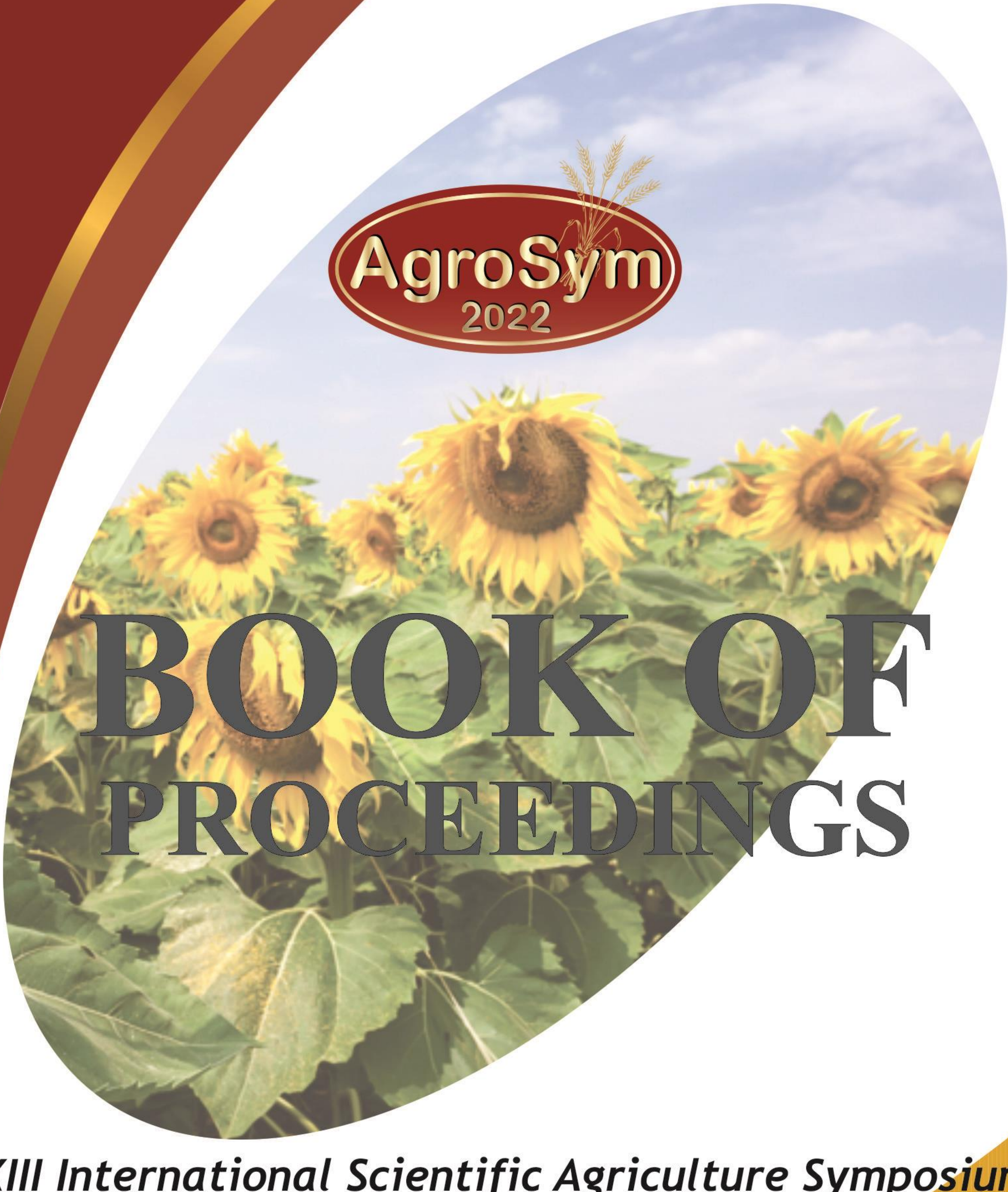

BOOK OF PROCEEDINGS

XIII International Scientific Agriculture Symposium
"AGROSYM 2022"
October 6-9, 2022

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PREFACE

Dear colleagues,

The Faculty of Agriculture of the University of East Sarajevo (Bosnia and Herzegovina), the Faculty of Agriculture of the University of Belgrade (Serbia), and CIHEAM - Mediterranean Agronomic Institute of Bari (CIHEAM Bari, Italy) organized from 6 to 9th October 2022 on Jahorina mountain (East Sarajevo, Bosnia and Herzegovina) the 13th International Scientific Agriculture Symposium "AGROSYM 2022". The symposium was organized for the third time in a hybrid format, with in-person participation (around 300 participants) and online participation (400 participants), because of the prescribed restrictions caused by the COVID-19 pandemic and the world's political situation.

AGROSYM 2022 made an important contribution to agriculture science and practice in different topics: plant production, plant protection, animal husbandry, environmental protection, organic farming, agroforestry, agroeconomy, and rural development. The Symposium topics cover all branches of agriculture as well as forestry and agroforestry. The scientific committee received around 700 papers and after review, it accepted 668 papers, 159 for oral presentations and 509 for poster presentations, which represents over 1500 authors from more than 80 countries worldwide.

During AGROSYM 2022, we had the opportunity to share the results of the current research at the international level and new information relating, inter alia, to biotechnology, world markets and agricultural knowledge and innovation systems in the European Union, especially in plenary papers. Based on many investigations, we can see that practices based on the concept of sustainable agriculture are gaining more and more attention. The goal for sustainable agriculture must be to meet society's needs, not only the production of goods such as food and fiber but also the maintenance or restoration of ecosystem services such as watershed protection, healthy soil and the biodiversity on which humanity depends.

Big thanks to all members of the scientific committee and the staff from the symposium secretariat for their continued efforts and hard work that made this symposium possible and successful. I should like to thank my colleagues from the organizing committee, particularly the Dean of the Faculty of Agriculture of the University of East Sarajevo, professor Vesna Milic, as a host and chairperson. Special thanks to His Excellence, Prof. Sinisa Berjan, for all he has done to bring this event together; it has been a considerable logistical exercise.

Finally, I would like to thank all the authors, reviewers, session moderators and colleagues for their help in preparing and editing these e-Proceedings. Special thanks go to the organizers, partners and sponsors for their unselfish collaboration and comprehensive support.

Editor in Chief

East Sarajevo, 25 October 2022

Dušan Kovačević

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CONTENTS

PLANT PRODUCTION

THE STUDY OF PHOTOSYNTHETIC GAS EXCHANGE PARAMETERS OF BREAD WINTER WHEAT UNDER VARIOUS WATER SUPPLY

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Abstract

Water scarcity is the main limitation for plant growth and productivity. Gas exchange parameters of field grown 21 winter bread wheat genotypes were studied by using LI-6400 XT Portable Photosynthesis System. The average values of photosynthesis rate (P_n), stomatal conductance (g_s), and transpiration rate (T_r) for all genotypes were found to be higher in the morning and afternoon hours in the irrigated variants compared to drought-exposed plants. In the morning hours, the average values of P_n, g_s, and T_r for all genotypes in the stressed and irrigated variants were, respectively, 11.8 and 21.1 µmol CO_2 $m^{-2}s^{-1}$, 0.103 and 0.395 mol H_2O $m^{-2}s^{-1}$, 2.58 and 7.31 mmol H_2O $m^{-2}s^{-1}$, and in the afternoon hours, 11.9 and 19.6 µmol CO_2 $m^{-2}s^{-1}$; 0.083 and 0.266 mol H_2O $m^{-2}s^{-1}$; 2.58 and 5.81 mmol H_2O $m^{-2}s^{-1}$. While the rate of photosynthesis was close at both measurements, in both stressed and irrigated variants, a decrease in stomatal conductance and transpiration rate (in the irrigated variant) was observed during the afternoon measurements. Genotypes with high T_r values were also found to have high stomatal conductance, while genotypes with low T_r had low stomatal conductance, indicating that T_r is mainly regulated by stomatal conductance. Stomatal conductance was more important in the course of photosynthetic processes during relatively mild drought conditions.

Keywords*: bread wheat, water supply, photosynthesis, stomatal conductance, transpiration.*

Introduction

Wheat, which is the main food for humans, is the most widely grown crop in the world. In modern times, when it is impossible to expand the sown areas, the most effective way to meet the growing needs of the population is to increase the productivity from a unit area (Bernardo, et al., 2014. The productivity of crops is limited by some abiotic factors, especially drought. Water is vital in plant metabolism at the cellular and plant level. When plants are grown under drought conditions, they undergo some morphological, physiological, and biochemical changes to increase their tolerance to drought. Genotypes that can make these changes and minimize losses during drought, i.e. the least damaged from drought, are known to be drought tolerant. One of the complex measures aimed to protecting field crops from drought is the creation of drought-tolerant varieties (Aliev, 2012). Photosynthesis is one of the main physiological processes increasing plant productivity, and over 90% of the dry biomass is formed at the expense of organic compounds formed during this process (Aliev, 2012; Maccaferri, *et al*., 2008; Shan, *et al*., 2012). Drought strongly affects the gas exchange parameters of cultivated plants, slows down the growth of leaves, disrupts the photosynthetic mechanism, accelerates the oxidation of lipids of chloroplasts, and causes changes in the structure of pigments and proteins (Aliyev and Huseynova, 2014; Anjum, *et al*., 2011; Menconi, *et al*., 1995). Water deficiency, first of all, leads to a decrease in stomatal conductance, thereby plants try to maintain a water regime by declining transpiration. As a result, the amount of carbon dioxide absorbed by the leaves is reduced (Allahverdiyev, 2015; Chaves *et al*., 2002; Cornic and Massacci, 1996). At the same time, it was found that the stomatal conductance in genotypes tolerant to water stress can be regulated by various mechanisms, thus ensuring absorption of carbon dioxide to a certain degree, which ultimately increases the efficiency of water use (Brestic and Zivcak, 2013). Thus, the process of photosynthesis plays an important role in the formation of plant productivity, and the speed of this process depends on the water supply of plants. Therefore, the comparative study of photosynthetic gas exchange parameters under water deficit and normal water supply conditions is of both scientific and practical importance.

