and abiotic harmful factors on the crown condition of trees was monitored in the form of an assessment of the damage intensity on trees. Damage from a certain harmful factor was assessed on a scale with an interval of 5% (0 = not damaged tree; 5%; 10%; 15% ... 100 % = dead tree).

Results and discussion

The results of defoliation degree assessment during the study period from 2017 to 2023, indicated that the most slightly and moderately defoliated trees, considering all trees on sample plots occurred in 2017 (Figure 1). This was primarily result of the summer droughts in 2017. The highest percentage of dead trees during the observed period was recorded in 2023 and was caused by storm.

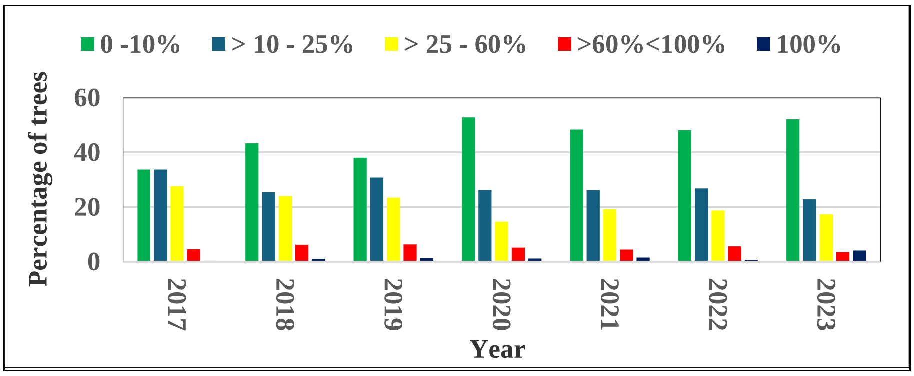

Figure 1. The distribution of all tree species by classes of defoliation (2017-2023)

In the period from 2017 to 2023, the percentage of sessile oak trees without defoliation and with slight defoliation was very high (Figure 2). Moderately defoliated trees were present in 2017 and 2019 when the strongest attack of early defoliator insects was determined. The decline of some sessile oak trees occurred due to physiological weakening caused by drought and insect attack. On dead trees were present most often the symptoms of the attack of *Cerambyx cerdo* L. It is very significant that after 2019, the share of trees with defoliation above 25% decreased, which is considered as a good indicator of the vitality of forest ecosystems (Potočić et al., 2008).

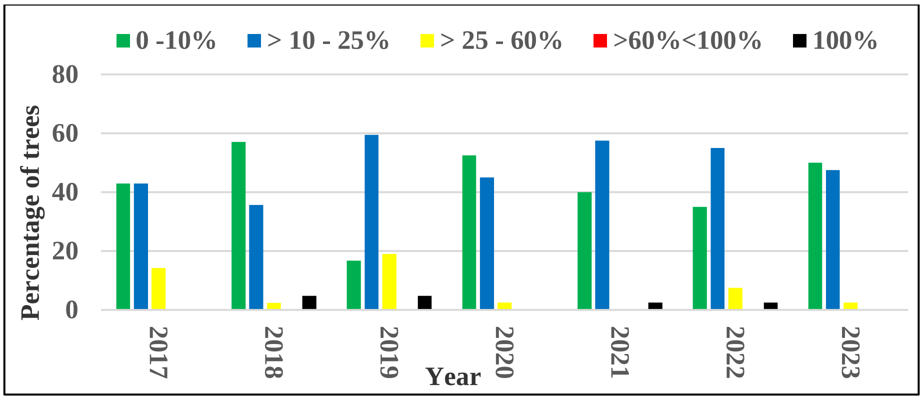

Figure 2. The distribution of *Quercus petrea* (Matt.) Liebl trees by classes of defoliation (2017-2023)

In the period from 2017 to 2023, a relatively low number of trees without defoliation (7.1% - 37.3%) was found for pedunculate oak (Figure 3). Also, in the period from 2004 to 2016 Drekić et al. (2016) recorded for the same species the dominant share of trees with slight and moderate defoliation. The dying of the pedunculate oak trees was recorded in 2021 and 2023. A strong attack of the invasive oak lace bug (*Corythucha arcuata* Say), which is according to Nikolić et al. (2019) a significant physiological pest of oaks, was regularly recorded on the trees of the pedunculate oak. The highest intensity of attacks of the oak lace bug was recorded in 2017 and 2021. Damage from early season defoliators insects was recorded in 2019 and 2020 on the plot in Morović.

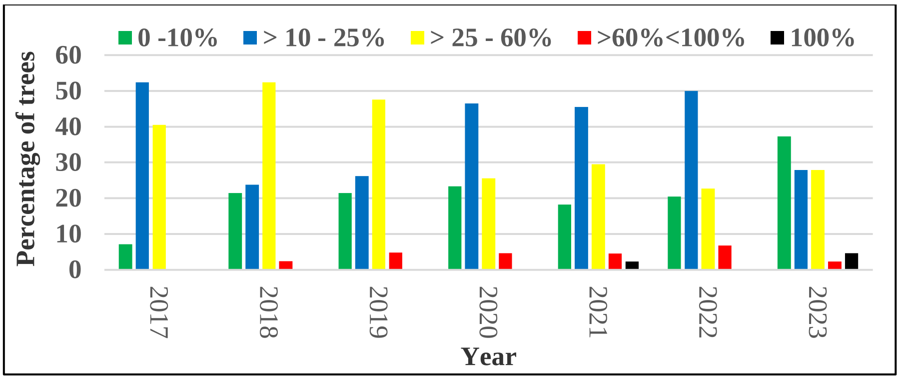

Figure 3. The distribution of *Quercus robur* L. trees by classes of defoliation (2017-2023)

Silver linden was present on the monitoring plots located on Mt. Fruška Gora and Mt. Vršački breg. For this species, during the observed period, dominateted trees without defoliation (Figure 4).

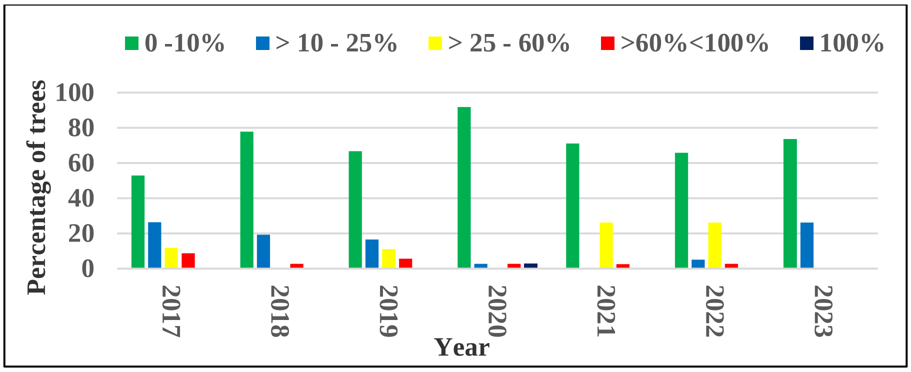

Figure 4. The distribution of *Tilia argentea* L. trees by classes of defoliation (2017-2023)

Due to the summer drought, a slightly greater percentage of trees with moderate and strong defoliation in a few years was seen in the shallow soil plot on Mt. Vršački Breg. Only one silver linden tree decline was recorded during the examined period in 2020, and that decline was caused by a windbreak. The condition of the evaluated silver linden trees can be characterized as very good, and this species shows a high degree of vitality.
For narrow-leaved ash in the period from 2017 to 2023, the presence of slightly and moderately defoliated trees was dominant (Figure 5). However, from 2020 to 2023, dieback of narrow-leaved ash trees was recorded every year. In the observed period 2017-2023 every year, defoliation caused by the ash weevil (*Stereonychus fraxini* De Geer) was recorded on the trees of the narrow-leaved ash. Defoliation was particularly intense in 2021 (12.5% leaf mass on average) and 2023, when it amounted to an average of 16.2% of the leaf mass of the trees. On some trees, the defoliation was over 30% of the leaf mass, which certainly had a negative impact on tree vitality and accelerated dying of the trees. The fungus *Ganoderma* sp. was also often found on dead narrow – leaved ash trees.

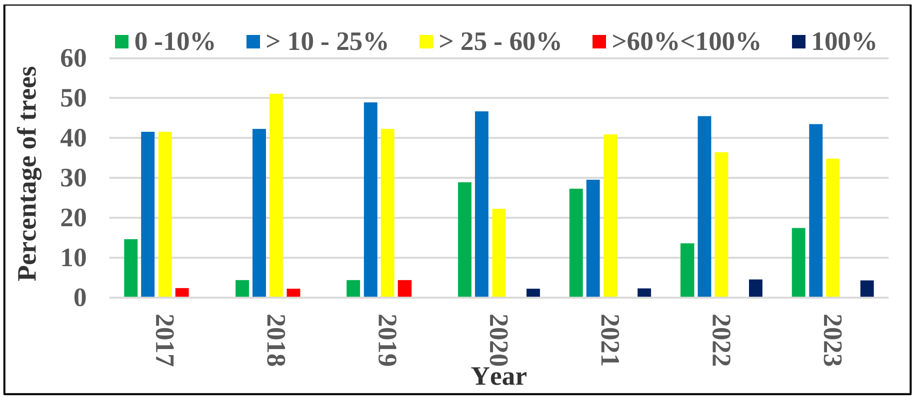

Figure 5. The distribution of *Fraxinus angustifolia* Vahl trees by classes of defoliation (2017 – 2023)

In the period from 2017 to 2023, dominant participation of Euroamerican poplar trees without defoliation was recorded (Figure 6).

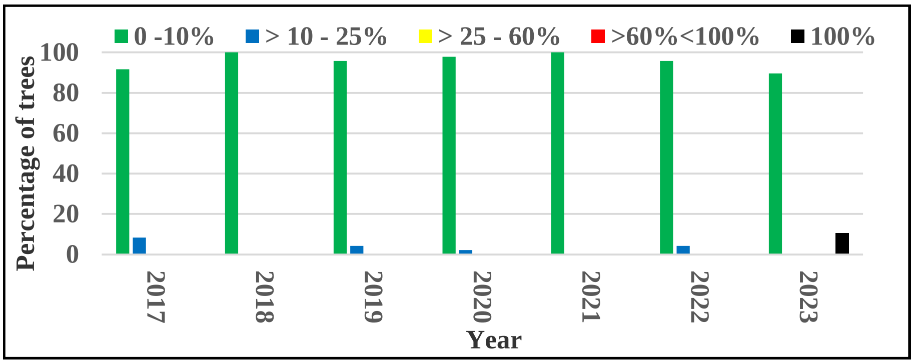

Figure 6. The distribution of *Populus x euramericana* (Dode) Guinier trees by classes of defoliation (2017-2023)

However, in 2023, because of the stormy wind that hit the forests in AP Vojvodina in the summer, a part of the Euroamerican poplar trees on the plot in Morović collapsed. Nevertheless, the condition of the poplars on the plots of the first level from the aspect of biotic factors in the observed period was good and no major damages from insects and plant diseases were recorded on observed plots.

In the observed period, black pine trees with a moderate intensity of defoliation were the most common, while the percentage of trees without defoliation was very low. The participation of trees with a high intensity of defoliation was also significant and this indicates the poor condition of the evaluated black pine stands, which is primarily the result of unfavourable habitat conditions and the old age of the stand on Deliblatska peščara (Figure 7). The poor condition of the black pine trees culminated in the complete decay of several trees in 2023 as a result of years of their gradual physiological weakening. In contrast to the period 2004-2016, when the health condition of black pine trees was significantly affected by pathogenic fungi (Drekić et al. 2016), in the period from 2017 to 2023, only an attack of fungus *Diplodia pinea* Desm was recorded on black pine trees.

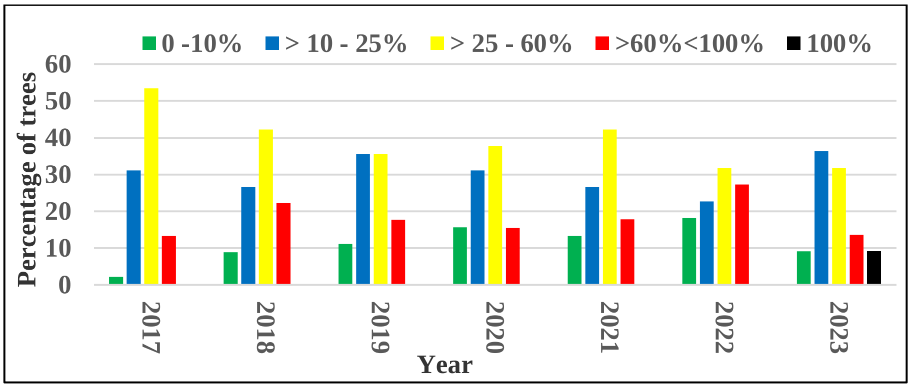

Figure 7. The distribution of *Pinus nigra* Arn. trees by classes of defoliation (2017-2023)

The results of monitoring of the tree crown condition on 13 plots of the first level indicated great influence of insects on the defoliation of pedunculate oak and narrow - leaved ash trees. Drought had a negative impact on the condition of the evaluated silver lime and pedunculate oak trees. Pap et al. (2017) state that a precipitation deficit of 39.0% was recorded in Vojvodina from November 2016 to March 2017, and in the vegetation period of 2017 was observed a deficit of 20.4% in relation to the multi-year average. In the vegetation period of 2022, a drought was also noted, and in the period from May to the end of August, a precipitation deficit of 150 mm of water sediment was recorded compared to the multi-year average for that part of the year. In the years immediately after the drought, an intensive occurrence of tree drying was recorded, which coincides with the statements of Dobbertin (2005), that the drought does not show its negative effect in the year when it is pronounced but in the following years. The analysis of the results of the tree crown condition on Level I plots showed a high degree of crown damage and drying for pedunculate oak which is similar as in previous periods (Drekić et al., 2013; 2016). Decline of oaks is widespread in Europe and has been going on for three centuries (Thomas et al., 2002). The authors state that decline occured as a result of the action of various biotic and abiotic harmful factors (Manojlović, 1924; Vajda, 1983;Thomas et al., 2002). The phenomenon of *Q. robur* dyeback in Serbia is a problem that has been present for a long time (Manojlović, 1924; Grbić et al. 1991; Medarević et al., 2009; Bauer et al., 2013). During the sanitation fellings in the period 1994-2011 in Morović forests in Serbia the average annual harvested volume of damaged and dead trees was 1.85 and 1.59 times higher than the current increment of the remaining trees in that period (Bauer et al., 2013). It's important to notice that a considerable decline of the narrow-leaved ash trees was observed which did not happen between 2003 and 2016. In the upcoming time frame, more research into the reasons for decline of this species should be conducted. Monitoring of forests is a prerequisite for understanding the state and changes that occur in forest ecosystems caused by the action of various harmful factors. Thus, continuous monitoring is the basis for the development and implementation of protection measures.

Conclusions

The most intensive damage in the form of defoliation was recorded for narrow - leaved ash, pedunculate oak and Austrian pine. In these three species was noted a low percentage of trees without defoliation and was recorded dieback of trees, as well. The results indicated a pronounced influence of insect attacks and drought on tree crowns defoliation and its variation. The problem of poor crown condition of the pedunculate oak and narrow - leaved ash is very pronounced, which indicates the need to investigate in detail the causes of this phenomenon and to suggest measures to improve the current situation. The most favorable situation is found for the Euroamerican poplar and silver linden in which the percentage of trees without defoliation was high. In order to collect the more data on the forest condition, it is necessary to add more sample plots which could be helpful in determination of the causal relationships between harmful factors and the resulting damage of forest ecosystems.

Acknowledgments

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INVESTIGATION OF ENVIRONMENTAL CONDITIONS FOR THE DEVELOPMENT OF FIR DECAYING FUNGI

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Abstract

In order to effectively combat wood-degrading factors, particular emphasis is placed on the research of the impact of fungi that cause the most destructive form of decay – brown cubical rot. Among these species, one of the most common in our region is the fungus *Fomitopsis pinicola* (Sw.:Fr.) P. Karst, which attacks both deciduous and coniferous trees and develops as both a parasite and a saprophyte. The aim of the study was to determine whether and to what extent basic parameters of the external environment influenced the successful colonization of nutrient substrates by the fungus *F. pinicola*, which was a probable indicator of infection under natural conditions compared to competing decaying fungi. The research focused on the impact of H-ion concentration on the growth and mass production of mycelium, as well as on changes in substrate pH under the influence of this fungus. Experiments were conducted using dikaryotic mycelium of *F. pinicola* isolated from fruiting bodies taken from fir trees in the Tara National Park. It was found that at constant substrate pH values, the mycelium of *F. pinicola* exhibited maximum growth on a slightly acidic substrate (pH 4.8). Investigations on non-buffered substrates showed that the *F. pinicola* mycelium tended towards a pH value of 2.3, at which the highest dry mass of mycelium was recorded.