Material and Methods

The research was conducted on 21 wheat genotypes differing in morphophysiological characteristics and productivity under the conditions of Mountainous Shirvan in Azerbajian with unstable moisture supply. The research has been conducted in 2012-2013 vegetation season. The objects of the research were 12 varieties and 9 lines of bread wheat differing in morphophysiological characteristics. Planting was conducted in the form of randomly placed blocks in 3 repetitions using experimental beds of 1.0 m^2 and the sowing rate was 450 seeds per 1 m^2. To make a difference in water supply, artificial drought conditions were created in early May by covering one block with a transparent polyethylene material, while the second block was irrigated. Photosynthetic gas exchange parameters-photosynthesis rate- P_n, stomatal conductance- g_s, carbon dioxide (CO_2) concentration in intercellular spaces- C_i, and transpiration rate- T_r were measured using LI-6400 XT Portable Photosynthesis System (LI-Cor Biosciences, Lincoln, USA) equipped with 6 cm^2 leaf chamber. Statistical analyses were performed with the JMP 5.0.1 program.

Results and Discussion

Photosynthetic gas exchange parameters were measured in the flag leaf of 21 bread wheat genotypes, during the milk ripening phase (Zadoks 73 growth scale) of drought-exposed and irrigated variants, in the morning (11^0-12^{00}) and afternoon (14^{00}-15^{00}) hours. The results of the measurements are presented in Tables 1 and 2. The analysis of variance showed a significant difference at the 0.01 level between the studied genotypes in all measurements according to the photosynthetic gas exchange parameters. As seen in Table 1, in the morning hours, the average rate of CO_2 assimilation, stomatal conductance and transpiration rate in the drought-exposed and irrigated variants were, respectively, 11.8 and 21.1 µmol CO_2 $m^{-2}s^{-1}$; 0.103 and 0.395 mol H_2O $m^{-2}s^{-1}$; 2.58 and 7.31 mmol H_2O $m^{-2}s^{-1}$. In this case, the highest values of P_n were observed in the drought-exposed variants of Tale 38 and Aran (17.1 and 16.6 µmol CO_2 $m^{-2}s^{-1}$), and in the irrigated variants of Gyrmyzy gul 1, Tale 38, 11thIWWYT №20, Sheki 1, and Gyzyl bughda (24.3, 23.9, 23.7, 23.7 and 22.9 µmol CO_2 $m^{-2}s^{-1}$) genotypes. The highest values of stomatal conductance were detected in the drought-exposed variants of 11thIWWYT №20 and Tale 38 (0.200 and 0.199 mol$H_2O$$m^{-2}s^{-1}$), and in the irrigated variants of the Gyrmyzy gul 1, Tale 38, 11thIWWYT №20 and Gyzyl bughda (0.517, 0.480, 0.442, and 0.456 mol H_2O $m^{-2}s^{-1}$) genotypes. At the time of the study, the transpiration rate was highest in both variants of the Tale 38 variety, and amounted to 5.06 and 9.06 mmol H_2O $m^{-2}s^{-1}$, respectively.

Table 2 shows the results of photosynthetic gas exchange parameters measured in the afternoon hours. An average values of CO_2 assimilation rate, stomatal conductance, and transpiration rate in drought-exposed and irrigated variants were, respectively, 11.9 and 19.6

µmol CO_2 $m^{-2}s^{-1}$; 0.083 and 0.266 mol H_2O $m^{-2}s^{-1}$; 2.58 and 5.81 mmol H_2O $m^{-2}s^{-1}$. The highest values of the parameters were observed in the drought exposed variants of Aran, Zirve 85, Sheki 1, Tale 38, and in the irrigated variants of Aran, Vostorg, 7thWON-SA №465 and 12thIWWYT №9.

Table1. Parameters of the photosynthetic gas exchange (morning measurements)

Genotypes	P_n µmol CO_2 $m^{-2}s^{-1}$		$g_s$mol H_2O $m^{-2}s^{-1}$		$T_r$mmol H_2O $m^{-2}s^{-1}$	
	drought	irrigation	drought	irrigation	drought	irrigation
Bezostaya 1	13.0	19.8	0.096	0.353	2.77	7.48
Gyzylbughda	11.2	22.9	0.077	0.456	2.26	8.33
Sheki 1	14.1	23.7	0.108	0.353	3.16	7.74
Sonmez 01	11.8	21.4	0.083	0.404	2.21	6.76
Aran	16.6	22.2	0.128	0.381	3.50	7.05
Vostorg	14.0	17.9	0.137	0.429	3.54	7.69
Murov 2	11.8	22.2	0.094	0.362	2.63	7.07
Gobustan	9.8	15.6	0.074	0.278	2.46	6.03
Tale 38	17.1	23.9	0.199	0.480	5.06	9.06
Fatima	13.8	19.0	0.147	0.397	2.74	6.68
Gyrmyzygul 1	8.8	24.3	0.065	0.517	1.47	8.07
Zirve 85	12.1	18.9	0.130	0.429	2.63	7.08
7^{th}WON-SA №465	8.1	20.9	0.062	0.451	1.52	7.59
Ferrigineum 2/19	10.6	20.3	0.069	0.395	1.86	7.08
11^{th}IWWYT №20	14.0	23.7	0.200	0.442	3.20	8.06
12^{th}IWWYT №6	9.7	21.8	0.078	0.372	2.16	7.67
12^{th}IWWYT №8	13.0	21.3	0.096	0.422	2.76	7.44
12^{th}IWWYT№9	11.8	19.3	0.088	0.255	2.52	5.77
12^{th}IWWYT №17	9.7	23.1	0.060	0.363	1.65	7.08
7^{th}WON-SA №477	8.8	22.3	0.079	0.438	2.02	7.55
4^{th} FEFWSN №50	8.9	18.3	0.086	0.312	2.14	6.16
Average	11.8	21.1	0.103	0.395	2.58	7.31
LSD	0.78**	1.05**	0.0096**	0.0301**	0.27**	0.52**
CV %	5.7	4.4	8.2	6.5	9	6.2

As seen, the average values of the rate of photosynthesis, stomatal conductance, and transpiration rate were higher in the irrigated variants compared to the drought-exposed variants at both measurement times, which is the result of reduced water content in the soil under drought. While the rate of photosynthesis was close at both measurement times in both drought-exposed and irrigated variants, a decrease in stomatal conductance and transpiration rate (irrigated variants) was observed. It should be noted that in g_s values, the decrease between morning and afternoon measurements was 19.4 and 32.7% in the drought-exposed and irrigated variants, respectively, and in T_r values, the decrease was only in irrigated variants and amounted to 20.5%. Thus, the less decrease in stomatal conductance in the drought-exposed variants compared to the irrigated plants was also reflected in the rate of transpiration. Thus, in the drought-exposed variants, the value of this parameter did not change, i.e. the current state of stomatal conductance was sufficient for a small amount of transpiration.

Table 2. Parameters of the photosynthetic gas exchange (afternoon measurements) (2012-2013 vegetaion season))

Genotypes	P_n µmol CO_2 $m^{-2}s^{-1}$		$g_s$mol H_2O $m^{-2}s^{-1}$		$T_r$mmol H_2O $m^{-2}s^{-1}$	
	drought	irrigation	drought	irrigation	drought	irrigation
Bezostaya 1	13.6	15.7	0.093	0.269	2.95	5.53
Gyzylbughda	13.6	18.9	0.098	0.228	2.93	5.46
Sheki 1	14.2	18.5	0.115	0.180	3.32	4.85
Sonmez 01	11.1	19.5	0.055	0.217	2.01	5.26
Aran	14.8	24.9	0.120	0.350	3.58	6.81
Vostorg	13.2	23.4	0.090	0.462	2.81	7.96
Murov 2	13.4	19.4	0.090	0.265	2.89	5.84
Gobustan	8.7	16.3	0.040	0.200	1.43	4.78l
Tale 38	14.1	20.8	0.130	0.300	4.13	6.28
Fatima	11.9	18.9	0.080	0.212	2.57	5.8
Gyrmyzygul 1	13.1	19.8	0.112	0.348	3.65	6.09
Zirve 85	14.3	18.0	0.164	0.264	4.44	6.72
7thWON-SA №465	9.1	21.4	0.049	0.308	1.54	6.73
Ferrigineum 2/19	11.8	19.6	0.071	0.286	2.26	6.06
11thIWWYT №20	12.9	20.6	0.095	0.218	3.04	5.93
12thIWWYT №6	9.4	21.3	0.062	0.232	2.00	5.54
12thIWWYT №8	7.4	16.5	0.044	0.248	1.43	5.03
12thIWWYT №9	13.8	24.0	0.086	0.417	2.55	7.27
12thIWWYT№17	8.3	20.4	0.047	0.204	1.33	4.74l
7thWON-SA №477	9.8	17.2	0.053	0.195	1.48	4.73l
4thFEFWSN №50	10.5	16.1	0.053	0.179	1.89	4.66l
Average	11.9	19.6	0.083	0.266	2.58	5.81
LSD	0.77**	1.0**	0.0093**	0.0222**	0.26**	0.48**
CV %	5.7	4.5	9.9	7.2	8.7	7.1

In the irrigated variants, a further weakening of stomatal conductance had a serious impact on transpiration and its value declined by 20.5%. Due to this condition of stomatal conductance, the values of photosynthesis rates are close in the drought-exposed and irrigated variants at both measurement times. It was previously reported that the water conductance of the stomata was 1.56-fold higher than the CO_2 conductance (Grant *et al*., 2005). In other words, a decrease in stomatal conductance affects the rate of transpiration more than the rate of photosynthesis. In our study, a decrease in stomatal conductance in the afternoon hours also had a smaller effect on the rate of photosynthesis than the rate of transpiration.

The rate of photosynthesis was on average 44.1% lower than in the drought-exposed variants compared to the irrigated variants in the morning, and 39.3% lower in the afternoon. The largest differences in morning measurements were observed between the variants of Gyrmyzy gul 1 (63.8%), 7thWON-SA №465 (61.2%), 7thWON-SA №477 (60.5%), 12thIWWYT №17 (58.0%), and 12thIWWYT №6 (55.5%), while the smallest differences were found in the genotypes Aran (25.2%), Vostorg (21.8%), Tale 38 (28.5%), and Fatima (27.4%). The largest differences in afternoon measurements were observed between the variants of Gobustan (46.6%), 7thWON-SA №465 (57.5%), 12thIWWYT №6 (55.9%), 12thIWWYT №8 (5.2%), and 12thIWWYT №17 (59.3%), while the smallest differences were detected in the genotypes Bezostaya 1 (13.4%), Gyzyl bughda (28.0%), Sheki 1 (23.2%), and Zirve 85 (20.6%).

During the morning measurements, stomatal conductance was more sensitive to water deficit and its value was on average 73.9% lower in the morning measurements and 68.8% lower in the afternoon measurements in the drought-exposed variants compared to the irrigated one. The largest differences between the variants in the morning measurements were observed in the genotypes Gyzyl bughda, Gyrmyzy gul 1, 7thWON-SA №465, Ferrigineum 2/19, 12thIWWYT №17 and 7thWON-SA №477 (83.1, 87.4, 86.3, 82.5, 83.5 and 82.5%, respectively). In the afternoon measurements, the largest differences in stomatal conductance between drought-exposed and irrigated variants were found in the genotypes Vostorg (80.5%), Gobustan (80.0%), 7thWON-SA №465 (84.1%), 12thIWWYT №8 (82.3%), 12thIWWYT №9 (79.4%) and 12thIWWYT №17 (77.0%) genotypes. Whereas, the smallest differences between the experimental variants were in the Sheki 1 (36.1%), Zirve 85 (37.9%) varieties.

In the morning measurements, the highest values of transpiration rate (T_r) were detected in the drought-exposed variants of Sheki1, Aran, Vostorg, Tale 38, and 11thIWWYT №20, respectively, 3.16, 3.5, 3.54, 5.06 and 3.20 mmol H_2O $m^{-2}s^{-1}$, while the smallest values of this parameter were observed in the genotypes Gyrmyzy gul 1, 7thWON-SA №465, Ferrigineum 2/19, and 12thIWWYT №17, which amounted to 1.47, 1.52, 1.86, and 1.65 mmol H_2O $m^{-2}s^{-1}$, respectively (Table 1). The genotypes with high T_r values were observed to have high stomatal conductance, while those with low T_r values had also low stomatal conductance, which indicates that T_r is mainly regulated by stomatal conductance. In the morning measurement, the difference in transpiration rate between the variants was an average 64.7%. In this case, the largest differences between the variants were observed in the genotypes Gyzyl bughda, Gyrmyzy gul 1, 7thWON-SA №465, Ferrigineum 2/19, 12thIWWYT №17 and 7thWON-SA №477. In the afternoon measurements of T_r, the average difference between the variants for all genotypes was 55.6%. The highest values of this difference were 61.8, 64.7, 70.1, 77.1, 71.6, 71.9, and 68.7%, respectively, in the genotypes Sonmez 01, Vostorg, Gobustan, 7thWON-SA №465, 12thIWWYT №8, 12thIWWYT №17, and 12thIWWYT №477. The smallest difference between the variants was observed in Sheki 12 (31.5%), Tale 38 (34.2%), Qyrmyzy gul 1 (40.1%), and Zirve 85 (33.9%) varieties, which is attributed to high values of T_r in the drought-exposed variants.

Conclusion

It has been found that the rate of photosynthesis is higher in both variants of Sheki 1, Aran, and Tale 38 and only in the irrigated variants of the genotypes Gyrmyzy gul 1, 11thIWWYT №20, Vostorg, and 12thIWWYT №9 compared to other genotypes. Stomatal conductance is more sensitive to water deficit. The rates of transpiration and photosynthesis depend on stomatal conductance. Besides, the rate of transpiration is more sensitive to stomatal conductance than the rate of photosynthesis.

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CARROT QUALITY DEPENDING ON THE TYPE OF ROOT

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Abstract

The anatomical structure of the carrot root is composed of: cork tissue, phloem and xylem. The best quality part of the carrot is the phloem because it contains biologically active substances important for human health. Phloem development is different depending on the type of carrot root. Depending on the shape of the carrot root, carrots are divided into four basic types: round, braunschweing, shantene and nantes. Taking into account the fact that the nutritional value is in the root rind, the aim of the study was to determine how much the shape of the root affects the participation of root rind, ie the quality of carrots. We examined three types of carrot root: spindle-type Braunschweig carrot, cone-type Shantane, cylindrical-type nantes. On a sample of 10 plants of different carrot root types, we analyzed: root mass and length, participation of root rind (%). The type Braunschweing has a statistically significantly higher share of root rinde (50.33%) compared to the type Shantene (42.66%) and type Nantes (44.33%). There was no statistically significant difference in root rind participation between the type Shantene and the type Nantes. The quality of the type Braunschweing is good because participation of root rind is 50.33%, while the other two tested types (shantene, nantes) have poor quality because the participation of root rind is below 50%.

Keywords: *carrot, type of root, quality.*

Introduction

Carrot have great importance in human nutrition. It is rich in vitamins and minerals. Carrot can be used fresh, cooked or processed. It is among the ten most important vegetable crops. It is especially important in the nutrition of children.The main component of the nutritional quality of carrots is the content of sugar, protein, carotenoids, essential oils and minerals. Nutritional characteristics of the crop carrots are rich sources of carotene, ascorbic acid and are known as vitaminized food with moisture, protein, fat, carbohydrates, sugars and fiber in the range of 84 to 95%, 0.6 to 2.0%, 0.2 to 0.7, 9.58 to 10.6%, 5.4 to 7.5% and 0.6 to 2.9%, respectively (Khanum et al., 2000; Hashimoto and Nagayama, 2004). High levels of provitamin A in carrots are important for treating skin diseases and poor eyesight. (Đurovka, 2008).

Carrots are consumed either raw or cooked and processed into value added products viz. canned carrots, chips, candy, kheer, halwa, powder, juice,beverages, preserve and intermediate moisture products (Haq Raees-ul and Prasad, 2014.). The vegetative part of carrots used in the diet is the root. The anatomical structure of carrot roots consists of phloem and xylem. The vascular system, xylem and phloem of the root are produced by the growth of secondary cambium towards inner side and outer side, respectively (Haq Raees-ul and Prasad, 2014.).

The bioactive components are mainly concentrated towards the exterior side of root (pholem) and the presence of handsome amount of the vitamins,bioactive components and minerals have led (Alasalvar et al., 2001; Dias, 2014) to rank it among top ten fruits and vegetables on the basis of nutrition. Taking into account that the nutritional value of carrots is in the rind of root, the aim of the study was to determine how much the type of root affects the quality of carrots, i.e. the participation of root rind.

Materials and methods

The tests were performed in the laboratory of the Faculty of Agriculture in East Sarajevo in 2022. We examined three types of carrot root: spindle-type Braunschweig carrot, cone-type Shantane, cylindrical-type nantes. The Braunschweig type of carrot has a cylindrical root, narrowed at the end. The head of the root is sunk, blunt at the top. The Shantane type of carrot has a cylindrical root, slightly narrower at the end. The tip of the root is blunt. The Nantes type of carrot has a root of a typically cylindrical form with a blunt tip.
On a sample of 10 plants of different carrot root types, we analyzed:
-root mass (g),
-root length (cm),
-participation of root rind (%).
The participation of root rind (%) was calculated using the formula Đurovka (2009):
% root rind = D-d/D x100
(D-total root diameter; d-diameter xylem)
The obtained results were processed by the variance analysis method for a mono-factor experiment (ANOVA). The significance of differences between individual environments was tested by LSD test.

Results and discussion

Average root mass is one of the most important factors that directly affects carrot yield (Boskovic-Rakocevic and Pavlovic, 2008). The mass of carrots of the type Shantene (87.17 g) is statistically significantly higher compared to the type Braunschweing (65.82 g). Compared to the cylindrical (78.72g) root type, the difference was not statistically justified.
Indicators of root length indicate maximum values in the type Braunschweing and minimum in the type Shantane. The differences are statistically significant.
The quality of the root depends on the participation of the root rind, because most of the nutrients are in the phloem of the root. Root quality is not good if the root rind content is below 50%. The quality is good if the share of root rind is 70-80%, medium 50-70% (Đurovka,2009; Đurovka,2008). The type Braunschweing has a statistically significantly higher share of root rinde (50.33%) compared to the type Shantene (42.66%) and type Nantes (44.33%). There was no statistically significant difference in root rind participation between the type Shantene and the type Nantes.
The results opposite to ours are presented by Rašević (2017) who examined the participation of root rind in three types of carrot roots (conical, spindle-shaped, cylindrical). Carrots in the type nantes had a statistically significantly higher root rind compared to the other two examined types. The quality of the type Braunschweing is good because participation of root rind is 50.33%, while the other two tested types (shantene, nantes) have poor quality because the participation of root rind is below 50%.