Keywords: *brown cubical rot, H-ion, pH, Tara, mycelium.*

Introduction

In protection of our most important tree species, particular emphasis is placed on measures to combat fungi that cause decay of the heartwood, the most valuable part of the tree. Among those species, one of the most common in our region is the fungus Fomitopsis pinicola (Sw.:Fr.) P. Karst, which develops as both a saprophyte and a parasite on coniferous and deciduous trees in temperate regions across Europe and Asia (Bishop, 2020), causing the most destructive form of decay – brown cubical rot. In this type of decay, the wood quickly cracks, becomes brittle, and loses its mechanical properties (Karadžić et al., 2020).
The research was conducted on the basis of samples collected in Tara National Park.
The basis for a rational fight against wood-destruction factors lies in understanding the basic physiological characteristics of the agents of destruction, which involves investigating the most important conditions that determine and enable fungi to initiate infection under natural conditions. In this regard, the influence of hydrogen ion concentration in the substrate was examined, as it is one of the basic parameters of the external environment in which the entire process of infection and decay development occurs over a certain period of time.

Material and Methods

The dikaryotic mycelium of the fungus *Fomitopsis pinicola* (Sw.:Fr.) P. Karst, with which the research was conducted, was isolated using standard methods from the fruiting bodies of fungi taken from fir trees in Tara National Park in Serbia.

The influence of constant substrate pH values on the growth of *F. pinicola* mycelium (on buffered, solid substrates)

To investigate the impact of different constant pH values on the development of *F. pinicola* mycelium isolated from fir, a buffered substrate was prepared. In order to ensure uniform nutrient distribution in certain parts of the buffer, the buffering system was prepared according to the recipe shown and using the Wolpert method, which has been used by several authors (Rypáček, 1957; Mirić, 1993). By mixing different volumes of 0.3 molar phosphate solutions – H_3PO_4, KH_2PO_4, and K_2HPO_4, substrates with different pH values were obtained, but with the same amount of phosphate, so that their different quantities would not affect the results. In that way, 5 series of phosphates of 187.5 ml each were obtained and then diluted in 5 Erlenmeyer flasks with a volume of 300 ml (Scheme 1).
1,000 ml of double-concentrated malt substrate (10 Bé sugar) and agar (4%) were prepared separately and poured into 5 Erlenmeyer flasks with a volume of 300 ml (187.5 ml each). This substrate was autoclaved separately from 0.3 molar phosphate solutions, and following the control of the pH value, they were mixed under sterile conditions. The pH control was performed after the sterilization to determine the stability of the buffer systems. In this way, a substrate of standard concentration (5 Bé sugar and 2% agar) was obtained, with physiologically equal representation of buffers (0.15 M).

Scheme 1. Recipe for preparation of buffered (solid) substrates for *F. pinicola* mycelium cultivation

Series number	Parts of 0.3 M solution (ml)			Parts of solution of the substrate malt (10 Bé and agar 4%) (ml)	pH of buffer	pH of substrate	
	H_3P0_4	KH_2PO_4	K_2HPO_4			after sterilization	at the end of experiment
1	32.5	155	-	187.5	2.9	3.2	2.8
2	11.5	176	-	187.5	3.3	3.8	2.9
3	-	186.7	0.75	187.5	4.5	4.8	2.9
4	-	156.2	31.3	187.5	6.0	6.0	5.0
5	-	42	145.5	187.5	7.2	7.2	6.9

The buffered substrate prepared in this way was poured (20 ml each) into plastic petri dishes (D=90 mm). For each pH value tested, 3 replications were used. Inoculation was performed in a laminar chamber using circular mycelial fragments (D=11 mm), which were placed along the edge of the petri dishes. Cultures were developing in a thermostat at a temperature of 21°C. Mycelial growth was marked at 24 hour increments in three directions - along the diameter and on both sides at an angle of 22.5°. The average daily growth was determined as the mean growth value in these 3 directions, and the number of days of measurement depended on the growth rate of the fungus at certain (constant) pH values of the nutrient substrate. To verify the stability of the buffer system (substrate pH control at the end of the experiment), at the same time a liquid medium was prepared, which was then inoculated and incubated under the same conditions.

The influence of *F. pinicola* mycelium on the change in pH of the nutrient substrate (on non-buffered, liquid media)

To examine the influence of the fungus *F. pinicola* isolated from fir on the change in pH value of the nutrient substrate, a non-buffered (liquid) medium was prepared using the method of Schmidt and Liese (Schmidt, 1994). A total of 2,600 ml of double-concentrated malt substrate (10 Bé sugar) was prepared with distilled water. From this quantity, 408 ml each was poured into 6 Erlenmeyer flasks with a volume of 500 ml (for 6 series), and according to the presented recipe (Scheme 2), the appropriate amount of distilled water and 1M HCl or 1M NaOH solution was

added. This resulted in the required amount of liquid malt nutrient medium of standard concentration (5 Bé sugar).
Before sterilization, the pH values of each series were measured. From each series, 120 ml of substrate was poured into 12 Erlenmeyer flasks with a volume of 300 ml (4 fungi with 3 replications each), so that substrates from 6 series were poured into 72 Erlenmeyer flasks which were sterilized in an autoclave for 20 minutes at a temperature of 120±1°C and a pressure of 1.4 bar. After sterilization, pH values were measured again, and they were treated as initial values. Inoculation with the fungus *F. pinicola* was performed in a laminar chamber, using mycelial fragments of circular shape (D=11 mm). For each series (initial pH value), substrates were inoculated in 3 Erlenmeyer flasks each.

Scheme 2. Recipe for preparation of non-buffered (liquid) substrates

Series number	Parts of solution (ml)		Distilled water (ml)	Parts of solution of double-concentrated malt substrate (10 Bé) (ml)	pH values of substrate	
	MHC1	MNaOH			before sterilization	after sterilization (initial pH)
I	9.60	-	400	408	2.2	2.2
II	0.56	-	408	408	2.9	2.8
III	-	-	408	408	4.2	4.2
IV	-	0.80	408	408	4.8	4.8
V	-	3.24	405.6	408	5.9	5.4
VI	-	25.60	389.6	408	6.4	6.2

The incubation lasted for 21 days at a temperature of 21°C. During the incubation period, pH change was measured every 7 days. For each measurement, 10 ml of substrate was withdrawn under aseptic conditions, using a sterile syringe with a needle, transferred into cuvettes, and pH values were measured with a digital pH meter.

Results and Discussion

The basic prerequisite for understanding the conditions enabling a fungus to colonize wood is knowledge of its fundamental physiological characteristics. It is important to note that different strains of the same fungus show clear differences under optimal environmental conditions (Dresh *et al*., 2015), whereas the fungi isolated from natural habitats and then transferred and cultured in laboratory conditions are under unusual conditions of existence, which causes their somewhat different physiological activity (Vučetić, 1985). This is because it is very difficult to replicate conditions in a laboratory that adequately reflect those of the external environment and vary only one factor without affecting others. Therefore, results obtained through even the most precise laboratory methods cannot directly apply to natural conditions, so they should only be accepted as probable indicators of potential occurrences.
Influence of pH values of the substrate
The pH symbol (the negative logarithm of the hydrogen ion concentration in mol/1 of water) is a measure of the acidity or alkalinity of a medium. The concentration of H ions affects the growth and development of all plant species, including the metabolism of wood-decay fungi (Rypáček, 1957). Substrate acidity can stimulate or inhibit the growth of saprophytic fungi, while changes in pH values have a significant impact on the rate of nutrient consumption and substrate decomposition.
Lilly & Barnett (1951) state that the majority of decay fungi have an optimum for growth in a slightly acidic pH range (pH 5 to 6). The acidity of the environment affects the enzyme system of fungi, which provides the organism's vital needs for food. Many decay fungi produce

organic acids in significant quantities, leading to substrate acidification. By decomposing wood (oxidizing and hydrolyzing wood constituents), epixylous fungi increase its acidity through oxalic acid formed in these processes. Rypáček (1957) states that a neutral substrate reaction suits most decay fungi during the substrate colonization phase, but that during mycelium development, as a result of fungal metabolism, initial pH values change towards acidic conditions. Rayner and Boddy (l988) state that the lower growth threshold of decay fungi is found in the pH range of 2 to 3, with an optimum between 4 and 6, where brown rot agents seek lower pH values than white rot agents. Jačevski (1933) states that epixylous fungi develop in a substrate with a pH between 2 and 8.5, with an optimum between 4 and 6, which represents the natural pH value of the majority of wood species.

Influence of the pH of the substrate on *F. pinicola* mycelial growth

Based on the results presented in Table 1, it can be observed that the mycelium of the tested fungus developed on all substrates with different tested pH values, and that the growth on substrates with certain constant pH values varied.

Table 1. Average daily mycelial growth of *F. pinicola* on buffered (solid) substrates (mm/day)

Series	pH of substrate		Growth of mycelia (mm/day)
	Initial pH	pH at the end of experiment	
1	3.2	2.8	2.14
2	3.8	2.9	2.67
3	4.8	2.9	2.46
4	6.0	5.0	1.86
5	7.2	6.9	0.89

The isolate of *F. pinicola* from fir had maximum growth in slightly acidic conditions with a pH of 3.8. The reduction in growth was more pronounced in slightly acidic substrate, while in a mildly alkaline substrate (pH 7.2), growth was almost completely reduced, amounting to only 0.89 mm/day. On strongly acidic substrate (pH 3.2), the growth of the *F. pinicola* isolate was faster, reaching 2.14 mm/day.

The substrate acidity that nearly halts mycelial growth is a mildly alkaline substrate (pH 7.2), where mycelial growth is extremely low. It should be noted that the range between the tested pH values of 3.2 and 7.2 is relatively large, and there is a possibility that the tested fungus would have higher mycelial growth at pH values between these two. Based on the control series in liquid media, which was set up to verify the stability of the buffer system, it was determined that at the end of the experiment, the fungus altered the initial pH values of the substrate, shifting the pH by 0.3 to 1.9 towards optimal values, which is within the tolerable range, so it can be considered that all buffer systems remained stable throughout the experiment.

The largest change of the pH, amounting to 1.9, was recorded in the 3rd series. Significant pH changes (0.9 and 1.0) were registered in the 2nd and 4th series. In the remaining series, pH changes were 0.3 and 0.4.

Influence of the mycelia of *F. pinicola* on the pH change of the nutrient substrate (non-buffered substrate)

Table 2 presents the change of pH in the standard concentration malt nutrient substrate under the influence of *F. pinicola* isolate from fir.

Table 2. Change of pH values of the substrate under the influence of the mycelia *F. pinicola*

Series	Initial pH	Change of substrate pH				Weight of mycelial
		After 7 days	After 14 days	After 21 days	Total change of pH	
I	2.2	2.4	2.1	2.1	- 0.1	0.328
II	2.8	3.0	2.3	2.2	-0.,6	0.387
III	4.2	4.5	2.2	2.2	-2.0	0.571
IV	4.8	4.2	2.5	2.3	-1.5	0.578
V	5.4	5.4	2.7	2.3	-3.1	0.451
VI	6.2	5.5	3.2	2.3	-4.1	0.366

Based on the results shown in Table 2, it can be seen that on the substrate with an initial pH value of 2.2, there was practically no pH change until the end of the experiment (pH was lowered by only 0.1). The greatest pH changes were recorded in substrates with initial pH values of 6.2 and 5.4 (pH decreased by 4.1 and 3.1, respectively, over 21 days). Except for the initial pH value of 2.2, which was almost unaffected by the *F. pinicola* mycelium, at all other initial pH values, the pH decreased to 2.2 and to 2.3 at the end of the experiment. This means that these were the pH values that the *F. pinicola* mycelium gravitated towards during development. For the initial substrate pH value of 2.2, it probably takes longer for the fungus to approach pH values of around 2.3 through its metabolic activities, as was the case in most of the tested series.

Considering that after 21 days of exposure to the fungus *F. pinicola*, the substrate pH at initial values of 2.8 to 6.2 was reduced to a narrow pH range of 2.2 to 2.3, this can be interpreted as a relatively favorable pH value for the development of *F. pinicola*. This is also evident in the dry weight of the mycelium, which was the highest in the 3rd and 4th series, where the substrate pH was reduced to pH 2.2 and 2.3 after 21 days.

Conclusions

The change in pH values of the substrate on which the *F. pinicola* cultures developed gravitated towards a slightly acidic reaction, indicating that it favors a slightly acidic substrate, like most decay fungi. This fact suggests that *F. pinicola* competes with other decay fungi to colonize the substrate with equal chances of success, at least in terms of H-ion concentration. Taking into account the results of the study of the influence of H-ion concentration on the growth and mycelial mass production of the *F. pinicola* fungus, as well as the change in substrate pH under the influence of this fungus, from the perspective of successful colonization of nutrient substrate in natural conditions it can be concluded that the examined species is neither favored nor inhibited by environmental factors compared to the competing decay fungi. The phenomenon of microbial competition on the same substrate, inhibition of growth, or the occurrence of antagonism may be due to the metabolism of the competing species of fungi, secretion of mycotoxins or antibiotics ahead of the growing mycelial front, and the sensitivity or reaction of competing species to them.

This is the phenomenon which the speed, course, and consequences of decomposition of wood will directly depend on – wood as a substrate and nutrition source, but also as a very important raw material for processing, which due to its organic origin, represents food for a large number of organisms and microorganisms. For this reason, it is necessary to investigate the competitive relationships between this and other competing species of decay fungi under controlled conditions in so-called mixed cultures, under conditions of moisture, temperature, and H-ion concentration that are suitable for all types of opposing fungi.

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EFFECT OF COMMERCIAL BIOFERTILIZER ON THE GROWTH PARAMETERS OF TWO-YEAR-OLD NORTHERN RED OAK (*QUERCUS RUBRA* L.) SEEDLINGS

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Abstract

Microbiological biofertilizers are gaining more and more attention in the global market as an environmentally healthier substitute for conventional chemical fertilizers. This paper investigated the effect of treatment of two-year-old northern red oak seedlings with commercial biofertilizer Slavol® during one growing season. The medium was prepared according to the producer’s instructions and applied foliar in late June. Seedling height and root collar diameter were measured as the prominent representatives of seedling quality growth parameters. The results confirmed the positive impact of the biofertilizer on seedling growth. Mann-Whitney U test showed a statistically significant difference in seedlings height ($U = 42.000$; $p < 0.001$) and root collar diameter ($U = 56.000$; $p < 0.001$) among the treated seedlings group at the beginning and the end of the experiment. In the control group, the statistically significant difference was noted for seedling root collar diameter ($U = 55.000$; $p < 0.001$), but not for seedling height ($U = 163.000$; $p = 0.624$) at the experiment beginning and end. The results indicate that Slavol® biofertilizer influenced two-year-old northern red oak seedling height enhancement at the end of the experiment, but not on seedling root collar diameter, which had significant growth in both control and treated plants.

Keywords: *northern red oak, biofertilizer, seedlings.*

Introduction

Northern red oak (*Quercus rubra* L.) is a deciduous tree species native to Northern America continent, and a common oak representative from the section *Lobatae* across Europe. Its introduction to this continent was done deliberately in the 17th century most likely from the northern part of its natural distribution and continued in the future (Marceron et al., 2017). During the 19th century, its usage for reforestation purposes and timber production started, in order to improve timber yields. It became one of the most common and commercially important alien species considering timber exploitation. Due to the symmetrical shape of a tree crown and large leaves that turn the foliage red in Autumn, red oak is also evaluated as a highly decorative species in urban parks, woods, and green areas. Acorns of red oak are a significant food source for a variety of birds and rodents found in those habitats.

Northern red oak in Europe is advantageous over native oak species for its modest environmental requirements, higher aridity and low-temperature tolerance (Isajev et al., 2006), and high adaptive potential. It produces plentiful acorns large in size and is more successful in regeneration due to its fast growth rate and tolerance to a wide range of different ecological factors in the habitat (Woziwoda et al., 2014). Nevertheless, in some European countries, it is considered to be an invasive alien species that suppresses native oaks, which combat with climate change issues. When established, red oak changes the environmental conditions by decreasing the understory light that further reduces its vegetation diversity and consequently impacting soil characteristics (Dyderski and Jagodziński, 2019). Moreover, the

abundant slowly decaying leaf litter of red oak is a challenge for decomposers, leading to the impediment of common ecosystem processes and the development of autochthonous species.
In Serbia around 60 ha of territory is under northern red oak (Živanović et al., 2023) and it is mostly located in city parks and urban gardens where is used as an ornamental. However, in the vicinity of Belgrade, significant stands of red oak can be found (Lazarević, 2020). Along with its contribution to the beauty of landscape, it is also used as material for processing in the wood industry.
Northern red oak seedlings are produced in nurseries under common practice that includes watering and chemical fertilizer and pesticide usage. Aspirations for a healthier environment led to the development of alternative strategies that will enable production of high-quality seedlings, but in sustainable and ecologically safe mode, and were supported both legally through management policies that regulate chemical utilization in forest nurseries. Biofertilizers are one of the well-known substituents in today’s global market, that are still rather applied in agriculture than in forestry. The tendency toward clean technologies encourages research in this direction, and numerous commercial products such as Slavol®, Rizokyl Simplex®, Aegis mycrogranule®, Bacillomix original®, etc. can be found in trade. Biofertilizers contain different microorganisms that help and support plant well-being through hormone production, effective nutrient and essential elements acquisition, induction of plant immunity, and are completely harmless. When studying their effect on plant organisms, different morphological and physiological growth parameters are being monitored, and in forestry seedling height and root collar diameter are most commonly measured.
Slavol® is an organic, microbiological fertilizer registered for foliar feeding of agriculture and horticulture species. Its liquid formulation contains bacteria that are nitrogen-fixators, phosphate solubilizers, and auxine producers.
In this study, we analyzed the effect of Slavol® biofertilizer application on two-year-old red oak seedlings and their growth parameters, seedling height, and root collar diameter.