Table 1. Individual root mass, root length, participation of root rind

Root type		root mass (g)	root length (cm)	participation of root rind (%)
type Shantane		87.17[a]	14.93[b]	42.66[b]
type Braunschweig		65.82[b]	22.90[a]	50.33[a]
type Nantes		78.72[b]	16.45[b]	44.33[b]
LSD	5%	20.09	3.69	6.25
	1%	27.04	4.96	8.41

Conclusion

Participation of the root rind affects the quality of carrots because it contains biologically active substances. Participation of the root rind is highly dependent on the type of root. Braunshchweing type of carrot has a statistically significantly higher percentage of root rind to type Shantene and type Nantes. Differences in participation of the root rind between type Shantene and type Nantes are not statistically significant.

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DETERMINATION OF PHYSICAL AND CHEMICAL PROPERTIES OF FRUITS OF DIFFERENT PEAR VARIETIES

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Abstract

Pear (*Pyrus communis* L.) is a deciduous tree from the rose family (Rosaceae). It grows up to 12 meters in height, forming a large, dense, round crown. The flesh of the fruit is whitish-yellow, wrapped in yellow or green rind, it is very juicy with a characteristic aroma. The quality of our domestic pear varieties is much lower than noble Western European ones. However, domestic varieties also have their advantages, because they adapt much better to poorer climatic and soil conditions, are more resistant to plant diseases and pests, have stable productivity, are more durable, and can have a high sugar content or total nutritional value. This research aimed to physically and chemically characterize three different varieties of pears grown in the area of Visoko (Bosnia and Herzegovina), and to evaluate the potentials of the varieties Butira, Viljamovka, and Santa Maria. After the completed analysis, it can be concluded that the variety of Butira had the highest fruit weight, while the height, width of the fruit, and length of the stalk had the highest value in the variety Viljamovka. The fruits of the Santa Maria variety had the highest average number of pits. After chemical analysis, the samples of the Viljamovka variety had the highest values of all examined parameters. The Butira variety had the lowest pH value and total acid content, while the Santa Maria variety had the lowest amount of soluble dry matter, as well as total inverts.

Keywords: *pear, pH value, dry matter, total inverts, total acids*.

Introduction

Pear (*Pyrus communis* L.) is a deciduous tree from the rose family (Rosaceae). It can grow up to 12 m, forming a large round canopy. The root can reach a depth of 2.5 to 6 m. Most of the root mass is in the first 1.5 m depth. The root itself is poorly branched, but they are grafted on pear seeds' generative rootstocks or on quince's vegetative rootstocks (Stančević, 1980). There are significant advantages of this method of grafting because the root penetrates deeper into the soil, and has greater resistance to low temperatures, drought, etc.

The leaves can be of different shapes, ellipsoidal, oval, round, and even feathery. The canopy is pyramidal with a dominant conductor, 10 to 30 meters high and 10 to 12 meters wide (Stančević 1980). Pear flowers are a group of metamorphic leaves that have the role of full reproduction. They are formed in the form of a canopy on which there can be 5 to 25 flowers. First, the lateral flowers are included, and then the apical flowers. They consist of calyx leaves, about five free pistils, and 15 to 30 anthers. In the middle of the coronal leaves are glands that secrete nectar. Each flower contains many pollen grains, and it is estimated that there are about 4,000 pollen grains in each pollen. The ripening time of the fruits depends on the variety, climatic conditions and altitude, and varies from the end of July to the middle of October (Vrbanac et al. 2007.). The choice of variety in most cases depends on the ecological conditions of the production area and consumption needs (Miljković 1991.).

Pear fruit consists of epicarp, fruit parenchyma, and endocarp. The fruit itself can be of different sizes, and its weight ranges from 2 g up to 2 kg. The flesh of the fruit is mostly white or yellow, it is soluble and very juicy with a specific aroma.
Microsporogenesis in pear anthers takes place from mid-February to the end of March and ends before flowering begins. Flower buds begin to form from late June to mid-August. The formation of flower buds begins earlier in lazy and autumn pear varieties, and one to two weeks later in winter varieties. According to Stancevic (1980), the pear usually blooms during April. It rarely begins to bloom in the second half of March or early May.
The quality of our domestic pear varieties is somewhat lower than in Western Europe. However, domestic varieties also have their advantages: they are much better adapted to poorer climatic and soil conditions, they are much more resistant to various plant diseases and pests, they are long-lasting and they can have high sugar content, the ie total amount of nutritional value.
A large number of pear varieties have a quality suitable for consumption in the fresh state, but a significant number is a very valuable raw material for the processing and food industry. It is used to make compotes, and juice concentrates and is also used in the production of mixed jams and marmalades. It is dried and pickled in domestic processing and is used in many other forms of processing in the diet of the population. Of the varieties for industrial processing, 'Vilijamovka' is the most used, primarily for the distillation of high-quality brandy (Gliha, 1997). It has a high nutritional value, such as vitamins A, B1, B2, B3, C, and minerals such as sodium, potassium, calcium, phosphorus and magnesium.

Material and Methods

The choice of sites for sampling different varieties of pears in this study was in the area of Visoko (Bosnia and Herzegovina), more precisely the place Mulići and on private property, where pears are traditionally grown on a plantation of 1ha. In this area, the most common varieties of pears are Viljamovka, Santa Maria, and Butira.

Photo 1: Sampling location

Viljamovka is a variety that ripens in late summer, at the end of August. The fruit is quite large, irregular in abundance, and slightly elongated. The flesh of the fruit is light yellow in color and contains a lot of fine acids and sugars, which makes it a very tasty and appreciated variety among a wide range of consumers.

Santa Maria comes to the harvest a little earlier than Viljamovka, in the middle of August. This variety has a specific appearance. Its color is greenish-yellow, with a blush on the side that has been exposed to the sun. The fruits are medium-sized, and the flesh of the fruit is white, quite juicy, and aromatic. This variety is widely used among the population and is valued.
The Butira variety is one of the better early pears and ripens in late July. The fruits are similar to the fruits of Santa Maria with a greenish-yellow color and a reddish part on the sunny side. The flesh of the fruit is white with a very sweet, juicy, and refreshing taste, with a very pleasant aroma. This variety is of exceptional quality and is one of the better varieties of pears.


Photo 2: Viljamovka


Photo 3: Santa Maria


Photo 4: Butira

The fruits were taken for analysis during the period of their full maturity, ie in the range of one month, from the end of July to the end of August 2021. 30 ripe fruits were taken from each variety, which were delivered for the determination of physical and chemical parameters.
Physical characteristics, average weight, height, fruit width, stalk length, and the number of stones was made in the laboratory of the Faculty of Agromediterranean, University "Džemal Bijedić" in Mostar. Measurements were performed on an individual sample, after which the average value was taken. Fruit weight was determined by weighing samples using a Mettler tolego P1210 technical balance. Fruit dimensions, height, fruit width, and stalk length were measured with a caliper, while the number of pits was determined by counting.
After the physical measurements, the samples were delivered to the laboratory "Control-H" for chemical analysis. Each of the investigated chemical parameters was determined by the method prescribed by law.
The pH value was determined by the Sl.l.SFRJ 29/83 technique, ie a method based on measuring the potential difference between two electrodes immersed in the tested liquid. The amount of sample sufficient for the electrodes to be immersed is taken for testing, depending on the apparatus used. The electrodes were immersed in the tested quantity of the sample to be tested and the pH-meter temperature correction system was adjusted to the measuring temperature. Further measurement was performed according to the instructions of the pH meter used. The pH reading was performed directly on the scale of the instrument, with an accuracy of 0.05 pH units to a constant value. At least two determinations shall be made on the same test sample.