Material and methods

Two-year-old northern red oak seedlings were produced from the acorns collected in the Autumn of 2021 from local woods in Košutnjak, Belgrade, Serbia. Acorns were sown in pots filled with white and black peat mixture (70:30 ratio) (Free Peat BV, Netherlands). Seedlings were watered daily and nurtured in half-shadow conditions in the nursery of the Institute of Forestry.
In late June of 2023, seedlings were treated with a Slavol® preparation applied on leaves, made according to manufacturer instructions (100 ml Slavol®/10 l water). Nineteen plants were chosen per treatment, and the control group was treated with tap water. Both treatments were repeated seven days later. No additional fertilizers or pesticides were used.
At the beginning of the experiment, seedling height and root collar diameter were measured. Seedling height was measured by a ruler with an accuracy of 0.5 cm. The seedling root collar diameter was estimated by Vernier caliper with an accuracy of 0.1 mm.
At the end of the same growing season, identical growth parameters were evaluated again by the same method.
Descriptive statistics and a Mann-Whitney U test were performed for statistical estimation of Slavol® impact on seedling growth. All statistical analyses were performed using the SPSS 27 software package (IBM, Armonk, NY, USA), and Microsoft Office Excel 2021 (Redmond, Washington U.S.).

Results and discussion

The growth of a total number of 38 two-year-old northern red oak seedlings was measured at the beginning and the end of the experiment. Descriptive statistics for estimated traits are presented in Table 1.

Table 1. Descriptive statistics values for measured seedling traits

		Beginning of the experiment		End of the experiment	
		D	H	D	H
Control treatment	average	4.72	21.24	7.45	44.68
	min	2.22	12	3.94	26
	max	8.24	32.5	11.59	80
Slavol® treatment	average	4.76	23.45	7.21	43.26
	min	2.6	8	2.95	18
	max	7.84	35.5	10.32	62

D - root collar diameter
H - seedling height

At the beginning and the end of the experiment, there was a statistically significant difference in seedlings' height ($U = 42.000$; $p < 0.001$) and root collar diameter ($U = 56.000$; $p < 0.001$) among the treated seedlings based on the Mann Whitney U test. In the control group, a Mann Whitney U test showed a statistically significant difference in seedling root collar diameter ($U = 55.000$; $p < 0.001$), while there was no significant difference in seedling height ($U = 163.000$; $p = 0.624$) at the beginning and end of experiment.
Based on the obtained results it can be concluded that the application of biofertilizer influenced the increment of seedlings' height, which did not significantly change in the control group, in comparison with the treated group. Although initial diameter and height are equally important in the first year after planting (Ivetić et al. 2016), seedling height is an important parameter for faster competition endangerment overcoming and later sylvicultural measures (Grossnickle and MacDonald 2018a). These findings point to the direction of regulating better growth conditions of young red oak seedlings and the potential for controlling further growth dynamics.
As stated in the manufacturer booklet, Slavol® is a biofertilizer for field crops, vegetables, and some horticultural species, as well as *Thuja* sp., *Cypress* sp., *Juniper* sp., and *Taxus* sp. It demonstrated the capability to promote growth and efficiency in earlier studies (Đorđević and Babović 2012; Djukic et al., 2012; Miskoska-Milevska et al., 2018; Miskoska-Milevska et al., 2020). Slavol® contains a mixture of plant-growth-promoting bacteria with the ability to produce auxin, solubilize phosphate, and fix nitrogen, hence naturally promoting plant growth. These pathways influence plant crucial metabolic processes, and the obtained results presented in this paper are therefore expected and consistent with literature data.
Mechanisms of action of plant-growth-promoting bacteria are well described in the literature (Olanrewaju et al. 2017) and in the situation where different legislations limit the utilization of chemical fertilizers and pesticides, these natural substituents present a potentive tool in seedling production.
Plant growth parameters measured in this paper are one of the most used morphological traits in forest seedling monitoring and quality estimation (Grossnickle and MacDonald, 2018b). Plant height is important for photosynthesis and competition with other vegetation in the field for sunlight, while root collar diameter gives insight into root growth, volume, and development. Roots play a crucial role in the acquisition of nutrients, deposition, and anchoring and are of great importance for plant well-being.

Conclusion

The biofertilizer potential of application is large and should be utilized for sustainable plant production both in agriculture and forestry. In this paper, we investigated the potential of Slavol® to enhance the northern red oak seedling growth, by measuring seedling height and root collar diameter. The results confirmed positive impact of the biofertilizer, and statistical analysis showed a significant difference in seedlings height and root collar diameter among the treated seedlings group at the beginning and the end of the experiment. In future research Slavol® effect should also be inspected in various environmental conditions and different application patterns.

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PLANTING ENERGY CROPS FOR BIOMASS PRODUCTION ON DEPOSOL SOIL

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Abstract

The paper shows the properties of deposol soil and the effect of growing different clones of soft-leaved trees on this type of soil. During 2023 an experiment was set up in controlled conditions with different poplar and willow clones on two types of deposol soil, while natural alluvial soil characteristic for the cultivation of these species, additionally enriched with nutrient substrate, was used as a control. Deposol soils were taken from city landfills in Novi Sad and Belgrade, while natural soil was taken from the surface of clone plantations of poplars and willows. The soil was placed in pots where poplar and willow cuttings were subsequently planted, and the experiment was conducted under controlled conditions of temperature and air humidity, with the necessary irrigation of the plants. The examined granulometric composition of deposol soil from the landfill in Novi Sad shows a total sand content of 63.84% and a total clay content of 36.16%, according to textural class this soil is sandy loam. The deposol soil from the landfill in Belgrade contained 39.96% of total sand and 60.04% of total clay, and is of the clay loam textural class, while the natural alluvial soil contained 89.12% of total sand and 10.88% of total clay and belongs to the textural class sand. The chemical properties of the tested soils are such that the deposol from the landfill in Belgrade is carbonated, moderately alkaline and moderately humus, and moderately supplied with nutrients, while the deposol from the landfill in Novi Sad is highly carbonated, highly alkaline and moderately humus, and poorly supplied with nutrients. The natural alluvial soil as a control is strongly carbonated, moderately alkaline and weakly humus, and poorly supplied with nutrients. Considering the shown properties of the soil that was used in the experiment for growing plants of various clones of poplars and willows, the soil from the landfill in Belgrade proved to be the least favorable for growing poplars and willows with poor reception and drying of the plants after the production cycle, better success in growing these species was achieved on the soil from the landfill in Novi Sad, while the best results were obtained from the control alluvial soil, which is also used for the cultivation of soft deciduous trees.

Keywords: *Deposol, Soil properties, Poplars, Willows, Biomass production, Bioenergy plantations.*

Introduction

Poplars and willows are known as fast-growing species that thrive on alluvial soils next to river courses (Živanov and Ivanišević, 1986). Their importance lies in the fact that, in addition to growing in plantations that provide an economic effect, they can thrive as pioneer species on various tailings, fire and polluted soils if there are minimum conditions for their survival and growth. Having in mind especially the soils that are polluted, as an alternative on such surfaces, Pilipović et al. (2023) indicates the use of woody plantations of poplar and willow in short rotation (SWRC) that would be used as bioenergetic plantations for phytoremediation of polluted areas. Kovačević et al. (2023), conducting research on the selection of planting material for short-distance plantings, suggests poplar planting material (0/1) where there are conditions for this type of planting. Also Stojnić et al. (2023) also indicate the possibility of raising black poplar clones by "shallow planting" with rootless seedlings (1/0) as a more economical method of planting. Studying landfill soils in the earlier period, Pekeč et al. (2023) found a heavier textural composition of deposol with a higher proportion of total clay compared to natural alluvial soil for poplar and willow cultivation, as well as a slightly higher content of humus and nutrients on these types of soil. Considering that poplars and willows, as fast-growing species, can also thrive on deposol soils, more like protective plantings between the landfill area and the surrounding soil, it is necessary to investigate the physical and chemical properties of the landfill soil and see what are the differences compared to natural alluvial soil for cultivation of these species. The aim of the work is to determine the characteristics of landfill soils, and the effect of growing poplars and willows on such lands in order to select poplar and willow clones that tolerate these conditions through the selection method. In the second phase of the research, exactly such clones would be used for field trials and the establishment of protective plantings around the area where landfills are located on deposol soils.

Material and method

During 2023, an experiment was set up under controlled conditions with a total of 26 poplar and 8 willow clones. The experiment was set up on two types of deposol soil, while natural alluvial soil characteristic for the cultivation of these species, additionally enriched with a nutrient substrate, was used as a control. The soil was cleaned of waste and sieved, and placed in pots where poplar and willow cuttings were subsequently planted, and the plant cultivation experiment was carried out under controlled conditions of temperature and air humidity, with the necessary irrigation of the plants. The investigated soil was from three locations: soil used for the production of poplar and willow plantations in Kać, soil from the landfill in Novi Sad and soil from the landfill in Belgrade in Serbia.

Soil samples were taken in a disturbed state to a depth of 30 cm and the following physical and chemical soil analyzes were performed:

The mechanical composition, using the Pipet method, prepares samples for analysis with Natrium pyrophosphate according to Thun, and the textural class of the soil is determined according to the classification of Tommerup;

The content of $CaCO_3$ was determined volumetrically using a "Scheibler" calcimeter;

The pH value was determined in a soil suspension with water, potentiometrically;

Humus content according to Tyurin, modified by Simakov;

Total nitrogen according to the Kjeldahl method;

Easily accessible phosphorus and potassium according to the AL method, Egner-Riehm-Dominigo.

Based on the analyzes performed, the characteristics of the tested soil were presented, and the effect of the success of growing poplar and willow clones on this type of soil was determined.

Results and discussion

Analyzing the granulometric composition of all three investigated locations, (table 1) indicate that the content of total sand in relation to the content of total clay on the soil for growing poplars and willows in the area of Kać is in the ratio 89.12 : 10.88%, this ratio decreases with the soil from the landfill in Novi Sad, where it is 63.84 : 39.96%, while for the soil from the landfill in Belgrade, the mentioned ratio is in favor of the total clay, and it is 39.96 : 60.04%. On the axis of the share of different granulometric fractions, the soil from the first location is of textural class sand, sandy loam from the landfill in Novi Sad, and clay loam from the landfill in Belgrade.

Table 1. Granulometric composition

Location	Horizon	Depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Total sand (%)	Total clay (%)	Texture class
Kać	(A)	0-30	42.09	47.03	7.68	3.20	89.12	10.88	Sand
Beograd	P_1	0-30	4.28	35.68	28.76	31.28	39.96	60.04	Clay loam
Novi Sad	P_1	0-30	22.08	41.76	19.24	16.92	63.84	36.16	Sandy loam

According to the chemical composition (table 2) it can be stated that the soil from the poplar and willow growing area in Kać is strongly carbonated (according to Belić et al. 2014), medium (moderately) alkaline according to the American soil classification in relation to chemical reaction, weakly humus according to Scheffer-Schachtschabel, (according to Belić et al. 2014) and poorly provided with nutrients (Manojlović and Čabilovski, 2019). The soil from the landfill in Novi Sad is also highly carbonated (according to Belić et al. 2014), but also strongly alkaline and moderately humus according to Scheffer-Schachtschabel (according to Belić et al. 2014) and poorly supplied with nutrients (Manojlović and Čabilovski, 2019). Compared to the previous two sites, the soil from the landfill in Belgrade is carbonate (according to Belić et al. 2014), medium (moderately) alkaline, moderately humus according to Scheffer-Schachtschabel, (according to Belić et al. 2014) and moderately provided with nutrients (according to Manojlović and Čabilovski, 2019).

Table 2. Chemical properties

Location	Horizon	Depth (cm)	CaCO3 (%)	pH (in H_2O)	Humus (%)	N (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
Kać	(A)	0-30	11.12	8.09	1.65	0.016	3.85	2.92
Beograd	P_1	0-30	6.82	7.95	2.14	0.133	12.66	10.28
Novi Sad	P_1	0-30	13.06	8.62	2.71	0.076	8.38	6.70

From the analyzed granulometric composition, we can see that the examined soils are different, and the alluvial soil is of sand textural class, the soil from the landfill in Novi Sad is sandy loam, and the soil from the landfill in Belgrade is clay loam. The chemical composition of the tested soils is mostly similar, with the fact that the natural alluvial soil is weakly humus, while the other two soils are slightly richer in organic matter and moderately humus. It can also be noted that the soil from the landfill in Belgrade is moderately supplied with nutrients compared to the remaining two examined soils that are poor in nutrients. Considering the difference in the textural composition of the tested soils, which results in different water-air properties of the soil, the textural composition of the soil had a significant impact on the reception and survival of plants during the production cycle. Of the total number of poplar and willow clones, on the control alluvial soil all plants survived completely, i.e. 26 poplar clones and 8 willow clones, and on the soil from the landfill in Novi Sad, 9 poplar and 4 willow clones survived, while on the soil with landfill in Belgrade, only 3 poplar and 3 willow clones succesed. Kovačević et al. (2008a), studying poplar clones, linked the height of the shoot with the formation of the root system immediately after cuttings, as well as with the survival of cutting. Bearing in mind that poplar as a species does not tolerate soils with a heavy mechanical composition, the soil of the deposol in Belgrade, which has a texture class of clay loam, influenced the survival of only 11.5% (3 clones of a total of 26 clones) of the total number of grafted cuttings, while due to the lighter mechanical composition that was found at the deposol in Novi Sad, the number of surviving poplars was three times higher, i.e. 34.6% (9 clones of a total of 26 clones). Kovačević et al (2008b) states that between genotypes and soil properties, a clear influence on the survival and growth of poplar cuttings was determined. Živanov et al. (1985) mentions soil with a granulometric composition of 30-50% dust and clay as favorable soil for growing poplars, while the investigated soil in Novi Sad has the mentioned content of 36.16% and the soil in Belgrade is above that limit and amounts to 60.04%. Observing the willow clones, which certainly better tolerate soils with a heavier mechanical composition, in the area of the deposol from Belgrade, the success rate of willow survival was 37.5% (3 clones of a total of 8 clones), while on the somewhat less heavy deposol soil in Novi Sad, the willow survival rate was 50 .0% (4 clones of a total of 8 clones). Orlović et al. (2005) states that willow as a species tolerates soil with a heavier granulometric composition as well as longer periods of flooding and notes that physiological parameters can be used in selection for the exuberance of white willow growth. Borisev at al. (2012) examining the impact of different levels of soil contamination on white willow indicates that it has the potential for remediation and redevelopment of contaminated areas with a moderate dose of pollution. Examining the granulometric composition of the soil, Vučić (1987) states that the appropriate content of total sand, dust and clay in the soil is in the ratio 40:40:20%, while the investigated deposol soil in Belgrade contains the ratio of the mentioned fractions 39:28:31%, which indicates a worse water-air properties, especially lower oxygen content for the development of the root system, while in the case of deposols in Novi Sad, the ratio of the mentioned fractions is 63:19:16%, which also affects worse water-air characteristics and results in weaker development of poplars and willows.

Conclusion

This paper were investigated two types of deposol soil and alluvial soil for the cultivation of soft decidous trees in controlled conditions. Apart from minor differences in the chemical composition of the examined soils, there are noticeable differences between the granulometric composition of these soils. The alluvial soil has a texture class of sand, the deposol from the Novi Sad area has a texture class of sandy loam, and the deposol from the area of Belgrade has a texture class of clay loam. Given this difference, the textural composition of the soil had a significant impact on the reception and survival of plants during the first year of the production cycle. Of the total number of poplar and willow clones, the best success was shown on sandy alluvial soil, with an increase in the proportion of total clay in deposol soil, the number of tolerant poplar clones decreases, while willow clones proved to be more tolerant on soil with a higher proportion of total clay.

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ANALYSIS OF FOREST DAMAGE: 20-YEAR STUDY IN SERBIA

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Abstract

Forest damage causes an alarming volume of affected trees. In this article we analyze the forest damage in the first 20 years of the XXI century on the territory of Serbia. The data are taken from the Statistical Yearbook. The results are presented according to the volume of damaged trees in five categories based on the main cause: by humans, by insects, weather disturbance, by plant diseases and forest fires. The results show that 1,8 million m^3 were damaged in the period under observation. The lowest volume of damage was recorded in 2006 with a total of 29.505 m^3 and the highest in 2016 with a total of 223.469 m^3. The most common cause of damage was weather disturbance (39%), followed by man-made damage (22%) and insects, plant diseases and fire (13% each). A trend towards an increase in the volume of damage can be observed for all types of damage, with the exception of fire damage. The largest volume of damage caused by humans was recorded in 2011 (28.285 m^3), by insects and plant diseases in 2016 (40.520; 37.249 m^3), by weather disturbance in 2015 (116.431 m^3), and by forest fires in 2012 (63.118 m^3). Forest damage is a complex phenomenon in which natural and anthropogenic factors play a role and lead to a loss of forest trees of up to 0,08% of the total volume and up to 3,4% of the total volume increment. This conclusion must be taken into account in future forest management plans.