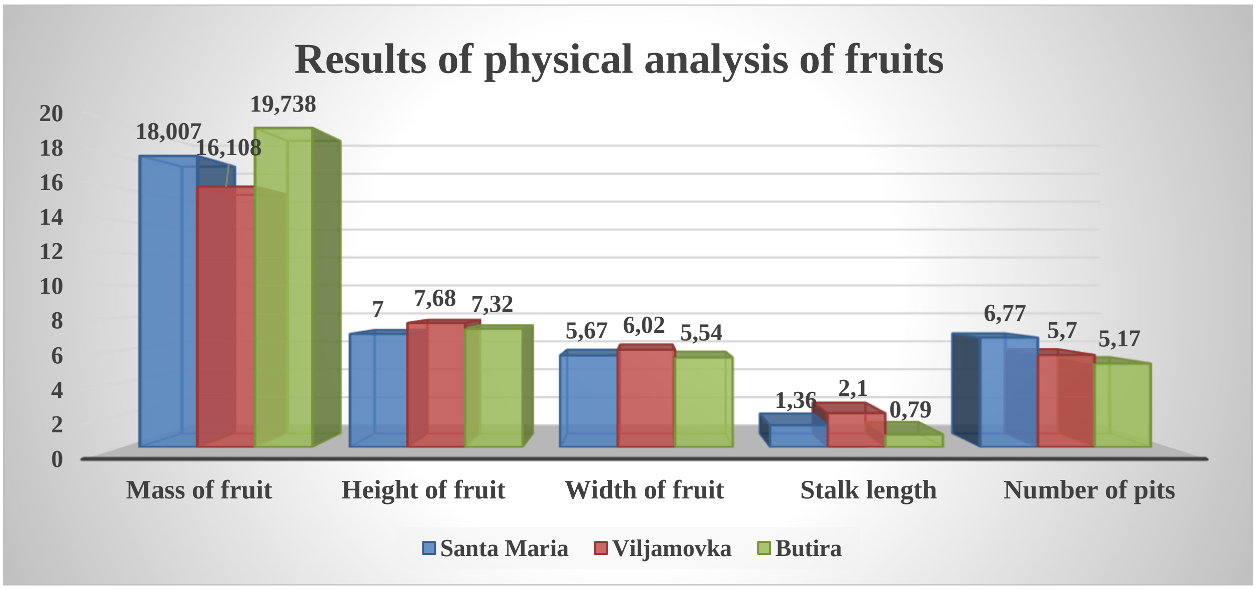
The amount of soluble dry matter was determined by the technique of BAS ISO 2173: 2008, ie by a refractometric method based on the physical law of refraction of light. The dry matter reading is performed on a refractometer scale at a temperature of 200 $^{\circ}$C.
The number of total inverts was determined by the technique Sl.l.SFRJ 29/83, this method is based on the principle that under certain conditions reducing sugars (natural invert) convert cuprous sulfate ($CuSO_4$) from Luff's solution to copper oxide (Cu_2O). The unspent amount of cupric ion retreats with thiosulfate solution. The non-reducing disaccharide (sucrose) must first be inverted or hydrolyzed to reduce monosaccharides by acid and then determined by Luff's solution. Thus, data on the total amount of sugar in the tested sample (total invert) are obtained. The difference between the total invert obtained and the natural invert gives the amount of reducing sugars formed by sucrose inversion.
The value of total acids was determined by the Sl.l.SFRJ 29/83 technique, by a potentiometric method based on titration with sodium hydroxide solution. The method is used to determine the total acidity in fruits and vegetables and fruit and vegetable products.

Results and Discussion

The following physical characteristics were determined on the collected fruits of pear varieties Viljamovka, Santa Maria and Butira: fruit weight, fruit height and width, stalk length and number of stones.

Table1: Average values of the results of physical analysis of fruits

Variety	Fruit mass g	Fruit height cm	Fruit width cm	Stalk length cm	Number of pits
Santa Maria	18.007	7.00	5.67	1.36	6.77
Viljamovka	16.108	7.68	6.02	2.10	5.70
Butira	19.738	7.32	5.54	0.79	5.17



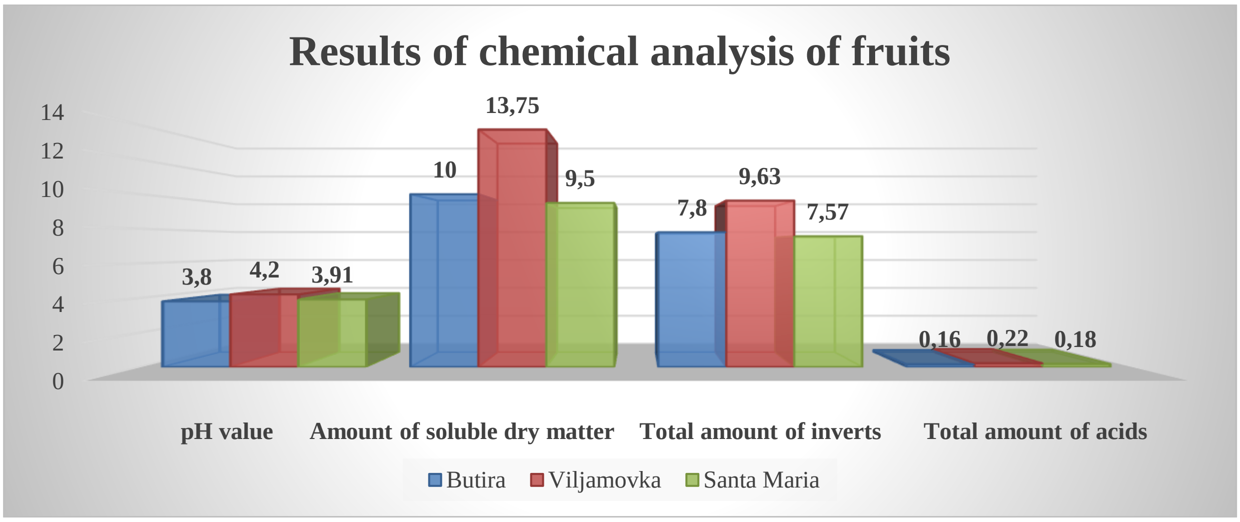
Graph 1: Results of physical analysis of fruits

Comparing the obtained results, it can be seen that the examined parameters in all three varieties show very approximate physical characteristics. According to the obtained values of fruit weight, we can see that the fruits of the Butira variety have the highest average weight of 19.738 g. Measurement of fruit height gave approximate values ranging from 7 cm in the Santa Maria sort to 7.68 cm in the Viljamovka variety. The width of the fruit is also quite

equable, from 5.54 cm in the Butira variety to 6.02 cm in the Viljamovka variety. As expected, the length of the stalk was the largest in the Viljamovka variety with 2.1 cm, because it is its varietal specificity, while the smallest was measured in the cultivar Butira. The number of seeds was quite equable in all three cultivars and ranged on average from 5.17 in the cultivar Butira to 6.77 in the cultivar Santa Maria.
From the chemical properties in the caused varieties of pears were determined: pH value, amount of dry matter, amount of sugar and the amount of total acids.

Table 2. Results of physical analysis of fruits

Sort	pH value	Amount of soluble dry matter	Amount of total inverts	Amount of total acids
Butira	3.80	10.00	7.80	0.16
Viljamovka	4.20	13.75	9.63	0.22
Santa Maria	3.91	9.50	7.57	0.18



Graph 2: Results of chemical analysis of fruits

From the results shown in Table 2 and Graph 2, it can be seen that the variety Viljamovka had the highest values of all tested chemical parameters. The fruits of the Butira variety had average values in the examination of the amount of dry matter and total inverts, while this variety had the lowest pH values and the number of total acids. The Santa Maria variety showed the lowest values of the amount of soluble dry matter and total inverts, while the results of pH and total acids had a mean value of the tested varieties.

Conclusions

After the performed analysis, and based on the results of determining the physical parameters, it can be stated that the highest fruit weight was in the variety Butira and the lowest in the fruit variety Viljamovka. The height and width of the fruit, as well as the length of the stalk, had the highest value in the variety Viljamovka. The fruits of the Santa Maria variety had the highest average number of pits.The results of the chemical analysis showed that the fruits of the Vilmovka variety had the highest values of all the examined parameters. The fruits of the Santa Maria variety had the lowest value of soluble dry matter and total inverts, while the fruits of the Butira variety had the lowest measured pH and the number of total acids.

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EXAMINATION OF THE VIABILITY OF BARLEY SEEDS USING THE TETRAZOLIUM TEST

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Abstract

For seed quality control of various plant species sinc most commonly used tetrazolium test because it allows a rapid evaluation of viability. Freshly harvested barley seeds show dormancy that can make the germination test ineffective for an immediate evaluation. Therefore, the development of more efficient methods, such as the tetrazolium test, is necessary. The objective of this research work was to study various procedures for performing the tetrazolium test on barley seeds. Seeds cultivar Simonida were used and subjected to the following treatments: seed preconditioning with direct immersion in H_2O 18 hours; preparation for staining -longitudinal cross-section of the seed through the embryo with disposal of one of the halves and staining the other half and second, seeds were longitudinally cross-sectioned through the embryo, staining the two halves. Two methods of staining on top of filter paper and direct immersion in the tetrazolium salt solution. Three concentrations of the tetrazolium salt solution (0.1%, 0.5%, and 1.0%) were used. It was concluded that the tetrazolium test on barley seeds may be accomplished with preconditioning by direct immersion in H_2O and staining on top of filter paper moistened with solution at a 0.5% concentration of the tetrazolium salt.

Key words: *Hordeum vulgare, tetrazolium salt, concentrations.*

Introduction

Barley (*Hordeum vulgare* L.) grown in a variety of environments, is the fourth largest grain crop globally, after wheat, rice, and corn. Barley is commonly used in breads, soups and health products, though it is primarily grown as animal fodder and as a source of malt for alcoholic beverages, especially beer. The overall importance of barley as a human food is minor but there is much potential for new uses exploiting the health benefits of whole grain and beta-glucans. To guarantee adequate agricultural production, the use of known quality seeds is fundamental; and their physiological potential have to be constantly monitored, starting from the preharvest, passing through the processing unit the end of the storage period. As emphasized by Tunes et al. (2009), the tetrazolium test is important on seed quality control, as it allows a fast estimate of the seed germination capacity, including the dormant ones. The test is based on the dehydrogenase enzymes activity that catalyzes the respiratory reaction in the mitochondria, correlating seed viability with changes on the color of living tissues. In this sense, there is an oxireduction reaction with the 2,3,5-triphenyl tetrazolium chloride (França-Neto, 1999), which results in the formation of a stable and non-diffusible compound of reddish coloration, the formazan. Such formation indicates respiratory activity in the mitochondria and allows delimiting living tissues from those tissues that remain unstained or exhibit abnormal coloration (Marcos-Filho, 2005). The test can be affected by given conditions such as: the presence of fungi, which can impair the germination test results; focuses on the embryos physic and physiological conditions of each seed; allows for rapidly evaluating the seed viability; enables the identification of different viability levels

for some species as common bean and soybean; is able to provide a diagnosis for the cause for seed viability loss; and requires simple and low cost equipment (França-Neto, 1999). The evaluation of seed viability by tetrazolium test is routinely used in quality control programs for various species, including soybean (França-Neto et al., 1998), corn (Dias and Barros, 1995), watermelon (Bhering et al., 2005), tomato (Santos et al., 2007), coffee (Zonta et al., 2009), cucumber (Lima et al., 2010), triticale (Souza et al., 2010), among other crops.
The International Seed Testing Association (ISTA, 2007) recommends the preconditioning of barley seeds by the immersion in H_2O during 4 and 18 h, at a 20 ºC temperature and then carry out the staining procedure for 3 h, at 30 ºC in a 1% tetrazolium chloride solution. In relation to splitting the seeds, a longitudinal cross-section of the embryo and ¾ of the endosperm, as well as a transversal cross-section, with the elimination of one of the halves, is recommended. The Rules for Seed Testing (Brasil, 2009) also recommend the preconditioning for 18 h, between sheets of paper towels moistened with H_2O and staining on top of filter paper moistened with a 0.5% tetrazolium salt solution. Seed quality control is improved with the use of the tetrazolium test in all phases of seed production such as harvesting, receiving, before and after seed processing and drying, during storage and before sowing. The test has been applied with success even before harvest: mature plants or plant parts are daily sampled from the seed production field about six to seven days before harvest; pods or other fruit structures are hand threshed, and seeds are then taken for analysis. The tetrazolium test will provide information on viability, vigor and weathering. Therefore, the objective of the present study was to investigate efficient procedures for evaluating the viability of barley seeds using the tetrazolium test.

Materials and methods

Experiment was carried out in the 2021 in Laboratory of the Faculty of Agriculture in East Sarajevo (Republic of Srpska, Bosnia and Herzegovina). To evaluate the viability of the seeds, three concentrations of 2,3,5-triphenyltetrazolium chloride (0.1%, 0.5% and 1%) were used. Barley seeds were used and subjected to the following treatments: seed preconditioning (direct immersion in H_2O 18 hours on 20ºC).
Seed preparation – seeds were longitudinally cross-sectioned through the embryo with disposal of one of the halves and staining the other half (Brasil, 2009; ISTA, 2007) and seeds were longitudinally cross-sectioned through the embryo, staining the two halves.
Staining procedures– seeds were placed in the dark for 2 and 3 h with a tetrazolium salt solution at the concentrations of 0.1%, 0.5%, and 1.0%. The staining method varied according to the type of preparation: immersion of one seed half into 5 mL of a 2,3,5 triphenyl tetrazolium chloride (TTC), in a 100 mL capacity Beaker, for staining at 30 ºC temperature 3 hours (Brasil, 2009; ISTA, 2007). The two seed halves were placed on top of a sheet of filter paper moistened with the tetrazolium salt solution equivalent to 2.5 times the mass of the paper and put for staining under 40 ºC temperature 2 hours. The seeds were removed from the chamber, washed under tap water. Seeds were classified as viable and non-viable according to the coloration of the embryonic axis, computing only the percentage of viable seeds. A completely randomized experimental design, with three replications, was used for the experiment. Data were subjected to analysis of variance (ANOVA) and the differences among treatments were analyzed with least significant difference (LSD) test.

Results and Discussion

Therefore, it was verified that all dilutions showed results in terms of the tetrazolium solution used; however, the biggest highlight is for the use of tetrazolium salt. In dead tissues, where there is no respiratory activity, dehydrogenase enzymes are inactive; therefore, the reaction with the tetrazolium solution does not occur and consequently, the seeds remain discolored. For seeds in deterioration process the development of the coloration is faster, generating a more intense and deep red tone, while the vigorous seeds have a bright appearance with pink to red coloration (Marcos - Filho et al., 1987; França-Neto et al., 1998). On Table 1, the results of the tetrazolium test on the viability of barley seeds, obtained with preconditioning by immersion in H_2O, for 18 h, and staining on top of filter paper moistened with tetrazolium salt solution and by immersion in that same solution are shown.

Table 1. Viability (%) determined by the tetrazolium test in barley seeds, carried out with preconditioning by immersion in H_2O for 18 h and staining on top of filter paper and by immersion in solution triphenyl tetrazolium chloride

Preconditioning by immersion in H_20 (18 h)	
Staining method	Viability (%)
By immersion (3h) 30ºC	40.88
On filter paper (2h) 40 ºC	46.55**
LSD 5%	1.72
LSD 1%	2.55

Means with *were significantly different at P<0.05
Means with **were highly significantly different at P<0.01

On Poaceae seeds as barley the vital areas for viability evaluation are: plumule; coleoptile; central region of the scutellum; radicle; and seminal roots region (Dias and Barros, 1995). In that way, the viable seed displayed a coloration ranging from light carmine to red at the vital areas and the non-viable. The preparation procedure using the two halves of seeds for staining on top of filter paper also allowed improving seed viability evaluation, since the two halves are analyzed. When only one of the halves is evaluated, doubts may be raised about seed viability, once cross-sectioning the embryo exactly in the middle it is difficult due to the small seed size. Such fact has also been verified by Dias and Alves (2009) for seeds of *Brachiaria* spp. and *Panicum maximum* (and verified by Souza et al., for seeds of black oats (2009), triticale (2010) and white oats (2010). Staining of seeds on filter paper for 2 hours at 40 ºC showed a statistically very significant difference compared to staining of seeds by immersion in glasses for 3 hours at 30 ºC.

Table 2. Viability (%) determined by the tetrazolium test in barley seeds, carried out with preconditioning by immersion in H_2O for 18 h and staining in 2,3,5 triphenyl tetrazolium chloride solution using different concentrations of the salt.

Preconditioning by immersion in H_20 (18 h)	
Concentration of the tetrazolium salt solution	Viability %
0.1%	35.6
0.5%	47.5**
1%	48.0**
LSD 5%	2.12
LSD 1%	3.15

Means with * were significantly different at P<0.05
Means with **were highly significantly different at P<0.01

The coloration of the seeds with tetrazolium salt 0.5% and 1% allowed to evaluate the viability without difficulty, it was observed that the embryo has a stronger and more prominent coloration in relation to the rest of the seed; however, in the concentration of 0.5% the coloration was more pronounced, but still the reading was made without difficulty. When the 1% solution was used, it was observed that the high concentration hindered the interpretation of the viability and caused excessive coloration. Tetrazolium salt concentrations of 0.5 and 1% showed a statistically highly significant difference results compared to a concentration of 0.1%.

Table 3. Viability (%) determined by the tetrazolium test in barley seeds, carried out with preconditioning by immersion in H_2O for 18 h and staining on top of filter paper moistened with a 2,3,5 triphenyl tetrazolium chloride solution and by immersion in that solution, using different concentrations of the salt.

Preconditioning by immersion in H_20 (18 h)		
	Staining method	
Conc. of tetrazolium salt solution	By immersion (3h) 30ºC	On filtet paper (2h) 40 ºC
	Viability (%)	
0.1%	30.6	40.6
0.5%	45.3**	49.6**
1%	46.6**	49.3**
LSD 5%	2.99	
LSD 1%	4.44	

Means with * were significantly different at P<0.05
Means with **were highly significantly different at P<0.01

When the treatment with 1% salt concentration was used, the coloration was adequate for the embryo, indicative of viability. Possibly the chemical constitution of the species is very different from each other, which refers to the need to use salt concentrations also differentiated. Craviotto et al. (2011) confirmed that the method with 0.5% tetrazolium at 30°C was considered efficient for soya coloring. In barley, treatment with 0.5% salt concentration on filter paper at 40°C proved to be better compared to other treatments.

Conclusions

The tetrazolium test is promising for the fast viability evaluation of barley seeds using the methodology of preconditioning by direct immersion in H_2O for 18 h, at 20 ºC, staining seeds on filter paper showed better results compared to dipping half of the seeds in a glass solution regardless of the method of staining. The tetrazolium test using the concentration of 0.5% at a temperature of 40 °C for 2 h on filter paper in an oven is efficient to evaluate the viability of seeds of *Hordeum vulgare* L.

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VARIABILITY OF GLUTEN PROTEINS IN WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Gluten proteins are formed from proteins of flour, gliadin and glutenins which in contact with water, begin to interact through the formation of chemical bonds. The aim of this study is identification of encoding genes polymorphisms of gliadin and glutenins in 10 bread wheat genotypes. For analysis used 30 seeds of 10 wheat genotypes for extraction of gliadins by 70% ethanole, and glutenins by 10% β-mercaptoethanol.The gliadins were separated by acid page electrophoresis (pH=3.1) on 8.33% polyacrylamide gel, while glutenins were separated by SDS-PAGE (pH-8.6) on 11.8% gel. Electrophoregrams were used for determining *Gli-1* and *Gli-2* alleles. The three alleles (***a, b, m***) at the *Gli-A1*, four alleles (***b, g, l, k***) at the *Gli-B1*, five alleles (***a, b, f, g, k***) at the *Gli-D1*, five alleles (***b, e, f, g, k***) at the *Gli-A2*, four alleles (***b, h, j, p***) at the *Gli-B2* and three alleles (***a, b, r***) at the *Gli-D2* locus were identified. For high molecular weight glutenin subunists (HMWGS) the three alleles (***a, b, c***) at the *Glu-A1*, three alleles (***b, c, d***) at the *Glu-B1* and two alleles (***a, d***) at the *Glu-D1* were identified. Gluten proteins varied according to composition alleles encoding gliadin and glutenins in analyzed wheat genotypes what related with established polymorphisms of each gliadin and glutenin loci.