Keywords: *natural disturbance, climate change, biotic, abiotic.*

Introduction

Forest damages are inherent part of natural ecosystems. Forest damage is defined as: "a disturbance to the forest which may be caused by biotic or abiotic agents, resulting in the death, or significant loss of vitality, productivity or value of trees and other components of the forest ecosystem" (TBFRA, 2000). Abiotic factors are weather conditions such as ice break, wind break, snow break, storms, droughts and human activities as well as fire. Biotic factors are insects and plant diseases. The frequency and intensity of extreme weather conditions and the occurrence of insects leads to an alarming volume of affected trees (Köhl, et al., 2024).
In a 50-year period from 1950 to 2000, an average of 35 million m^3 of damaged trees per year was observed (Schelhaas, et. al, 2003). The chronology of forest damage illustrates the vulnerability of forests. In the 1980s, large-scale forest damage occurred as a result of transboundary air pollution (Kandler, Innes, 1995) with significant science policy consequences (Poduška, 2018). In the 1990s, severe storms damaged up to 180 million m^3 of wood in Central Europe and boreal forests (Lassig, Mocalov, 2000). In the last few decades, we have seen a new combination of storms and resulting insect outbreaks. According to the FAO report, storms, insects and diseases damaged around 40 million ha of forest in 2015 (FAO, 2020).

The problem with the analysis of forest damage is the lack of a historical overview of disturbances in European forests, which is somewhat fulfilled by a comprehensive analysis of forest damage and disturbances since 1950 (Schelhaas, et al., 2003); Patacca, et al., 2023) and the Database on Forest Disturbances in Europe (Patacca et al., 2021). In Serbia, the analysis of the development of the extent of damage caused by natural hazards in the forests of Serbia and the influence of temperature and precipitation showed that the extent of damage caused by natural hazards decreases with increasing temperature (Ranković, et al., 2016). Other different causes of damage such as radiation, war and tree competition can be found in the literature. Analysis of forest damage caused by radiation (Witherspoon, 1965) and war (Westing, 1975) and damage caused by competition between trees (300.000 ha), which was observed in Finland (UNECE/FAO, 2000) is the least represented in the scientific literature and was not considered in this study.

Materials and methods

The data from monitoring of forest damages caused by natural disturbances can be found in several data bases: Database of European Forest Insect and Disease Disturbances, Database on Forest Disturbances in Europe, European Forest Fire Information System, International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests.
In this study as relevant source of data on forest damages we used National data base derived from Statistical Office of Republic of Serbia. The data was collected from Statistical Yearbooks in 20-years period (2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020). The data in Statistical Yearbooks was submitted by public forest enterprises: "Srbijasume" and "Vojvodinasume", and public enterprises for management national parks and units that are parts of other enterprises and cooperatives engaged in forestry. Data on damages to forests are collected by causes of damages and are expressed in m3.

Results and discussion

The results are presented for forest damages according to the causative agent, i.e. human caused, insects, natural inclemency - which represents damages from weather events, followed by damages caused by plant diseases, and fire. The volumes of damaged trees are present in two categories. abiotic and biotic, as well. Damage from human caused and damage from fire combined in the category of abiotic damage. Biotic damage is caused by insects and plant diseases.
Table 1. shows volume of damages per causative agent and total damages in 20-years period.

Table 11. Forest tree damages (m3)

m3 / Year	Humam caused damages	insects	natural inclemency	plant diseases	Fire ha	TOTAL
2000	11.792	5.966	35.684	3.900	7.944	63.223
2001	14.590	6.738	19.064	9.519	459	52.890
2002	12.158	5.796	5.280	7.033	969	43.052
2003	19.634	10.384	8.812	5.690	1.402	82.041
2004	15.212	1.043	9.544	11.857	202	39.158
2005	12.220	3.302	20.090	2.741	52	38.881
2006	9.918	1.402	9.172	7.933	494	29.505
2007	17.046	2.160	10.549	5.003	22.161	40.576

m3 / Year	Humam caused damages	insects	natural inclemency	plant diseases	Fire ha	TOTAL
2008	22.229	3.877	15.706	4.611	575	53.572
2009	14.090	3.021	19.937	8.313	1.210	47.293
2010	13.881	4.070	14.902	5.093	503	38.003
2011	28.285	3.281	8.141	3.358	2.036	67.635
2012	27.590	2.583	18.619	5.765	7.460	117.675
2013	17.859	13.056	29.960	17.556	561	85.774
2014	26.524	29.861	41.107	14.769	284	122.517
2015	24.393	32.680	116.431	13.218	827	191.781
2016	21.760	40.520	86.826	37.249	296	223.469
2017	26.327	27.303	44.074	17.504	1.050	126.623
2018	24.685	16.506	74.495	16.798	303	133.191
2019	27.378	11.765	58.028	13.876	1.079	114.444
2020	25.723	11.886	75.396	26.477	3.635	141.640
TOTAL	413.294	237.200	721.817	238.263	53.502	1.852.943

Table 1 shows the volume of damage per causative agent and the total damage over a period of 20 years. In the 20-year period observed, the total forest damage amounted to 1.852.943 m3. This corresponds to an average volume of 88.235 m3 of damaged trees per year. Over a 20-year period, state forests lose 2 m3 per hectare. The average annual damage in Serbia is 0,09 m3/ha, while the damage in Europe is significantly higher and amounts to 0,21 m3/ha) (Schelhaas, *et. al*.,, 2003). Forest damage caused by severe weather condition here called Natural inclemency caused 39 % of the total damage (721.817 m^3) over the 20-year period, followed by damage caused by humans (22 %) and damage caused by insects, plant dieses and fire (13 % each).The results are presented for forest damage according to the causative factors, i.e. damage caused by humans, insects, natural inclemency - which stands for damage caused by weather events, followed by damage caused by plant diseases and fire. The volume of damaged trees is divided into two categories: abiotic and biotic damage. Man-made damage and fire damage are summarised in the category of abiotic damage. Biotic damage is caused by insects and plant diseases.

Human-caused damage ranged from 9,918 m^3 in 2006 to 28,285 m^3 in 2011. The average human-caused damage was 19,681 m^3 per year. Total man-made damage in the last 20 – years amounted to 413,294 m^3, which corresponds to 22% of all damage in this period. The man-made damage mostly occurs in the form of illegal shelters, due to economic instability and the impossibility of guarding and protecting the forest in certain places, such as the security zone with Kosovo. The administrative authorities of the public forestry company Srbijašume state that the value of illegal timber over a 20-year period amounts to 22 million euros (CINS, 2019).

The damage caused by insects was highest in 2016 with 40,520 m^3 and lowest in 2004 with 1,043 m^3. The average damage caused by insects amounted to 11,295 m^3 over the 20-year reporting period. The total damage caused by insects amounted to 237,200 m^3, which corresponds to 13% of the total damage over the 20-year reporting period. The tree species most frequently attacked by insects were spruce, Austrian pine, oak and beach (Češljar, *et al*., 2014). The most common insect damage is caused by bark beetles attacking the most valuable spruce trees. Bark beetle outbreaks occur after wind breakage, when remedial measures such as the removal of uprooted trees come too late (Marković, 2012).

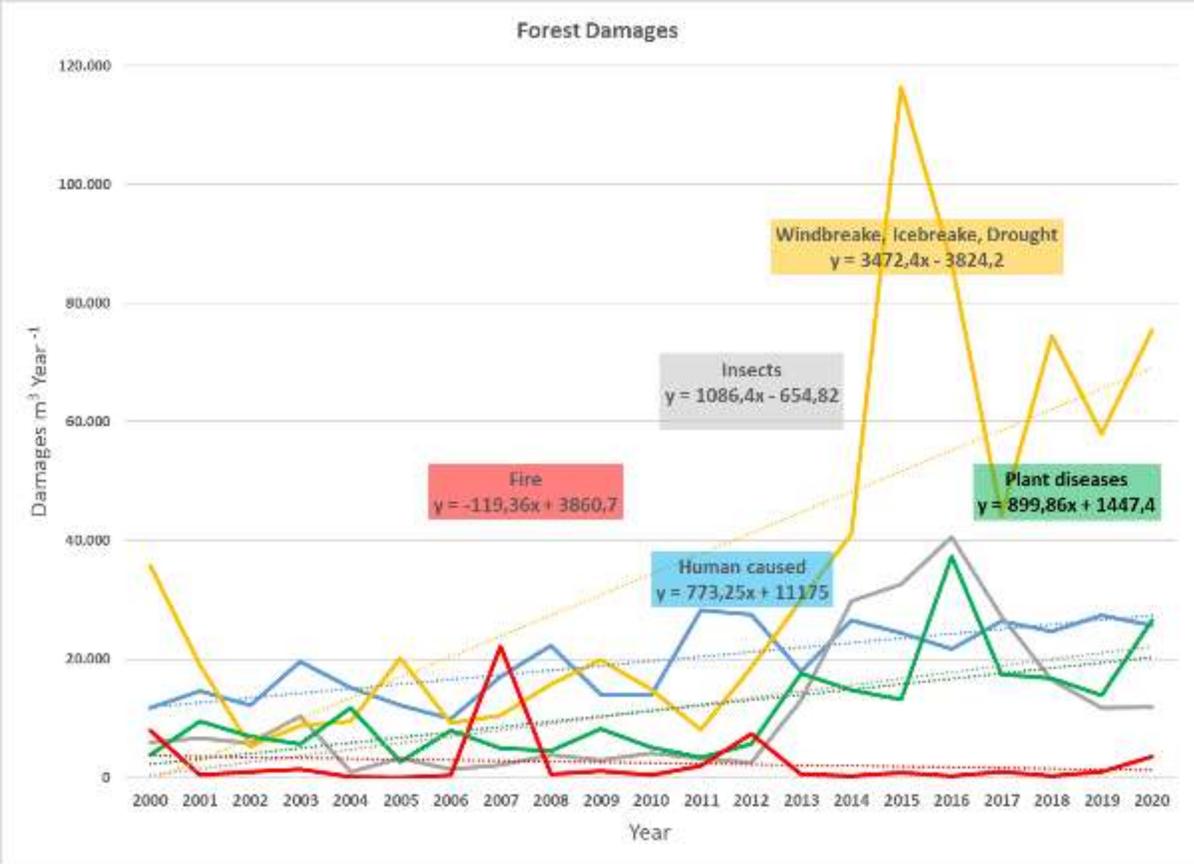

Figure 5. Forest damages

The extreme weather conditions referred to here as natural inclemency caused the most forest damage in 2015 with 116.431 m^3 and the least damage was observed in 2002 with 5,280 m^3. The main cause was the ice breakage in the winter of 2014/2015, which according to the reports of Public Forest Enterprise “Srbijašume” caused 456.326 m3 of damage, which for some reason is not visible in the Statistical Yearbook report. The average damage caused by natural inclemency were 34.372 m^3 in the reporting period. In Europe, storms are the main cause of damage and are responsible for around 51% of all damage recorded in the period 1950 – 2000 (Schuck, Schelhaas, 2013). After the wind simulation, it was pointed out that this extent of damage due to weather phenomena (ice, wind) could also be a consequence of novel silvicultural regimes leading to a relatively low tree height to stem diameter ratio (h/d) (Schelhaas, *et.al*., 2007). In even-aged stands, a low h/d ratio could have a direct effect on stand stability due to the low stand density. Low stand stability in uneven-aged stands depends on thinning, which favors taller trees with relatively small diameters.

The lowest fire damage was observed in 2010 with 57 m^3 and the highest in 2012 with 63.118 m3. The average damage caused by fire amounted to 11541 m^3. The total damage in the observed period amounted to 242,369 m^3. Such large discrepancy in forest fire damage can be explained by the fact that forest fires are considered both a damaging event (Sil, *et al*., 2019) and a beneficial event (Pausas, Keeley, 2019). The cause of forest fires is mostly human activity, with some benefiting from the fire, but there are also situations where the fire leads to significant losses (Poduška, Stajić, 2024). The global estimate of fire damage from the decade of 1960 is up to $7.06 billion (Ritchie *et al*., 2022). The greatest damage to trees in the state forests is in the region of southern and eastern Serbia. (Marčeta, Milanović, 2018).

The forests were damaged the most in 2016 with 223,469 m^3, and the least damage was observed in 2006 with 29,505 m^3. To gain a better insight into the causes of the damage, we have depicted abiotic and biotic damage in Figure 2.

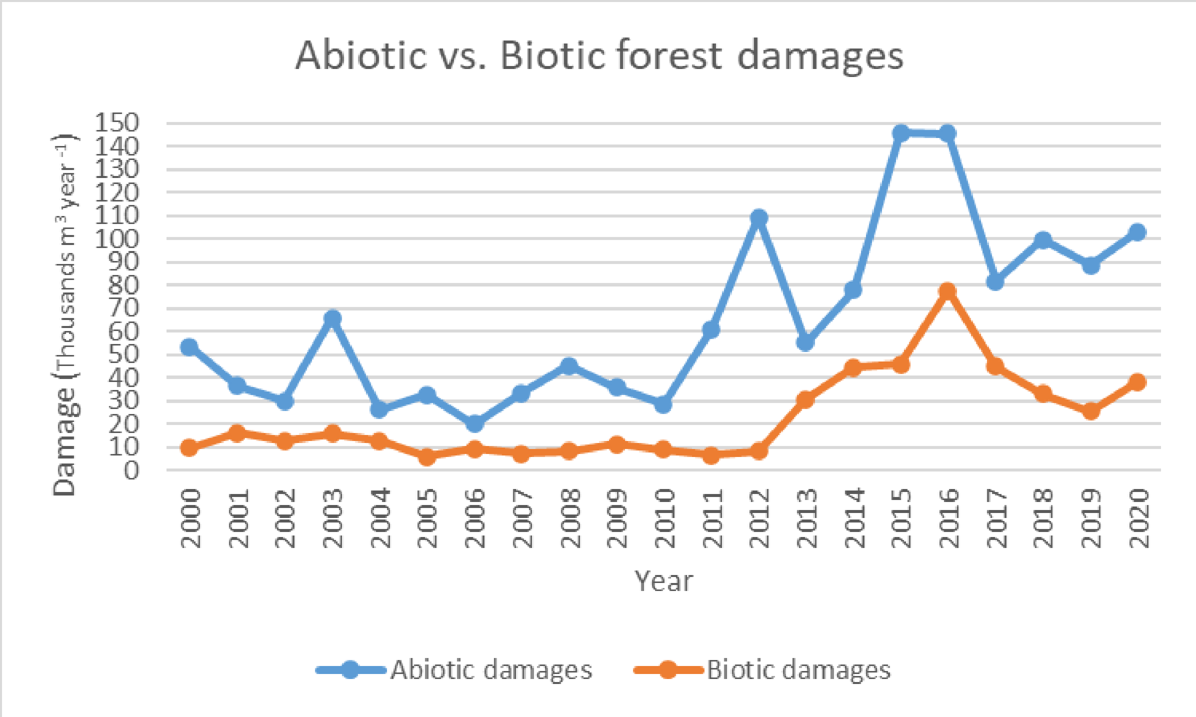

Figure 6. Abiotic vs. biotic forest damages

Forest damage caused by abiotic factors was observed to a greater extent than biological damage. The ratio between abiotic and biotic damage is 13 times higher in 2012, which is mainly due to natural inclemencies. The year 2012 was one of the driest years on record, which led to forest trees drying out (Radulović, *et al*., 2014).

Conclusion

In the assessed 20-year period from 2010 to 2020, the total damage to the trees in state owned forest in Serbia amounted to 1.852.943 m^3.

- Abiotic damage, which is caused by the influence of humans, fire and weather, accounts for the largest share of damage, accounting for 65% of all damage in the period from 2000 to 2020.
- Damage caused by natural disasters accounted for 39% of total forest tree losses.
- Human damage accounted for 22% of total tree losses.
- Fire damage accounted for 13% of total tree losses.
- Plant diseases caused 13% of total tree losses.
- Insects caused 13% of the total tree losses.

The patterns of damage in forests can be linked not only to the negative effects of climate change, but also to forest management and planning practices associated with silvicultural measures that have a significant impact on stand instability, as one of the objectives of such measures is to reduce the h/d ratio.

Acknowledgement

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SILVICULTURE IN THE FUNCTION OF SUSTAINABLE FOREST MANAGEMENT - CASE STUDY OF SE „SRBIJAŠUME“

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Abstract

Silviculture implies forest cultivation procedures in stands with the task of fulfilling the goals of forest management. Silviculture is the basic activity of SE "Srbijašume", which includes the procedure of professional and planned implementation of auxiliary measures, restoration, care, and establishment of new forests, which will optimally and permanently fulfill economic and general forest functions. The long-term strategic goals of SE "Srbijašume" in the field of silviculture are: improving the condition of existing forests and increasing the area under forest. Active forest management has its own requirements, which can only be met if, among other things, adequate planning and implementation of cultivation measures are ensured. The paper will show the structure of planning and execution of types of silviculture works on the example of SE "Srbijašume" in the past period, both in state and private forests. SE "Srbijašume" plans and carries out silviculture works in state forests on an area of 775,474 ha, but also performs professional - advisory work related to silviculture in the private owner's forests on an area of 1,222,624 ha, which includes about 3.4 million cadastral plots and about 1,000,000 owners. The paper will assess whether the level of planning (at the strategic and operational level) of silviculture works represents a sufficiently secure framework to ensure sustainable forest management in Serbia.
The paper's second goal is to perform an analysis (trend of growth, decline) on the extent of planning and execution of works on silviculture in the observed periods, but also an analysis of these phenomena in relation to ownership. The paper will give certain recommendations on how to improve the current situation in the field of silviculture in relation to planning, execution, and their impact on forest management for the conditions of Serbia through the example of SE "Srbijašume".