Keywords: *wheat, gliadin, glutenin, allele, polymorphism quality.*

Introduction

Gluten is complex group of proteins consisting gliadins and glutenins, approximately in equal amount (Wrigley et al., 200). Gliadin and glutenins are deposited in endosperm of grain which, are important in determining quality of flour, dough and bread (Knezevic et al. 2017). Hydrated gliadin and glutenins interact through the formation of chemical bonds and begin to stick to each other and forms a very extensible, elastic structure that is responsible for the gas-holding ability of bread dough. and determines the viscoelasticity, strength, resilience and stretchability of the dough (Menkovska et al., 2002; Shewry, 2007; Torbica et al., 2007). Gliadins are a heterogeneous group of proteins which contain different type of polypeptide molecules (α-, β-, γ- and ω-gliadins), globular conformation, with intra disulfide bonds single chains (Bietz, 1997) and most of them have molecular mass (16kDa to 50kDa). Gliadin are encoded by genes located on the short arm of 1. and 6. group of A, B and D chromosomes (Sozinov and Poperelya, 1980) i.e. loci *Gli A*1, *Gli B*1, *Gli D*1, *Gli A*2, *Gli B*2 and *Gli D*2 respectively, which characterized families of multiple alleles (Metakovsky, 1991;

Metakovsky et al, 2018).. Polymorphisms of gliadin alleles in Russian, French, Yugoslav, Italian, Spanish, wheat cultivars were established (Metakovsky et al, 1991; 1994; 1997; 2000). The glutenins contain two types of polypeptides, one type with low molecular weight 20kDa to 50kDa (LMW GS) and shorter, and another with high molecular weight 50kDa to 200kDa (HMW GS) or more. The glutenin proteins characterize intermolecular disulfide bonds between polypeptide. The HMW-GSs are encoded by three loci, *Glu-A1*, *Glu-B1*, *Glu-D1*, located on long arm of chromosomes (Payne et al., 1987; Knežević et al., 1993), and LMW-GSs are encoded by genes located on the short arm of *Glu-A*3, *Glu-B*3, *Glu-D*3.
The aim of this study was identification (i) alleles at *Gli-1*, *Gl-2* loci encoding gliadin proteins (ii) *Glu-1* loci encoding high-molecular weight (iii) determination variability of gliadin allele composition and (iv) determination variability of glutenins allele composition in analyzed wheat genotypes.

Material and methods

The 10 genetically divergent wheat genotypes (G-3626-1, G-3618-2, G-3606-4, G-3636-3, G-3627-1, G-3621-1, G-36-6-5, G-3607-5, G-3606-6, G-3632-1) were included for analysis variability of gliadin and glutenins on the base of identification encoding gene alleles.
At least 30 single seeds were used for extratction gliadin proteins in 70% ethanole at room temperature for one hour. After that samples centrifuged at at 5000 rpm for 20 min. For separation of gliadins used acid PAG electrophoresis method developed by Novoselskaya et al. (1983). Gliadin extract (20 µl) were loaded on the gel was performed in 8.33% polyacrylamide (12.5 g acrilamid, 0.62 g N,N'-methylenebisacrylamide, 0.15 g ascorbin acid, 200 µl 10% ferosulfate heptahydrate, diluted in 150 ml Al-lactate buffer pH=3.1) Electrophoresis was performed during 2.5 to 3 hours, in electric field under constant voltage from 550 V and in 5 mM aluminum lactate buffer. The separated gliadin bands were stained in 0.05% ethanol solution of Coomassie Briliant Blue R250 by adding 250 ml 10% threechloroacetic acid (TCA) and after that gels photographed. Gels and photographs were used for determination of gliadin blocks alleles according to method Metakovsky (1991).
For gluttenin extraction used residue of the same kernel sample, which treated by 120 mM Tris-HCl, pH=6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiled for 5 min. The sample were centrifuged at 12000 rpm for 10 min. Protein resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) Laemmli, (1970) with 11.8% gel and electrophoresed at 20mA for 2h. Gels were stained by Commassie Briliant blue dye resloved in 10% TCA and 250ml methanols. After staining, the electrophoregrams are used for analysis and determining HMW-GS and identification of *Glu-1* alleles (Payne and Lawrence, 1983).

Results and discussion

Gliadin alleles variability encoding gliadin proteins. The sudy of gliadin allele composition at *Gli-A1* and *Gli-A2* loci showed differences among the analyzed wheat genotypes. In ten wheat genotypes were identified 24 alleles at six *Gli*-loci, three of them (***a, b, m***) at *Gli-A1*, four alleles (***b, g, l, k***) at *Gli-B1*, five alleles (***a, b, f, g, k***) at *Gli-D1*, five alleles (***b, e, f, g, k***) at *Gli-A2*, four alleles (***b, h, j, p***) at *Gli-B2* and three alleles (***a, b, r***) at *Gli-D2* locus (table 1).
In analysis in some genotypes identified heterozygosity of some gene loci. The two different alleles at two loci identified in the genotype G-3606-4 at the locus *Gli-D1* (***b***+***g***), at *Gli-A2* (***g+e***) and in genotype G-3627-1 at the locus *Gli-A1* (***m***+***a***), at *Gli-D1* (***b***+***a***). Also, in two genotypes identified two different alleles at one locus and with the genotype G-3607-5 at the locus *Gli-A2* (***k+g***) and in genotype G-3606-6 at the locus *Gli-D2* (***a***+***b***) table 1.

The hetrozigosity indicates that wheat genotypes are not genetically homogenized for specified loci, which requires further selection in order to achieve genetic homozygosity of specified loci. The gliadin allele polymorphisms of each ***Gli-1*** and ***Gli-2*** loci was established in numerous invetigation of wheat varieties (Knežević et al., 2006; 2007; 2008; Knezevic et al., 2017, Metakovsky et al., 2018; 2021; Utebayev et al., 2019).

Table1. Gliadin and glutenin allele of winter wheat genotypes

Genotype	Gli- alleles						High molecular weight glutenin subunits			Glu-1 alleles			Glu-1 quality score
	A1	B1	D1	A2	B2	D2	1AL	1BL	1DL	**A1**	**B1**	**D1**	
G-3626-1	a	b	a	k	b	a	2*	7+9	5+10	**b**	**c**	**d**	**9**
G-3618-2	m	l	k	b	?	a	2*	7+8	5+10	**b**	**b**	**d**	**10**
G-3606-4	a	k	b+g	g+e	h	a	N	7+9	2+12	**c**	**c**	**a**	**5**
G-3636-3	b	l	a	k	j	b	N	6+8	2+12	**c**	**d**	**a**	**4**
G-3627-1	m+a	b	b+a	b	p	r	1	7+9	5+10	**a**	**c**	**d**	**9**
G-3621-1	a	l	b	f	h	a	2*	7+9	5+10	**b**	**c**	**d**	**9**
G-3606-5	b	g	b	g	b	b	2*	7+8	5+10	**b**	**b**	**d**	**10**
G-3607-5	b	b	f	k+g	b	b	N	7+9	2+12	**c**	**c**	**a**	**5**
G-3606-6	b	k	g	e	h	a+b	N	6+8	2+12	**c**	**d**	**a**	**4**
G-3632-1	a	l	a	f	b	b	1	7+9	5+10	**a**	**c**	**d**	**9**

Glutenin alleles variability encoding high-molecular glutenin proteins However, in those ten wheat genotypes were identified eight alleles at the *Glu-1* loci, three of them (***a, b, c***) at the *Glu-A1*, three (***b, c, d***) at the *Glu-B1* and two alleles (***a, d***) at the *Glu-D1* locus (table1).
The relationship between *Glu-1* alleles of the HMWG subunits and the bread-making quality was determined (Payne, 1987; Lafiandra, et al., 1987;Metakovsky et al., 1990). For each allele at the three *Glu-1* loci, assigned mark for contribution to quality score in assesing bread making quality. The highest mark 4 determined for alleles ***d*** at *Glu-D1*, while mark 3 is for alleles ***a, b,*** at the *Glu-A1*, as well for ***b, i, f*** at the *Glu-B1*. Mark 2 determined for alleles ***a,*** at the *Glu-D1* and ***c***, at the *Glu-B1*, while the lowest mark 1 determined for alleles ***c***, ***Glu-A1,*** and ***a, d, e,*** at the ***Glu-B1*** (Payne and Lawrence 1983).
In our study the highest *Glu*-1 quality score varied between 4 and 10. The highest value of *Glu*-1 quality score established in two genotypes G-3618-2 and G-3606-5, while the lowest in G-3636-3 and G-3606-6 (table 1).
Frequency of identified alleles at Gli-1, Gli-2 and Glu-1. The frequency of identified gliadin alleles was different. At the ***Gli-A1*** locus the highest frequency computed for two alleles ***a, b*** (40.0%), while the lowest had allele ***m*** (20%). At the ***Gli-B1*** locus the highest frequency had allele ***l*** (40.0%), lower had alleles ***b*** (30%) and ***k,*** (20%), and the lowest had allele ***g*** (10%). At the ***Gli-D1*** locus the most frequent was allele ***b*** (40.0%), lower frequency had allele ***a*** (30%)**,** and the lowest frequency had alleles ***f, g, k*** (10%). At the ***Gli-A2*** locus the most frequent was allele ***k*** (30.0%), while three alleles ***b, f, g*** had frequency (20.0%) and the lowest frequency had allele ***e*** (10%). At the ***Gli-B2*** locus the most frequent was allele ***b*** (40.0%), while the lowest and equal frequency had alleles ***j, p*** (10%). At the ***Gli-D2*** locus the most frequent was allele ***a*** (50.0%) and the lowest frequency had allele ***r*** (10%), while high frequent was allele ***b*** (40%) table 2.
The frequency of glutenin alleles varied at all three loci. At the ***Glu-A1*** locus the highest and equal frequency found for alleles ***b, c*** (40.0%), while the lowest had alleles ***a*** (20%). At the ***Glu-B1*** locus the most frequent was allele ***c*** (60.0%), while the lowest and equal frequency

had alleles ***b, d*** (20.0%). At the ***Glu-D1*** locus the highest frequency had allele ***d*** (60.0%), while the lowest frequency had alleles ***d*** (40%) table 2.