Keywords: *silviculture, sustainable forest management, SE "Srbijašume".*

Introduction

The relationship with the forest in modern times is incredibly intricate, encompassing ecological, economic, and social aspects. Numerous phenomena in the world and Serbia (climate change, economic conditions, increased demand for forest products and services) have a negative impact on the forest. The impacts of such events can also be observed in privately owned forests, which can lose stability and productivity in some cases. Additionally, the permanent removal of forests from their centuries-old habitats has been documented.
Nowadays, the importance of forests can best be explained through the functions of forests (Vasić, 2018). Sustainable management of natural values, preservation of natural balance and diversity, and increasing the quality of the environment is an imperative to modern society (Baković, 2013).

Silviculture is a scientific and professional discipline that deals with the establishment, care and regeneration of forests with the aim of optimal and permanent fulfillment of the economic and generally useful functions of forests. Silviculture aims to establish and maintain a forest stand in the shortest time and at minimal cost while preserving the soil's productivity. The goal is to create a forest that optimally and permanently serves economic and general utility functions. It was "set up" to "ensure" silvicultural procedures in forest stands, with the task of fulfilling the goals of forest management and gives us an answer to the question: How to act in a specific forest to fulfill the defined goals of forest management?
SE "Srbijašume" is a leading company for silviculture, protection and sustainable management of forests in the Republic of Serbia. For SE "Srbijašume", silviculture is one of the most important disciplines. The company aims to integrate silvicultural operations with appropriate forest management systems. A good circumstance for the forests managed by SE "Srbijašume" is that they are adequately managed following the modern principles of forest management planning. This also allows for the creation of annual silvicultural plans that are in coordination with valid development plans, forest management plans, forest management executive projects, and other planning documents.
The aim of this paper is to assess whether the different levels of planning of silviculture works represent a sufficiently secure framework to ensure sustainable management of forests in Serbia. Also through the paper, an analysis of the scope of planning and execution of works on silviculture in a ten-year period was carried out, as well as an analysis of these phenomena in relation to ownership. The paper gives certain recommendations on how the current situation in the field of silviculture in relation to planning, execution and their impact on forest management can be improved for the conditions of Serbia through the example of SE "Srbijašume".

Material and Methods

Considering the research problem, this paper uses an analytical and synthesis methodological approach. International and domestic professional literature related to this field was used for the preparation of this paper. The scientific methods that were most used in researching the problem included: the method of analysis and synthesis, the comparative method, and finally the method of induction and deduction.
On the basis of ten-year plans for silviculture works (regardless of ownership) and their execution, trends were derived in the function of proper analysis and drawing adequate conclusions.

Results and Discussion

In modern conditions, the importance of forests and the evaluation of their benefits are determined by the numerous and often contradictory expectations or demands of individuals, the economy, and sometimes society as a whole (Baković, 2018).
SE "Srbijašume" manages forests and forest land on a total area of 892,707 hectares, with 775,474 hectares covered by forest and 117,233.08 hectares not covered by forest (Source: Internal documentation of SE "Srbijašume", Belgrade, dated March 1, 2024. The data originates from the forest stand inventory of SE "Srbijašume"). The proportion of forested and non-forested areas managed by SE "Srbijašume" is 87% to 13%, which is considered relatively favorable. SE "Srbijašume" is responsible for managing 40% of the total 2,252,400 hectares of forested areas in Serbia (Banković et al., 2009).
SE "Srbijašume" plans to conduct silvicultural works through 85 types of activities based on collecting data on the condition of habitats and stands. These activities are being classified

into 55 types of works for the preparation of the Annual Business Program and to monitor their implementation.
In Table 1, there is an overview of the planned silvicultural works according to the valid forest management plans for a 10-year period. The works are grouped into 4 categories: auxiliary measures, regeneration treatments, establishment treatments, and intermediate treatments (forest care measures).

Table 1. Silvicultural plan according to valid forest management plans for a 10-year period (2014-2023) in SE "Srbijašume"

Forest Estate - code on the server	Type of work (in 10 years) 2024						Total
	Natural regeneration	Artificial regeneration	Total regeneration	Establishment of new forests	Forest care measures	Auxiliary measures	
	ha						
12	285	2,308	2,593	66	32,119	6,523	**41,301**
30	9,330	2,246	11,576	112	32,052	5,055	**48,795**
31	7,827	1,050	8,878	547	22,837	2,081	**34,343**
32	2,830	669	3,499	29	17,502	971	**22,001**
33	2,139	162	2,301	11	13,844	442	**16,598**
34	9,105	978	10,083	59	19,041	2,583	**31,766**
35	3,111	275	3,386	87	13,061	268	**16,801**
36	10,368	806	11,175	464	22,594	576	**34,809**
37	15,465	713	16,178	548	26,735	673	**44,134**
38	7,851	1,054	8,905	868	29,256	867	**39,897**
39	11,430	490	11,920	228	12,252	603	**25,003**
40	5,253	532	5,784	88	19,901	524	**26,297**
41	8,618	581	9,200	238	31,588	1,224	**42,250**
42	3,571	673	4,244	96	15,137	1,033	**20,510**
43	3,504	288	3,792	136	12,152	314	**16,394**
44	4,674	731	5,406	130	13,088	1,525	**20,149**
45	7,884	2,702	10,586	579	26,346	3,553	**41,064**
Sum	**113,245**	**16,259**	**129,505**	**4,287**	**359.507**	**28,815**	**522,113**

The silvicultural plan in SE "Srbijašume" according to the valid principles of forest management amounts to 522,113 ha for the next 10 years, i.e. 52,211 ha on average per year.
In the past 10 years at "Srbijašume," the average annual performance of silvicultural works has been planned for 46,662 hectares, about 89% of the legal planning possibility. This level of planning compliance can be considered very good. An important indicator of sustainable forest management is the area covered by forests (Baković, et. al, 2016), where it should be pointed out that afforestation (artificial regeneration and afforestation of bare land) in SE "Srbijašume" has a growing trend in the last 10 years. The ratio of planned and executed silvicultural works annually during the period 2014-2023 was 96%, which can be considered very good. The former gains particular significance if the objective reasons that influenced the scope of execution are considered, i.e. if they are taken into account, the volume of execution would rise to around 100%. Reasons that affect the scope of the works include:

1. Climate change - a short deadline for the execution of works. Unfavorable weather conditions lead to disruptions in execution dynamics. A short period for carrying out planting works. In spring, the snow melts late. There is a sudden rise in temperature and the beginning of vegetation growth. Autumns are extremely dry, and planting conifers is often interrupted. Dry autumns extend the vegetation period. Leaf fall occurs late (or not at all) and then snow generally falls in early December. Autumn planting of deciduous species will either begin promptly or not at all.
2. In the last decade, poor seed production in Serbia has significantly impacted the artificial regeneration of forests. Despite proper planning, a significant portion of the planned forest regeneration work (15,210 ha planned by SE "Srbijašume") has not been carried out. An aggravating circumstance is that the mast years of the main tree species are absent, and it should be emphasized that seed production is closely related to climate change.
3. Force majeure - drought, which was recognized by the relevant Ministry of the Republic of Serbia as an objective reason in the last few years, resulted in the drying of a significant number of seedlings planted in the last decade, as well as older seedlings. This meant that the types of work from the group of weed removal (manual, mechanical) and hoeing were not performed, even though they were properly planned because there was no need to perform these types of work.
4. Lack of labor force and poor technical equipment of service providers. Some contractors are very poorly equipped technically, which has caused frequent and long breakdowns of work machines.
5. Safety of employees. Part of the planned works along the administrative line towards the autonomous province of Kosovo-Metohija could not be carried out due to security concerns from 1999 to the present, despite proper planning, etc.

Table 2. Planned and implemented silvicultural works according to the Annual Business Program in SE "Srbijašume" for the period 2014-2023

Year	Auxiliary measures			Natural and artificial regeneration			Establishment of new forests (afforestation)			Forest care measures			Total silvicultural works		
	plan	execution		plan	execution		plan	execution		plan	execution		plan	execution	
	ha	ha	%	ha	ha	%	ha	ha	%	ha	ha	%	ha	ha	%
2014	1,241	1,133	91	10,173	9,075	89	124	128	103	32,645	32,510	100	44,183	42,846	97
2015	1,467	1,451	99	9,564	9,819	103	97	188	195	35,037	33,607	96	46,165	45,066	98
2016	1,316	1,292	98	9,486	9,707	102	137	130	95	33,279	31,987	96	44,217	43,117	98
2017	1,469	1,248	85	10,812	10,285	95	207	219	105	34,662	32,010	92	47,149	43,762	93
2018	1,667	1,388	83	11,022	10,664	97	381	270	71	34,622	33,395	96	47,693	45,718	96
2019	2,243	1,548	69	12,098	11,742	97	336	279	83	33,058	31,347	95	47,734	44,916	94
2020	2,149	2,119	99	12,073	12,137	101	209	193	93	33,763	33,862	100	48,194	48,312	100
2021	2,570	2,581	100	12,705	11,805	93	258	215	83	32,801	30,664	93	48,334	45,266	94
2022	1,831	1,661	91	12,672	12,400	98	317	264	83	33,500	31,811	95	48,320	46,135	95
2023	1,761	1,710	97	12,886	13,356	104	250	228	91	29,736	29,003	98	44,633	44,297	99
Sum	**17,713**	**16,131**	**91**	**113,491**	**110,992**	**98**	**2,316**	**2,114**	**91**	**333,102**	**320,197**	**96**	**466,622**	**449,434**	**96**

The private owner's forests in the Republic of Serbia (in the part covered by SE "Srbijašume") cover 1,222,624 ha (61% of the total forest area). The volume in private owner's forests is about 139.8 million m^3 (51% of the total volume in the area covered by SE "Srbijašume"), while the annual volume increment is about 3.4 million m^3 (50% of the total volume increment in this area). The state enterprise for forest management "Srbijašume" provides professional advisory services for private forest owners on behalf of the Republic of Serbia. These forests are managed based on the forest area development plan and the forest management program (Article 20 of the Law on Forests, "Official Gazette of RS", No. 30/10, 93/12, 89/15, and 95/18 - other law).

The forest area development plan is adopted for a 10-year period and encompasses all the forests within a single forest area. Forest management programs are also valid for 10 years, and are adopted for forests owned by natural persons (private forests owned by natural persons) in the territory of one or more municipalities. Until the adoption of the forest management program, these forests are managed based on the temporary forest management programs, which are adopted by the forest user, and approved by the Ministry of Agriculture, Forestry and Water Economy (Article 118 of the Law on Forests). When it comes to the aforementioned planning documents, it should be noted that there are currently no valid forest development plans, while the vast majority of forest management programs have expired, which can be assessed as an unacceptable planning circumstance.

The temporary forest management program serves as a substitute for the missing forest management program that was supposed to provide an overview of the current state of the forests, define management goals, outline measures to achieve these goals and determine the extent of silvicultural work and forest utilization. These temporary programs are drafted for planned municipalities as an interim solution. Implementation of the silvicultural plan in the forests of private owners (natural persons) for the period 2014-2023 is shown in Table 3.

Table 3. Execution of silvicultural plan in the forests of private owners for the period 2014-2023

Year	**Natural regeneration**			**Artificial regeneration**			**Forest care measures**			**Total**		
	Annual plan	**Execution**	**%**	**Ann. plan**	**Exec.**	**%**	**Ann. plan**	**Exec.**	**%**	**Ann. plan**	**Exec.**	**%**
2014	2,376	893	38	928	366	39	71,228	27,030	38	74,532	28,289	38
2015	2,300	906	39	863	264	31	70,321	25,685	37	73,484	26,855	37
2016	2,481	621	25	758	179	24	71,097	31,808	45	74,336	32,608	44
2017	2,483	599	24	753	65	9	71,072	16,796	24	74,308	17,460	23
2018	2,656	1,774	67	698	46	7	71,831	31,410	44	75,185	33,230	44
2019	2,467	2,037	83	711	83	12	74,070	25,697	35	77,248	27,817	36
2020	1,799	1,317	73	704	25	4	68,486	19,709	29	70,989	21,051	30
2021	1,846	1,410	76	718	10	1	62,725	30,219	48	65,289	31,639	48
2022	2,426	1,984	82	704	9	1	55,997	24,662	44	59,127	26,655	45
2023	2,239	1,436	64	722	1	0.1	57,124	16,623	29	60,085	18,060	30
Total	**23,073**	**12,977**	**56**	**7,559**	**1,048**	**14**	**673,951**	**249,639**	**37**	**704,583**	**263,664**	**37**
Average	**2,307**	**1,298**	**56**	**756**	**105**	**14**	**67,395**	**24,964**	**37**	**70,458**	**26,367**	**37**

The basic goal of forestry plans in private forests is to improve the condition of existing forests and increase the area under the forest (with the consent of the forest owner).

The ratio of planned and executed afforestation works in private forests in Serbia is unsatisfactory, which hinders the fulfillment of the set goal.

It is important to note that, in addition to the reasons mentioned earlier that affect the implementation of plans in state forests managed by SE "Srbijašume", the implementation of plans in private forests is primarily affected by various limiting factors that result in little interest from the forest owners in carrying out silvicultural works. The main limiting factors for sustainable forest management by private owners in the area of central Serbia and the entire Republic of Serbia are (Vasić et al., 2023):

- small property;
- unfavorable property structure (multiple small surfaces that are distant from each other);
- a large number of owners;
- unresolved property and legal relations;
- incompatibility of cadastre data to the actual situation on the terrain;
- absence of strategic and operational management planning documents;
- recognition and appreciation by the owner exclusively produce forest functions, which gives management a monofunctional character and which, in some situations, leads to excessive use, etc.

The above is in agreement with the statements of Baković (2018), according to which "The level of ecological knowledge and ecological awareness in Serbia is insufficient, but it is constantly increasing. Based on the same source, increasing ecological awareness is generally beneficial for forest ecosystems and society overall. This calls for a new approach to forest management planning, a fresh perspective on forest utilization plans, silvicultural plans, and the implementation of new technologies for establishing new forests (such as sowing, planting, etc.).

Conclusions

The overall planning and execution of silvicultural works in state forests, following the model of SE "Srbijašume" in Serbia, can be described as very effective. This is evidenced by the fact that the average completion rate of these works compared to the planned over the past 10 years has been 96%. SE "Srbijašume" plans and executes silvicultural works comprehensively through 85 types of activities. These activities are used to create the annual business program and monitor their execution. This includes analyzing the dynamics of execution and conducting economic and financial cost analysis, which involves 55 types of activities. To create annual silvicultural plans, SE "Srbijašume" prepares Silvicultural Guidelines, which ensures that annual forestry plans are prepared uniformly.

Forests owned by private individuals generally have lower quality compared to state-owned forests, with a significant decrease in the implementation of silvicultural works, only 37% in the last 10 years. This becomes crucial when comparing the area of private forests, which amounts to 1,196,583 ha (as of 31/12/2023), where SE "Srbijašume" provides expert-advisory services, with respect to 775,474 ha of state forests managed by the organization.

One of the main reasons for the incomplete execution of planned silvicultural work each year is the shortage of skilled labor, uncertainty, and training. This leads to the need to provide labor for fieldwork, as well as consistent funding for silvicultural works and further investment in innovation. To successfully carry out the planned silvicultural works, it is essential to prioritize the allocation of human and financial resources. This includes securing a skilled labor force and implementing various initiatives (such as incentives and awareness-raising programs for forest owners) to boost interest and participation in these activities within private forests. Also, the incomplete realization of the planned works on silviculture on

an annual level is, to a certain extent, affected by climate changes and the safety circumstances of employees along the Administrative Line to AP KiM and others.
The lack of strategic planning documents such as forest development program for the Republic of Serbia, forest area development plans, and forest management programs for private owners, makes it difficult to set clear directions for the development of silvicultural plans at the national level. This also makes it challenging to manage forests, regardless of ownership. The absence of by-laws following the Law on Forests hinders a systematic approach to the development of forestry plans, and especially its implementation.
Considering the importance of the missing bylaws in forest management, as well as the absence of strategic and operational level planning documents, these should be given full priority.
Recognizing the importance which silviculture has on sustainable forest management, nature protection and environmental protection, absolute priority in the Republic of Serbia should be given to elimination of the aforementioned risks for the full implementation of the planned works.

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SILVOPASTURE SYSTEM COMMUNITY LIVESTOCK PERMIT HOLDERS PERCEPTIONS AND CHALLENGES IN THE LIMPOPO PROVINCE, SOUTH AFRICA

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Abstract

A silvopasture system integrates trees, forages, livestock and related animals and plant species on the same land management unit(s). However, limited understanding, incorrect information, and a negative mindset could hinder its competitiveness. Hence, the study was aimed to document the perceptions of the constraints to silvopasture system competitiveness. The major objectives were: (1) to identify and describe the socio-economic characteristics of the selected SSCLPH. (2) to determine the perceptions and challenges among SSCLPH. Quantitative and qualitative designs were used as a questionnaire and stakeholder discussion and field observations were part of the data collection. A purposive and snowball sampling techniques were used to select 59 SSCLPH from 12 villages. The results indicated that an insufficient source of water was perceived by all as not the most important factor causing a decrease in silvopasture system competitiveness. This is mainly due to the plantation area having high rainfall hence the communities indicated that they moved their livestock to the plantation area due to its good climate. Furthermore, infrastructure was perceived by all as the most important factor causing a decrease in silvopasture system competitiveness. The SSCLPH indicated that in some parts of the plantation, there was no fencing or fencing was damaged hence poaching and accidents occur. Some SSCLPH (68%) further emphasized that they did not have any market challenges as their grass-fed livestock were in demand in their areas. However, all SSCLPH agreed that crime did cause a decrease in silvopasture system competitiveness, as poaching and infrastructure (fencing) were identified as their main challenges. In conclusion, the identified perceptions and challenges are in line with some of the researcher's field observations, and it is thus recommended that stakeholders should take note of the perceptions identified by the SSCLPH in an attempt to increase silvopasture system competitiveness in South Africa.

Keywords: *Silvopasture System, Agroforestry, Perceptions and Challenges, Limpopo Province, South Africa.*

Introduction

According to (Pattanayak *et al.,* 2003) in most parts of the world, agroforestry adoption has proceeded slowly despite apparent benefits. This has prompted significant research focusing on the factors that affect adoption. Studies have shown that, in addition to market, biological, and demographic factors, farmers' subjective perceptions of a technology play a key role in agricultural technology adoption (Adesina *et al.,*1995, Ajayi, 2007, Khan *et al.,* 2008). Their experiences and social networks can in turn, influence adopters' perceptions with other people (Kearns, 1992, German *et al.*, 2006). In addition, farmers' perceptions over time of an agroforestry technology can have an important impact on adoption and disadoption. A silvopasture system integrates trees, forages, livestock and related animals and plant species on the same land management unit (s). Hence, growing livestock in commercial plantations provides several benefits such as extra fertilization or weeding and diversifying the income.

However, limited understanding, incorrect information, and a negative mindset could hinder the competitiveness of this practice. Furthermore, a study by Shrestha *et al.,* (2004) indicated that the silvopasture system had the strengths and opportunities of providing a strong sense of stewardship and satisfaction to adopters, aiding in environmental protection, and helping to diversify income. This case study of the silvopasture system community livestock permit holders (SSCLPH) attempts to explain the constraints to silvopasture system competitiveness by analyzing their perceptions and challenges. Hence, the study was aimed to document the perceptions of the constraints to silvopasture system competitiveness in Limpopo Province. The major objectives were: (1) to identify and describe the socio-economic characteristics of the selected SSCLPH and (2) to determine the perceptions and challenges among SSCLPH.

Material and Methods

Study Area

The location of Vhembe District in the Limpopo Province is situated in the far north of the South African borders. Vhembe district borders Zimbabwe in the north and Mozambique in the east. Agriculture in the Vhembe district is one of the main economic drivers that contribute to the whole province and the nation at large. Various studies indicated that approximately 90% of rural communities found in the Vhembe district (South Africa) depend mainly on agriculture to sustain their livelihoods and generate income (VDM, 2014). In addition, Vhembe district’s land is primarily used for grazing (VDM, 2023). According to VDM (2023), the district has 23 commercial forestry companies with 23 203 planted hectares which are composed of 7 173 ha of gum and 15 066 ha of pine species. The study area, Entabeni plantation covers an area of 11 177 ha, equivalent to 50 % of the commercial forestry area in Vhembe district. There are 34 small timber growers specializing in pine and eucalyptus trees, with the average land under plantation of 259 ha (VDM, 2023). The estimated yield of commercial plantations is 238 9909 tons while for small timber growers is 26 780 tons (VDM, 2023). In addition, 59 SSCLPH from 12 villages participated in the study and were spread on the SAFCOL Entabeni plantation as indicated in Figure 1.

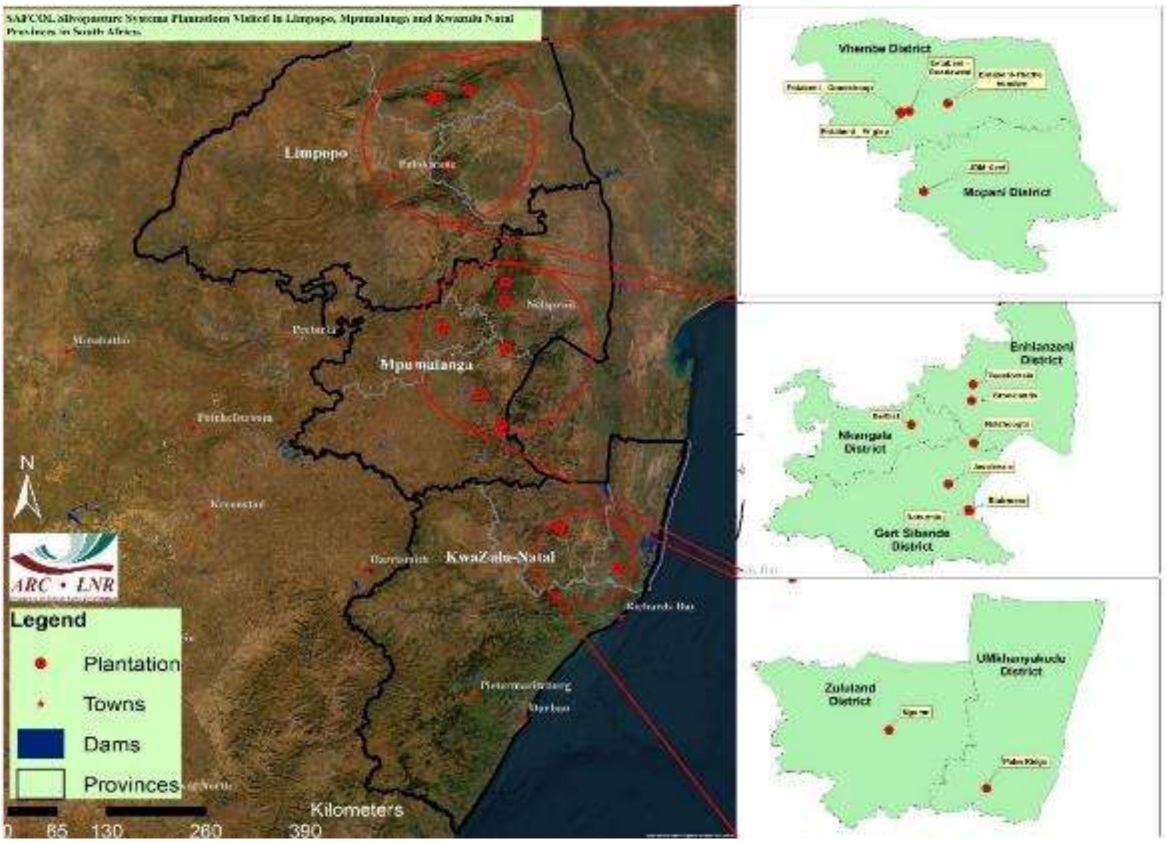

Figure 1: Silvopasture System Community Livestock Permit Holders Locations (ARC – NRE, 2023)

Study Design

The research employed both qualitative and quantitative methods concurrently and this was applied with the aim on establishing the limitations, balance and strength of the data. Furthermore, the methods included participatory action research as the community growers and stakeholders benefitted while the research was ongoing. Data collection methods were via tele-interviews, site observations, past research, web and governmental reports. Pre and post-intervention questionnaire was developed and pilot-tested with researchers working on community development within the Agricultural Research Council (ARC). A closed and open-ended questionnaire with the following sections was used: Socio-economic, food security, sustainability, perceptions, market information and observations. Closed-ended questions provide a question immediately and ask participants to choose from a list of possible responses and are quantitative, allowing the researcher to gather numerical data for statistical analysis. Open-ended questions alternatively are those that provide participants with an allowance to construct their response about the subject matter. The latter will include focus group discussions and field observations and it took a maximum of 20 minutes to interview each community grower. The ARC & SAFCOL team conducted face-to-face interviews with the same 59 SSCLPH. The 59 SSCLPH were interviewed in their native language for better understanding. Agricultural Research Council (ARC) technicians were recruited and trained on the PAR (Participatory Action Research) approach and data collection and analysis.

Sampling Procedure and Analytical Technique

Purposive and snowball sampling techniques were used on selected 59-silvopasture system community livestock permit holders in the Vhembe. These SSCLPH were spread on the South African Forestry Company Limited (SAFCOL) land and each SSCLPH was allocated an area for livestock grazing. The list of SSCLPH was supplied by SAFCOL and the sample size was agreed upon with the stakeholders. Furthermore, data collected was analyzed quantitatively using the Statistical Package for Social Sciences (SPSS) Windows version 21. A descriptive analysis was conducted.

Results and Discussion

Silvopasture System Livestock Community Permit Holders Perceptions and Challenges

Perceptions were asked on seven factors namely: (1) Production (2) Demand (3) Related & Supporting Industries (4) Government Support (5) Organisational Strategy, Structure & Rivalry (6) Market and (7) Chance. The results indicated that the production factors (Table 1) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH disagreed (68%, 66% and 62% respectively). Among the thirteen factors of production: An insufficient source of water (100%) was perceived by all as not the most important factor causing a decrease in silvopasture system competitiveness. This is mainly due to the plantation area's high rainfall (+750mm per annum) hence the SSCLPH indicated that they moved their livestock to the plantation area due to its good climate. However, quite several SSCLPH perceived production factors as causing a decrease in silvopasture system competitiveness (32%, 34% and 38% respectively). Among the thirteen factors of production: Infrastructure (100%) was perceived by all as the most important factor causing a decrease in silvopasture system competitiveness. The SSCLPH indicated that in some parts of the plantation, there is no fencing or fencing is damaged hence poaching and accidents occur. This situation is in line with Calle (2008) who emphasised that the primary barriers to silvopasture adoption noted by the adopters were the high cost of establishment and the lack of information and knowledge about the system.

Table 1. Production factors causing a decrease/no decrease in silvopasture system competitiveness

Views The following production factors are causing the decrease in silvopasture system competitiveness	**Responses (59 silvopasture system permit holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree	%
Cost of production	-	66%	-	34%	-	100%
Labour	-	62%	-	38%	-	100%
Cost of unskilled labour	-	68%	-	32%	-	100%
Quality of unskilled labour	-	68%	-	32%	-	100%
Availability of unskilled labour	-	68%	-	32%	-	100%
Cost of skilled labour	-	68%	-	32%	-	100%
Availability of skilled labour	-	68%	-	32%	-	100%
Administration cost associated with labour matters	-	68%	-	32%	-	100%
Insufficient source of water	-	100%	-		-	100%
Infrastructure	-		-	100%	-	100%
Lack of knowledge	-	68%	-	32%	-	100%
Lack of Technology	-	68%	-	32%	-	100%
Capital / Finance	-	68%	-	32%	-	100%

The results indicated that the demand factors (Table 2) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH strongly disagreed (68%, 66% and 64% respectively). Among the five demand factors: Distance to market (68%), Market information (68%) and Market for silvopasture (68%) were perceived as not the most important factors causing a decrease in silvopasture system competitiveness. Some SSCLPH emphasized that they do not have any market challenges as their grass-fed livestock are in demand in their areas. However, quite several SSCLPH perceived demand factors as causing a decrease in silvopasture system competitiveness (32%, 34% and 2% respectively).

Table 2. Demand factors causing decrease/no decrease in silvopasture system competitiveness

Views The following demand conditions are causing the decrease in silvopasture system competitiveness	**Responses (59 Silvopasture System Permit Holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree	%
Distance to market	68%	-	-	32%	-	100%
Market information	68%	-	-	32%	-	100%
Cost to the market	64%	-	-	34%	2%	100%
Quality of products	66%	-	-	32%	2%	100%
Market for Silvopasture	68%	-	-	32%	-	100%

The results indicated that the related and supporting industries factors (Table 3) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH strongly disagreed (68% and 64% respectively). Among the four related and supporting industries factors: Financial institutions (68%) and Research institutions (68%) were perceived as not the most important factors causing a decrease in silvopasture system competitiveness. Several SSCLPH indicated that they were receiving research assistance from the Department of Forestry, Fisheries and Environment (DFFE), the University of Venda (UNIVEN) and the Agricultural Research Council (ARC). However, quite several SSCLPH perceived related and supporting industries factors as causing a decrease in silvopasture system competitiveness (32%). Only 4% of the SSCLPH were not sure. According to Maponya et al. (2023) several silvopasture adopters also lamented the lack of support from agricultural institutions, the amount of labour involved in managing silvopasture, and tree mortality.

Table 3. Related and supporting industries causing decrease/no decrease in silvopasture system competitiveness

Views The following related and supporting industries are causing the decrease in silvopasture system competitiveness	**Responses (59 Silvopasture System Permit Holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree	%
Financial institutions	68%	-	-	32%	-	100%
Research institutions	68%	-	-	32%	-	100%
Suppliers	64%	-	4%	32%	-	100%
Electricity suppliers	64%	-	4%	32%	-	100%

The results indicated that government factors (Table 4) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH disagreed (68% and 66% respectively). However, quite several SSCLPH perceived government factors as causing a decrease in silvopasture system competitiveness (30% and 32% respectively). Among the six government factors: Land reform policy (100%) was perceived by all as the most important factor causing a decrease in silvopasture system competitiveness. This is not surprising as the

SSCLPH indicated that they were constrained by the unavailability of land in their villages hence the plantations offered more land for their livestock to graze.

Table 4. Government support causing decrease/no decrease in silvopasture system competitiveness

Views The following production factors are causing the decrease in silvopasture system competitiveness	**Responses (59 Silvopasture System Permit Holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree	%
Poor interaction and support between the Government	68%	-	2%	30%	-	100%
Indirect support	68%	-	-	32%	-	100%
Trade policy	68%	-	-	32%	-	100%
Land reform policy		-	-	100%	-	100%
Labour policy	66%	-	2%	32%	-	100%
Fiscal policy	68%	-	-	32%	-	100%

The results indicated that the related and supporting industries factors (Table 5) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH strongly disagreed (68% and 66% respectively). Among the five organization strategies, structure & rivalry factors: Adaptability (68%), Culture (68%) and Structure (68%) were perceived as not the most important factors causing a decrease in silvopasture system competitiveness. However, quite several SSCLPH perceived related and supporting industries factors as causing a decrease in silvopasture system competitiveness (32 and 34%). Only 2% of the SSCLPH were not sure.

Table 5. Organisation strategy, structure & rivalry causing decrease/ no decrease in silvopasture system competitiveness

Views The following production factors are causing the decrease in silvopasture system competitiveness	**Responses (59 Silvopasture System Permit Holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongly agree	%
Adaptability	-	68%	-	32%	-	100%
Culture	-	68%	-	32%	-	100%
Structure	-	68%	-	32%	-	100%
Flexibility	-	66%	2%	32%	-	100%
Pricing strategy	-	66%	2%	34%	-	100%

The results indicated that the market factors (Table 6) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH disagreed (66%). All four market factors: Market power of suppliers (66%), Market power of buyers (66%), Threat of substitutes (66%) and Threat of new substitutes (66%) were perceived as not the most important factors causing a decrease in silvopasture system competitiveness. However, quite several SSCLPH perceived market factors as causing a decrease in silvopasture system competitiveness (32% and 28% respectively). Furthermore, a few SSCLPH (2% and 6%) were not sure about their perception.

Table 6. Market causing decrease/no decrease in silvopasture competitiveness

Views The following production factors are causing the decrease in silvopasture system competitiveness	**Responses (59 Silvopasture System Permit Holders)**					
	Strongly disagree	Disagree	Not sure	Agree	Strongl y agree	%
Market power of suppliers	-	66%	2%	32%	-	100%
Market power of buyers	-	66%	2%	32%	-	100%
Threat of substitutes	-	66%	2%	32%	-	100%
Threat of new substitutes	-	66%	6%	28%	-	100%

The results indicated that chance factors (Table 7) do not cause a decrease in silvopasture system competitiveness as the majority of the SSCLPH disagreed (68%, 66% and 62% respectively). Among the nine factors of chance: Frost (100%) was perceived by all as not the most important factor causing a decrease in silvopasture system competitiveness. In addition, all SSCLPH agreed that crime and drought do cause a decrease in silvopasture system competitiveness. This is in line with the results presented in Table 7 that poaching is a challenge as the result of lack of fencing and again they moved to the plantation area due to its good rainfall as compared to their villages, which are dry (Maponya et al., 2021).

Table 7. Chance causing decrease/no decrease in silvopasture system competitiveness

Views	**Responses (59 Silvopasture System Permit Holders)**					
The following production factors are causing the decrease in silvopasture system competitiveness	Strongly disagree	Disagree	Not sure	Agree	Strongl y agree	%
Economic stability	-	68%	-	32%	-	100%
Aids	-	68%	2%	30%	-	100%
Political stability	-	64%		36%	-	100%
Price stability	-	68%		32%	-	100%
Crime	-	-	-	-	100%	100%
Drought	-	-	-	-	100%	100%
Floods	-	68%	10%	22%	-	100%
Fires	-	68%	12%	20%	-	100%
Frost	-	100%	-	-	-	100%

Selected Silvopasture System Site Visits Pictures

Picture 1: Silvopasture System Visited in Mpumalanga Province, South Africa

Picture 2: Silvopasture System Visited in Limpopo Province, South Africa

Picture 3: Silvopasture System Visited in Limpopo Province, South Africa

Conclusion and Recommendations

The Sustainable Development Goals encouraged all countries to address 17 social, environmental, and economic goals that promote prosperity while protecting the planet and silvopasture system can support the attainment of these goals. The study highlighted that the promotion of silvopasture system is important because it offers the prospect of increasing production and hence raising the community income and food security. Recognizing and tackling main perceptions and factors that determine the competitiveness of the communities in silvopasture practices are relevant to the adoption involving economic as well as sociological considerations. In conclusion, identified community perceptions namely production factors, demand, related, supporting industries, government support, firm strategy, structure, rivalry, market, and chance are in line with some of the researcher field observations and it is thus recommended that stakeholders should take note of the perceptions identified by the SSCLPH in an attempt to increase silvopasture system competitiveness in South Africa.

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SILVOPASTURE SYSTEM COMMUNITY LIVESTOCK PERMIT HOLDERS FOOD SECURITY STATUS IN THE LIMPOPO PROVINCE, SOUTH AFRICA

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Abstract

Agroforestry is a land use system that includes the use of woody perennial and agricultural crops and animals in combination to achieve beneficial ecological and economical interactions for food, fiber and livestock production. Furthermore, a silvopasture system integrates trees, forages, livestock and related animals and plant species on the same land management unit(s). Hence, growing livestock in commercial plantations provides several benefits such as extra fertilization or weeding and diversifying the income. However, some researchers emphasized that the obstacle to the local development of the silvopasture system is the lack of scientific data. In the present study, research was conducted with the overall aim to determine the food security status of the silvopasture system community livestock permit holders. The major objectives were: (1) to identify and describe the socio-economic characteristics of the selected silvopasture system community livestock permit holders (SSCLPH). (2) to determine the food security status and food insecurity levels among SSCLPH. Quantitative and qualitative designs were used as a questionnaire written in English, stakeholder discussion and field observations were part of the data collection. A purposive and snowball sampling techniques were used on selected 59-silvopasture system community livestock permit holders from 12 villages in the Vhembe district. These SSCLPH were spread on the South African Forestry Company Limited (SAFCOL) Entabeni plantation and each SSCLPH was allocated an area for livestock grazing. The study also employed the following food security indicators: Food Availability, Food Accessibility and Food Diversity. The socio-economic data were also coded, captured and analyzed using Statistical Package for Social Science (SPSS version 21). The SSCLPH indicated that they received income from various sources: social grants (22%), agriculture (20%), business (31%) and employment (27%). In addition, there are 761 cattle costing R15000 – R20000 each and 44 goats costing R1500 – R3000 each grazing in the plantation. Hence, in terms of food security and food insecurity levels: 68% of SSCLPH were food secure and 32% were mildly food insecure. In conclusion, the SSCLPH indicated that they could sustain their livelihoods as the land allocated and provided by SAFCOL enabled their livestock to graze, hence they could also access livestock products (manure, milk and meat etc.) and sell livestock in the informal and formal markets. It is thus recommended that the silvopasture system should be adopted and promoted throughout South Africa.

Keywords: *Silvopasture System, Agroforestry, Food Security, Limpopo Province, South Africa.*

Introduction

Agroforestry is a land use system that includes the use of woody perennial and agricultural crops and animals in combination to achieve beneficial ecological and economical interactions for food, fiber and livestock production. A properly managed agroforestry system provides multiple benefits and contributes to improved livelihoods and income generation.

Agroforestry systems are also area and climate-specific hence, it is key to develop locally relevant agroforestry systems and consider the biophysical and socio-economic context on a case-by-case basis. South Africa is known as a semi–arid country vulnerable to water stress, particularly drought. A silvopasture system integrates trees, forages, livestock and related animals and plant species on the same land management unit(s). Furthermore, according to Jose *et al.,* (2019), silvopasture, has gained popularity worldwide in recent years as an environmentally friendly alternative land use system that is economically viable. According to Davila–Solarte *et al.,* (2019) growing livestock in commercial plantations provides several benefits such as extra fertilization, reduction of wildfire risks, weeding and diversification of income. However, Leal *et al.,* (2019) emphasized that the obstacle to the local development of silvopasture is the lack of scientific data. The researchers observed that most aspects of the silvopasture system were studied well, but the climate change and social dimensions would require more research efforts to address the emerging challenges.
In addition, the benefits of the silvopasture system include the production of high-value timber products in the long term while obtaining short-term economic benefits from the livestock and forage components. The silvopasture system is also area and climate-specific hence, it is key to develop the locally relevant systems and consider the biophysical and socio-economic context on a case-by-case basis. This created a need for South African Forestry Company Limited (SAFCOL) to assist in addressing some of their neighbouring communities’ socio-economic challenges; hence land was allocated to nearby communities to engage in the silvopasture system. In addition to the biophysical consideration, soil health influences the quality and nutrient status of the grass-fed livestock and mulch from fallen tree branches, leaves and livestock manure contribute to soil health by enriching the soil with organic matter. So, grass grown under a silvopasture system with noble nutrient status will improve the quality of cattle feeding under the system. Furthermore, this grass-fed livestock attracts good prices at the market as compared to grain-fed livestock. This will be beneficial to the communities whose livestock are feeding in the plantations as it will provide a good income source and allow them to be food secure.
In the present study, research was conducted with the overall aim to determine the food security status of the SSCLPH. The major objectives were: (1) to identify and describe the socio-economic characteristics of the selected SSCLPH and (2) to determine the food security status and food insecurity levels among SSCLPH.

Material and Methods

Study Area

The location of Vhembe District in the Limpopo Province is situated in the far north of the South African borders. Vhembe district borders Zimbabwe in the north and Mozambique in the east. Agriculture in the Vhembe district is one of the main economic drivers that contribute to the whole province and the nation at large. Various studies indicated that approximately 90% of rural communities found in the Vhembe district depend mainly on agriculture to sustain their livelihoods and generate income (Vhembe District Municipality (VDM), 2014). In addition, Vhembe district’s land is primarily used for grazing (VDM, 2023). According to VDM (2023), the district has 23 commercial forestry companies with 23 203 planted hectares which are composed of 7 173 ha of gum and 15 066 ha of pine species. The study area, Entabeni plantation covers an area of 11 177 ha, equivalent to 50 % of the commercial forestry area in Vhembe district. There are 34 small timber growers specializing in pine and eucalyptus trees, with the average land under plantation of 259 ha (VDM, 2023). In addition, 59 silvopasture system community livestock permit holders participated in the study and were spread on the SAFCOL Entabeni plantation as indicated in Figure 1. Furthermore, SSCLPH were from 12 villages.

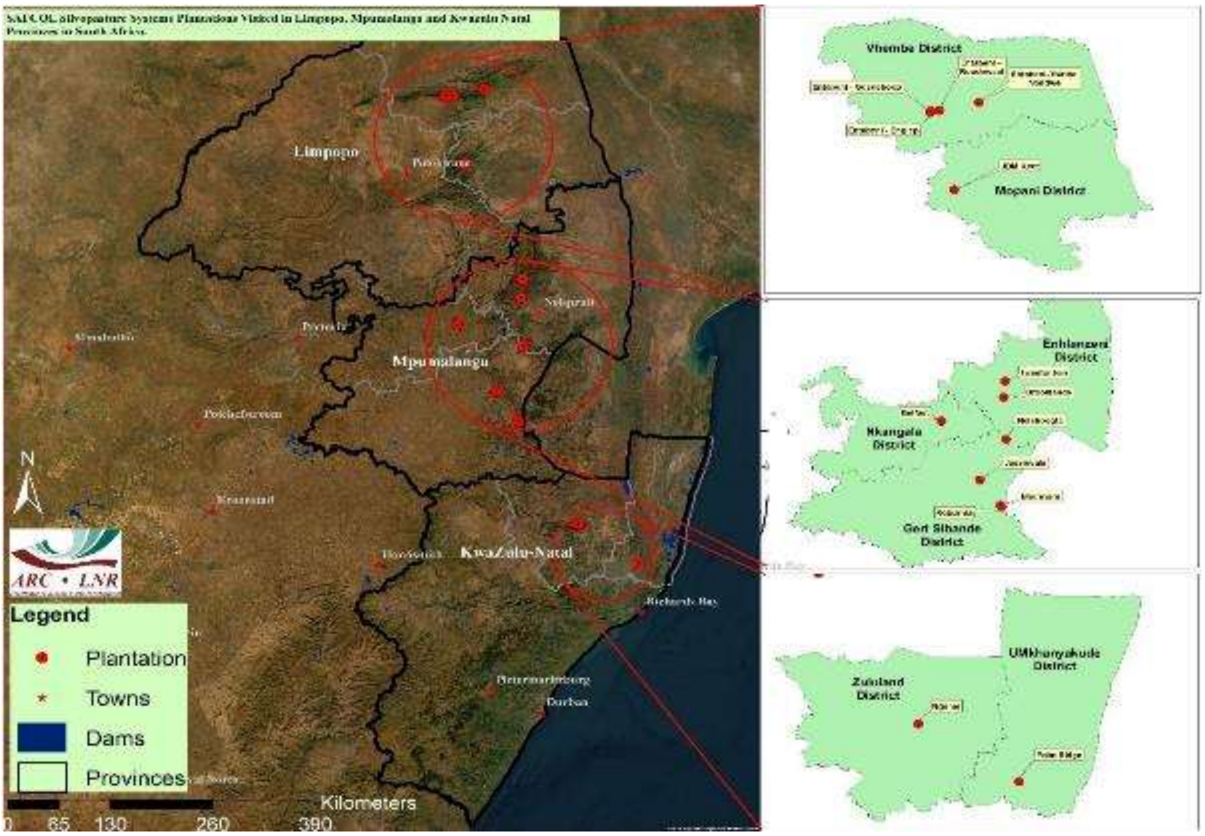

Figure 1: Silvopasture System Community Livestock Permit Holders Locations (ARC – NRE, 2023)

Study Design

The research employed both qualitative and quantitative methods concurrently and this was applied with the aim of establishing the limitations, balance and strength of the data. Furthermore, the methods included the participatory action research approach as the community growers and stakeholders benefitted while the research was ongoing. Data collection methods were via tele-interviews, site observations, past research, web and governmental reports. Pre and post-intervention questionnaire was developed and pilot-tested with researchers working on community development within the Agricultural Research Council (ARC). A closed and open-ended questionnaire with the following sections was used: Socio-economic, food security, sustainability, perceptions, market information and observations. Closed-ended questions provide a question immediately following a response and ask participants to choose from a list of possible responses and are quantitative, allowing the researcher to gather numerical data for statistical analysis. Open-ended questions alternatively are those that provide participants with an allowance to construct their response about the subject matter. The latter included focus group discussions and field observations. The ARC & SAFCOL team conducted face-to-face interviews with the same 59 community growers and it took a maximum of 20 minutes to interview each community grower. The 59 community growers were interviewed in their native language for better understanding. Agricultural Research Council (ARC) technicians were recruited and trained on the PAR (Participatory Action Research) approach, data collection and analysis.

Sampling Procedure and Analytical Technique

Purposive and snowball sampling techniques were used on selected 59-silvopasture system community livestock permit holders in the Vhembe District. These SSCLPH were spread on the South African Forestry Company Limited (SAFCOL) land and each SSCLPH was allocated an area for livestock grazing. The list of SSCLPH was supplied by SAFCOL and the sample size was agreed upon with the stakeholders. Furthermore, data collected was analyzed quantitatively using the Statistical Package for Social Sciences (SPSS) Windows version 21. A descriptive analysis was conducted.

Food Security Approach

The study employed the following food security indicators: Food Availability, Food Accessibility and Food Diversity. The community growers were also categorized as follows: (1) **Food secure***:* Community growers did not worry about food access; they rarely experienced anxiety about not having enough food. These are community growers that were able to have a full meal three times in a day without food running out, in the past 30 days. (2) **Mildly food insecure:** Community growers were anxious about not having sufficient food. They usually consume an inadequate diet or eat food that they do not prefer. These community growers experienced food insecurity once or twice in the past 30 days (3) **Moderately food insecure:** Community growers began sacrificing quality continuously by consuming inadequate diet and eating less preferred food. They started reducing the quality of food intake by decreasing meal sizes. These community growers experienced food insecurity three to ten times in the past 30 days (4) **Severe food insecure:** Community growers experienced high incidences of food insecurity. The condition of reducing meal sizes and the number of meals worsened each day. The three most severe conditions of going a whole day without eating, going to bed hungry or running out of food in the past 30 days occurred 'often'. These community growers experienced food insecurity more than ten times in the past 30 days.

Results and Discussions

Selected Silvopasture System Livestock Community Permit Holders Socio-Economic Characteristics

The majority of SSCLPH interviewed were male (83%) and 17% were women. In terms of educational attainment, 31% of permit holders had no matric and 25% of SSCLPH had matric education, and 44% of permit holders had tertiary education. In addition, the SSCLPH indicated that for agroforestry practice they relied mostly on their indigenous knowledge system (IKS). Results on land acquisition indicated that the permit holders were allocated land by SAFCOL for livestock grazing. The age distribution of the growers indicated that the majority were in the age group of >56 (83%). There were 0% of youth involvement, 36 – 45 (7%) while 46 – 55 had 10%. This situation is worrisome and indicates the urgent need to attract the young generation into agroforestry as an important priority. The SSCLPH experience is spread across: 1 – 5 years (9%); 6 – 10 years (39%); 11 – 20 years (32%) and 21 – 49 years (20%). The results also showed the SSCLPH household size: About 81% of SSCLPH had 1-5 members and 19% of SSCLPH had 6 – 10 members. A total of 97 % of the SSCLPH were married as compared to 3% who were single. The SSCLPH indicated that they all received income from various sources: social grants (22%), agriculture (20%), business (31%) and employment (27%). Most of the permit holders' income levels are more than

R2501 and their expenditures (Electricity, Transport, Clothes and Food) were more than R601 except for water at R200.

Silvopasture system community livestock permit holders food security status

In terms of food accessibility: Most SSCLPH indicated that they could sustain their livelihoods as the land allocated and provided by South Africa Forestry Company Limited (SAFCOL) enabled their livestock to graze. For instance, it was rare for the permit holders or their household members to go to sleep at night hungry because there was not enough food. And it was rare for the SSCLPH or household members to go a whole day and night without eating anything because there was not enough food. The SSCLPH indicated that they received income from various sources: social grants (22%), agriculture (20%), business (31%) and employment (27%). In addition, there are 761 cattle costing R15000 – R20000 each and 44 goats costing R1500 – R3000 each grazing in the plantation. Hence, in terms of food security and food insecurity levels: 68% of SSCLPH were food secure and 32% were mildly food insecure (Table 1). This food security approach is in line with the study conducted in the Limpopo and Mpumalanga Provinces, South Africa by Maponya *et al.,* (2022), which addressed the food security index indicators; (1) Availability (Sufficient) (2) Accessibility (Physical, Social & Economic Access) (3) Utilisation (Dietary Needs, Safe & Nutritious) (4) Stability (Short Term) (5) Sustainability (Environmental, Social & Economic) (6) Agency (People & Food Preferences).The study used standard questions to ask participants to address food security status (percentage food secure/insecure) and levels (Severe, Mild & Moderate).

Table 1. Silvopasture system community livestock permit holder food security status and extent of food insecurity

Variable	**Category**	**Community Grower (s)**	**%**
Food Security Level	Food Secure	40	68%
	Food Insecure	19	32%
Extent of Food Insecurity	Mild	19	32%
	Moderate	0	0%
	Severe	0	0%

In terms of food availability: Most of the SSCLPH (40 Never and 19 Sometimes) indicated that they do not run out of food before they get money to buy more. Most of the SSCLPH can buy or have enough food and they are often not hungry (40 never). Their children are also getting enough to eat and do not go to bed hungry (59). The SSCLPH can often and always eat enough every day. **In terms of food diversity**, all the SSCLPH (59) have access to the following food groups: cereals, white tubers and roots, vitamin A-rich vegetables, fruit, dark green leafy vegetables, other vegetables, legumes, meat and fish, eggs, dairy, oil and fat, sugar, and spices, condiments and beverages. The Household Dietary Diversity Score (HDDS) reflects the number of different food groups consumed by the community groups. The community growers were asked whether any household member consumed a food item of one of the nine predefined food groups at least once in the last 7 days. This included consumption of the food item at home or home-prepared but consumed outside the home. The nine food groups are (Labadarios *et al.,* 2009): cereals and tubers, vitamin A-rich vegetables and fruit, other vegetables and fruit, legumes, meat and fish, eggs, dairy, oil and fat, sugar, and beverages. These food groups should reflect the combination of nutritional needs for a healthy diet. This dietary diversity score can be used to assess changes in diet before and after intervention.

Conclusion and Recommendations

The study findings highlighted and concluded that the silvopasture system can bridge the gap that often separates agriculture and forestry by building integrated systems that address both environmental and socio-economic objectives, income generation, food security and market access. The study indicated that rainfall in the study area is well above the agriculture and forestry threshold to sustain commercial forestry activities (+750mm per annum). Hence, the silvopasture site will allow production of all components (Trees, Livestock and Pasture). In addition, there are 761 cattle costing R15000 – R20000 each and 44 goats costing R1500 – R3000 each grazing in the plantation. Hence, the silvopasture system community livestock permit holders' food insecurity was 32% (mild) and 68% (food secure). The silvopasture system community livestock permit holders have access to different food groups and can often and always eat enough every day. In conclusion, the silvopasture system community livestock permit holders indicated that they could sustain their livelihoods as the land allocated and provided by SAFCOL enabled their livestock to graze, hence they could also access livestock products (manure, milk and meat etc.) and sell livestock in the informal and formal markets. It is thus recommended that the silvopasture system should be adopted and promoted throughout South Africa.

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DETERMINATION OF ARBUSCULAR MYCORRHIZAL FUNGI SPECIES AT DIFFERENT DEPTHS OF SOIL BENEATH THE PURE SESSILE OAK (*Quercus petraea* [Matt.] Liebl.) CANOPIES WITHIN A TEMPERATE FOREST STAND

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Abstract

Arbuscular Mycorrhizal Fungi (AMF) develop symbiotic relationship with terrestrial roots of plants, which host them. Hence, the AMF supply macronutrients to those roots whereas they obtain carbon from their organic compounds. Therefore, the AMF play crucial role in material and nutrient cycles, that are continuous within the ecosystem. In the scientific literature, the diversity and species analyses directly from the soils beneath the forest stand canopies are quite limited, and they have been conducted particularly within the tropical forests. However, these similar studies have not yet been conducted for the AMF, which are host directly at the roots within soils rhizosphere zones under the projection of the temperate forest trees. Thereby, this study aims to analyze and determine the AMF species, which are host directly at roots within the different depths of soils rhizosphere zone, under the projection of tree canopies in a pure sessile oak (*Quercus petraea* [Mattuschka] Liebl.) stand within a temperate forest ecosystem from the Western Black Sea Region of Turkey. The soil samples taken from two different depths of the rhizosphere where the sessile oak tree roots predominantly spread were analyzed with the stereo microscope. Thus, the identification of the AMF species was based on the AMF species guidebook. As a result of the analyses for the topsoil (0-30 cm) beneath the pure sessile oak canopies, it was determined that the AMF spores consisted of *Rhizophagus aggregatus*, *Claroideoglomus claroideum*, *Gigaspora margarita*, *Glomus diaphanous* species, whereas for the subsoil (30-60 cm), the determined AMF spores consisted of *Gigaspora gigantea*, *Diversispora eburnea*, *Glomus multicaule*, *Scutellospora coralloidea* species.

Keywords: Sessile oak (*Quercus petraea* [Matt.] Liebl.), Arbuscular Mycorrhizal Fungi (AMF) species, temperate forest ecosystem, rhizosphere zone of soils, topsoil and subsoil.

Introduction

Arbuscular Mycorrhizal Fungi (AMF) generally establish a mutual relationship with their host plants by penetrating the outer membrane of the roots of those vascular plants, providing macronutrient elements such as phosphorus, sulphur and nitrogen to these host plants and obtaining carbon from their organic compounds (Schaetzl and Anderson, 2005). AMF can establish a symbiotic-mutualistic relationship with approximately 80% of all terrestrial plants, thanks to their arbuscules penetrating the cortical (wall) of the plant's root cells (Smith and Read, 2008). While the AMF provide mineral nutrients, especially nitrogen (N) and phosphorus (P), to the plants, it continues its vital activity by taking carbohydrates, one of the products of photosynthesis, from them (Smith and Smith, 2011). Therefore, the AMF are closely related to the cycle of plant nutrients and matter (Waring and Running, 2007), directly or indirectly, to many biogeochemical processes of the ecosystem (Rillig, 2004), and finally to the health and balance of the ecosystem. However, the AMF can contact the roots of a wide variety of plants in almost all terrestrial biomes and ecosystems, from forests to pastures and grasslands, agro-ecosystems and deserts (Öpik et al., 2006). Changes in soil properties and plant compositions in these biomes and ecosystems can directly or indirectly affect the AMF composition (Zobel and Öpik, 2014; Zubek et al., 2016).

As in tropical forest trees, there is also a symbiotic relationship between the roots of the temperate forest trees and AMF. The AMF also have an effect on the growth of these temperate forest trees, especially on their root development (Williams et al., 2011), leaf and root physiology (Yang et al., 2014). As a matter of fact, temperate forest trees contribute to the composition, number of spores and species diversity of AMF (Bahnmann et al., 2018). Due to the relatively diversity of regions with temperate climates on earth (Bonan, 2016), from region to region the temperate forest tree species vary, at the roots of which the AMF species and their symbiotic relationships have been determined (Thomas and Packham, 2007). Sessile oaks (*Quercus petraea* [Mattuschka] Liebl.) are common in the forests extending from the west to the east of Europe (Bréda and Granier, 1996; Ducousso et al., 1996). They are also very common in the forest stands of the Black Sea region, occurring at the west and extending to the east of Türkiye (Atalay, 2008). On the other hand, although the international literature does discuss the status of symbiotic relationships with the AMF penetrating the roots of the understory vegetation in the sessile oak forests (e.g., Clapp et al., 1999) or the amount of plant nutrients in the soils under this sessile oak forest cover (e.g., López- Mondéjar, 2018) and even there have been studies trying to reveal the change in the growth rates of sessile oak seedlings with AMF inoculation (e.g., Helgason et al., 2007), the studies on the AMF species in the rhizosphere zone of the roots of the sessile oak trees located in the forest ecosystems, which are their general growing environments, are very limited. Therefore, studying such symbiotic relationships between the AMF species and sessile oaks (*Quercus petraea* [Mattuschka] Liebl.), which is one of the most important and widespread species of temperate forests and especially in Türkiye, will both determine the development, growth and production of sessile oaks under the influence of the AMF, and also contribute to the knowledge and benefit for the sustainability of the sessile oak forests. As a matter of fact, being able to identify the species of the AMF in the rhizosphere zone of the roots of sessile oaks, will make a great contribution to the international and national scientific literature. To achieve this goal, the study aims to identify and analyze the AMF species present under the canopy of a pure sessile oak forest. Consequently, the AMF species in the rhizosphere of the roots of pure sessile oak trees were identified and characterized.

Materials and Methods

The pure sessile oak (*Quercus petraea* [Mattuschka] Liebl.) stand selected as the study field, is located within the border of the Abdipaşa Forest Management Directorate from the Ulus Forest Administration of Bartın Province in Turkey. The trees of this pure sessile oak stand, shown with the symbol “Mzab3”, are in their juvenile-dense (a) and pole-post (b) ages. In other words, their average diameter at the 1.30 m breast height is 10 cm, and the stand is almost fully dense with a "3" cover, that is, a cover degree of 71% or more (TGDF, 2021). The hillside with dominant southeast aspect and with average 35° slope, where this “Mzab3” stand with a perimeter of approximately 1.7 km and an area of approximately 17 hectares is located (Figure 1). The altitude of this hillside ranges from 300 m asl. to 500 m asl. The average annual air temperature in the region is 9.5°C, and the average annual total precipitation is 1388 mm (TSMS, 2022). In the study field, grey-brown podzolic soils

(TMAF, 2005) have developed on the parent materials of sandstone-mudstone geological formations (TGDMER, 2007).
In order to identify the AMF species, soil samples were taken from the two different soil depths (0-30 cm and 30-60 cm) at the rhizosphere zones of the roots of the sessile oak trees; beneath the projections of their canopies. The research was conducted in 2023. Spores of arbuscular mycorrhizal fungi (AMF) were analyzed according to the wet sieving method (Gerdemann and Nicolson, 1963; Jenkins, 1964; Sieverding, 1991), and diagnosed morphologically (such as referring to color, shape, size, breakage pattern, hyphal structure) under the microscope on a species basis (Schenck and Perez 1990; INVAM 2020).

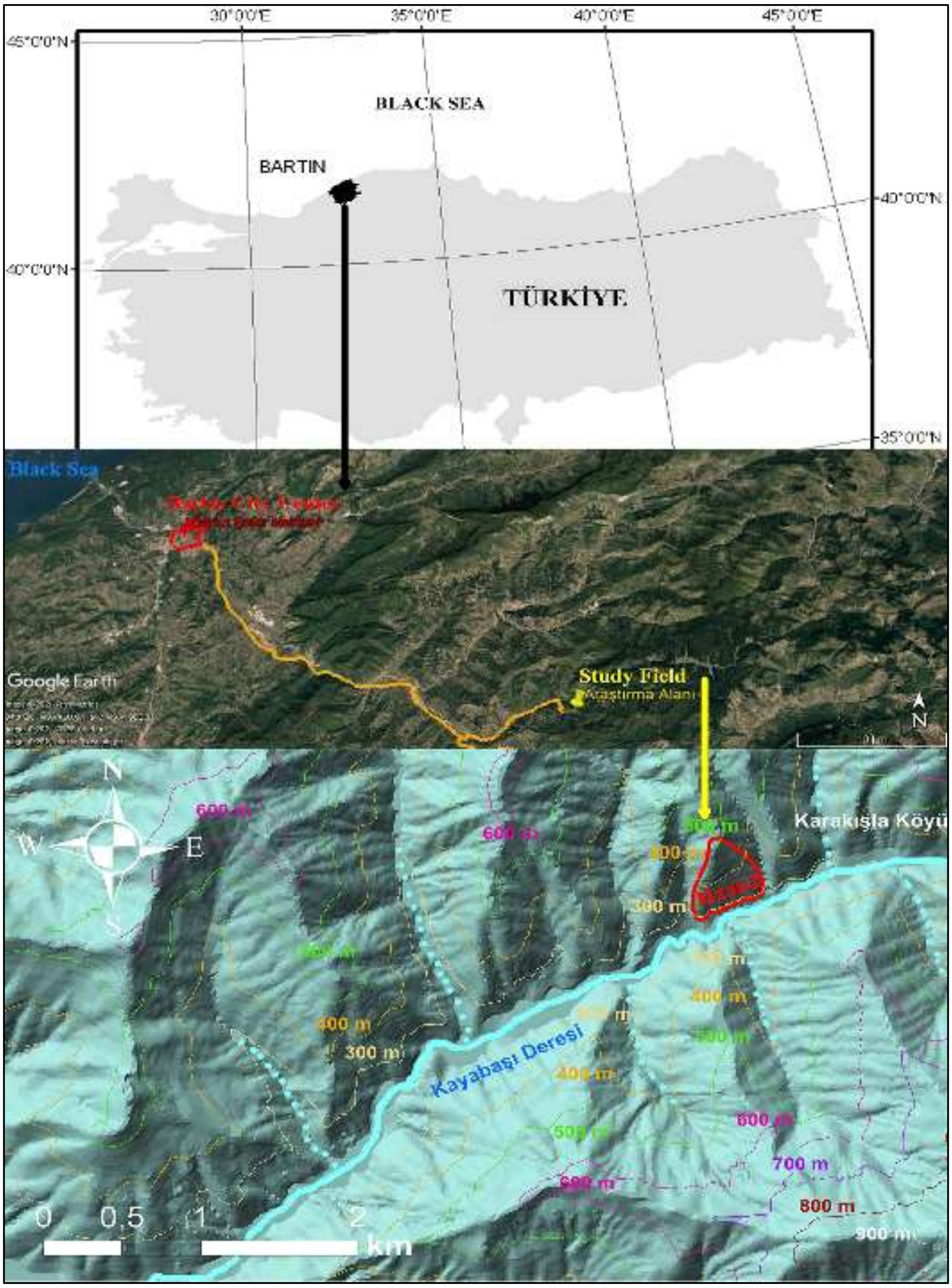

Figure 1. Location of study field within the region and Türkiye

Results and Discussion

As a result of the analyses, the AMF spores identified within the topsoil (0-30 cm) of the rhizosphere zone of the tree roots beneath the pure sessile oak canopies, were belonging to the *Rhizophagus aggregatus* (1), *Claroideoglomus claroideum* (2), *Gigaspora margarita* (3), *Glomus diaphanous* (4) species. On the other side, within the subsoil (30-60 cm), the AMF spores were belonging to the *Gigaspora gigantea* (5), *Diversispora eburnean* (6), *Glomus multicaule* (7), *Scutellospora coralloidea* (8) species (Figure 2).

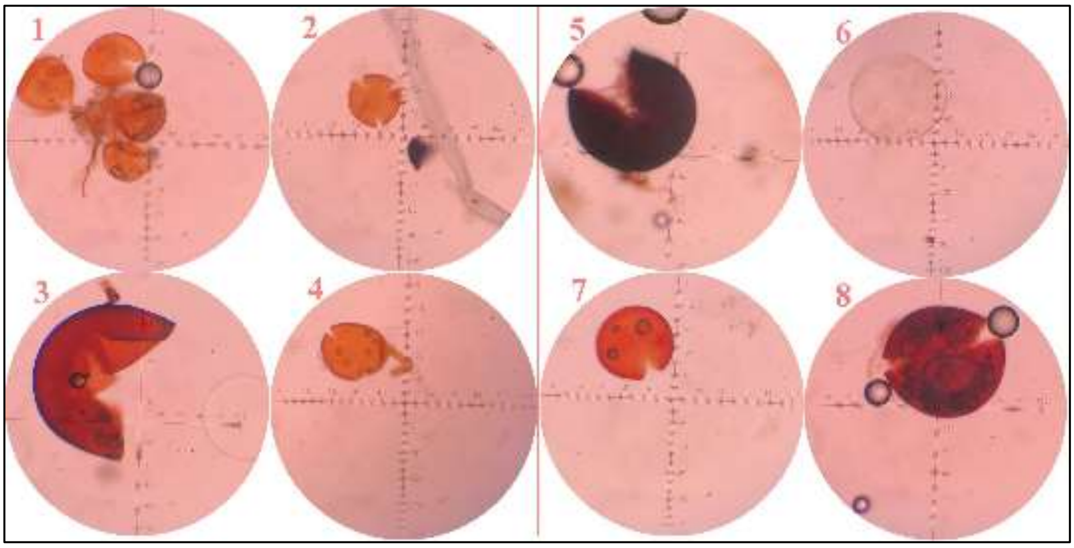

Figure 2. AMF spore samples from the topsoil (0-30 cm) and subsoil (30-60 cm) at the rhizosphere zone of the tree roots of pure sessile oak stand within the study field

The shape of the *Rhizophagus aggregatus* species is either globose or oblong or even irregular, whereas the colour of the spore is hyaline, and the spore has unit two-layered outer wall (Souza, 2015). In a study by Djighaly et al. (2018), *Rhizophagus aggregatus* was one of the AMF species, with which *Casuarina equisetifolia* L. and *Casuarina glauca* Sieb. were inoculated to improve their tolerance to salinity. However, these two tree species established relatively moderate growth because of *Rhizophagus aggregatus* inoculation compared to the other AMF species. The shape of the *Claroideoglomus claroideum* species is either globose or subglobose, whereas the colour of the spore is cream or light yellow, and the spore has evanescent (first two layer) and laminated (third layer) outer wall (Souza, 2015). In a study by Moradi Behbahani et al. (2017), *Claroideoglomus claroideum* was one of the AMF species, which were recorded at the rhizosphere of *Tamarix arceuthoides* in a riparian forest after the sand mining disturbances. The shapes of the *Gigaspora margarita* and *Gigaspora gigantea* species are either globose or subglobose, whereas their spore colours are hyaline to white, and yellow to green respectively, and their spores have unit (first layer) and laminated (second layer) outer wall (Souza, 2015). In a study by Klinsukon et al. (2021), *Gigaspora margarita* was used as growth promoter and successful biocontrol of leaf blight disease in eucalyptus seedlings. The shape of the *Glomus multicaule* species is either globose or subglobose or even ellipsoid or triangular, whereas their spore colour is brown, and their spore has unit one-layered outer wall (Souza, 2015). In their study, Ong et al. (2012) determined that the soil chemical properties had direct effect on the abundance of AMF, particularly on the *Glomus* species. The shape of the *Diversispora eburnea* species is either globose or subglobose or even irregular, whereas their spore colour is white and cream, and their spore has unit (first layer) and laminated (second layer) outer wall (Souza, 2015). The shape of the *Scutellospora* *coralloidea* species is either globose or subglobose, whereas their spore colour is red-brown, and their spore has unit (first layer) and laminated (second layer) outer wall (Souza, 2015).

Conclusion

Habitats of the pure sessile oak stands (*Quercus petraea* [Mattuschka] Liebl.), which is one of the oak species commonly found within the forest ecosystems of Türkiye, and especially in the Black Sea region are prevalent. However, they have been gradually transforming into the mixed stands during the recent years with the invasion of other coniferous and deciduous tree species from the nearby stands and habitats. With this study, the effect of arbuscular mycorrhizal fungi (AMF) species, which exist within the rhizosphere zone and, which penetrate the tree roots of the already pure sessile oak stands, was revealed to some extent. Therefore, these results can reveal some of the reasons for the actual forest stand transformations, which have led to the decrease of the pure sessile oak stands within the region.

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