The different frequency of the *Gli-1*, *Gli-2* and *Glu-1* allele may be the result of a directed selection of the genotype according to some desirable component of quality and yield or adaptability to biotic and abiotic factors, which also indicates the associability of the identified gliadin alleles with desirable traits as for example: frost, resistance, resistance to diseases, grain hardness, flour and dough quality, lipid composition and starch properties etc. In some cases, high allele frequency is results of using parent varieties that carry low genetic variability at certain loci. Differences in allele frequencies are interpreted in a similar way in other studies (Knezevic et al., 1998; 2017; Lookhart et al., 2001; This et al., 2001).

Table 2. Frequency of alleles at *Gli-1*,*Gli-2* and *Glu-1* loci

Gliadin alleles												Glutenin aleles					
Gli-A1		*Gli-B1*		*Gli-D1*		*Gli-A2*		*Gli-B2*		*Gli-D-2*		*Glu-A1*		*Glu-B1*		*Glu-D1*	
Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%
a	40	*b*	30	*a*	30	*b*	20	*b*	40	*a*	50	*a*	20	*b*	20	*a*	40
b	40	*g*	10	*b*	40	*e*	10	*h*	30	*b*	40	*b*	40	*c*	60	*d*	60
m	20	*k*	20	*f*	10	*f*	20	*j*	10	*r*	10	*c*	40	*d*	20		
		l	40	*g*	10	*g*	20	*p*	10								
				k	10	*k*	30	?	10								

Conclusion

The variability of gluten proteins, based on identified alleles at gliadin and glutenin loci. The polymorphism of each *Gli-1*, *Gli-2* and *Glu-1* locus was identified. A different number of alleles were identified at each locus. In ten wheat genotypes, at the six gliadin loci were identified 24 different alleles, while at three *Glu-1* loci were identified eight different alleles. The highest polymorphisms were established at the *Gli-D1* and *Gli-A2* locus, on which five different alleles were identified in analyzed ten wheat genotypes. Frequency of identified gliadin alleles varied between 10 and 50% and for glutenin alleles between 20 and 60%. The most frequent alleles are *Gli-A1a, Gli-B1l, Gli-D1b, Gli-A2k, Gli-B2b, Gli-D2a*, *Glu-A1b, Glu-B1c* and *Glu-D1d*. Composition of gliadin and glutenin alleles was different and specific for each wheat genotype and can be used as reliable marker for quality traits in breding program considering.

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NUTRITIONAL AND PRODUCTION PROPERTIES OF TRITICALE DEPENDING ON THE AMOUNT OF NITROGEN FERTILIZER

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Abstract

The great possibility of using triticale for different purposes, as well as the pronounced variety differences, impose the need for a more complete study of its varieties with the aim of their more efficient use in wide production. The aim of this paper is to present the results regarding the yield and protein content in grain obtained at four triticale varieties, studied during two years, under different levels of fertilization with nitrogen. In this regard, four triticale varieties were conducted in northern Montenegro to research the possible influences of nitrogen levels on grain yield and grain protein content. Nitrogen was used in the following quantities: N_0 (control), N_1 (60 kg ha-1), N_2 (80 kg ha-1), N_3 (150 kg ha-1), where 1/3 of the planned amount was used together with sowing, and the rest of the planned amount in top dressing in the early spring. The results of the research showed the differences between varieties in response to a variation in N level. The highest performance of grain yield of four cultivars was associated with the highest amount of nitrogen (150 kg ha^{-1}).
The cultivar Triumph achieved the highest grain yield in both years of testing. The lowest grain yield in the first year was in the varieties Kg-20 and Favorit, and in the variety Kg-20 in the second year.The lowest protein content in the grain was recorded in the variety Triumph.

Key words: *Triticale, Nitrogen, Variety, Grain yield, Protein content.*

Introduction

Created with the idea to combine the positive characteristics of the parental species (wheat and rye), triticale has a high genetic potential for yield with a pronounced tolerance to biotic and abiotic stresses (Epure et al., 2015). Characterizing it as a species that is suitable for cultivation in areas where soil characteristics and climate conditions are a limiting factor, but also for intensive technological conditions, many triticales are classified as grains species that belong to the future (Dumbravă et al., 2014).
In addition to having the potential to give good yields in moister stressed conditions (Drăghici, 2012), also it has the ability to produce increased grains and biomass yields under various types of soils and climatic conditions (Burdujan et al., 2014). Dumbravă et al. 2016 underlined that triticale reacts more favorably with higher yields than those obtained with wheat and corn where intensive technologies are applied in limited soil and climatic conditions.
Increased grain yield and protein content are two important goals in cereal production (Gulmezoglu and Aytac, 2010). Total protein content is the most important chemical parameter of a complex grain quality. This property is important whether the grain is intended for human or domestic animal consumption, because the nutritional value of the grain, and thus the endproduct, depends on the protein content.

Basically, the protein content in the grain primarily depends on the nitrogen content in the soil. The lack of nitrogen in the soil has a negative impact on plant growth, causing lower protein content in the grain, which ultimately results in lower grain yield and lower protein yield per unit area (Zheng et al., 2009). Ivanova and Tsenov (2014 a,b) pointed out that the conditions of the year and the level of fertilization are decisive factors for the formation of the yield in new varieties of triticale.

The protein content in the grain is the result of a complex interaction between grain yield and temperature, nitrogen and water availability (Gulmezoglu and Aytac, 2010). As stated by Filipčev et al. (2005) and Đekić (2010) protein content in grain is a property of genotype and its inheritance is controlled by factors of complex nature, and the content largely depends on environmental conditions.

Climatic elements, especially the amount of precipitation and temperature, have a strong influence on the protein content of grain, and the difference in protein content of grain in different years is due to different amounts and distribution of precipitation during grain filling, which is considered a key environmental factor (Garrido-Lestache et al., 2004). The aim of this study was to determine the effects of varieties and different doses of nitrogen on the grain yield and grain protein content of triticale during two years.

Materials and Methods

Field experiment was conducted during two years (2018/19 and 2019/20) on the territory of the municipality of Bijelo Polje in the north of Montenegro. The experiment was set up by random block system in three repetitions, with the size of an elementary plot of 10 m^2 (5 x 2 m). The previous crop in the experimental fild was corn. Four winter triticale varieties were used as research material (Kg-20, Favorit, Triumph and Knjaz). The experiment included control (without fertilization) and three variants of nitrogen fertilization in quantities of 60 (N_1), 80 (N_2) and 150 kg ha^{-1} (N_3) where 1/3 of the predicted amount of nitrogen fertilizer was introduced into the soil together with the basic land cultivation while the rest of the nitrogen fertilizer is given in top dressing at the beginning of March at Zadoks growth stage 30 (pseudo stem erection). Nitrogen was applied in the form of mineral fertilizer KAN (27% N). In both years of research, sowing was done manually in the optimal time, in the second decade of October, where the row spacing was 12.5 cm, and the distance in the row was 3 cm. Harvesting of triticale was performed in the phase of full maturity, during which the yield from each individual plot was measured, corrected to 14% moisture and converted to yield in t ha^{-1}. Before harvest, plant samples were taken for analysis (50 plants from each plot). Classes were hardened, dried and ground to determine the total nitrogen content using the Kjeldahl method. The protein content was calculated by multiplying the obtained values for the concentration of total nitrogen in the grain by a coefficient of 6.25.

Grain yield and protein content in the grain are presented in average values and statistically processed by the method of analysis of variance using the WASP 2.0 statistical package. The significance assessment for the obtained values for grain yield and protein content in grain was tested using the LSD test.

Soil and climatic conditions

Before starting the experiment, soil samples were taken and their chemical analysis was performed. Based on the obtained results, it was determined that the soil on which the experiment was performed was weakly carbonate with the content of total carbonates from 5.14 to 5.18, acid reaction pH_{H2O} = 6.23, quite humus (3.87-4.29) and with low content of phosphorus (5.56-7.84 mg 100 g^{-1} soil) and potassium (4.56-4.03 mg 100 g^{-1} soil).

Table 1. Middle monthly air temperature (°C) and precipitation amount

Year	Month										Average-Sum
	X	XI	XII	I	II	III	IV	V	VI	VII	
Middle monthly air temperature (°C)											
2018/19	13.5	7.0	0.8	-1.2	2.9	7.0	11.8	13.2	20.6	20.5	9.6
2019/20	12.5	9.7	3.2	-0.6	4.6	6.7	12.0	16.1	18.7	21.3	10.4
1991-2020	10.8	5.5	0.8	-0.4	1.6	5.7	10.4	14.6	18.4	20.1	8.7
Precipitation amount (mm)											
2018/19	38.0	119.7	49.1	71.9	60.2	13.6	44.0	62.3	125.1	97.7	681.6
2019/20	45.4	142.3	189.9	17.3	52.6	73.2	37.6	74.3	40.3	117.8	790.7
1991-2020	85.7	100.0	89.9	63.6	77.6	67.7	70.9	81.4	72.4	69.3	778.5

Results and disscusion

The data in Table 1 clearly show that the years in which our research was conducted differed with respect to meteorological conditions both among them and in relation to the multi-year average characteristic of Bijelo Polje. Average air temperature for the observed period 2018/19. and 2019/20. was higher by 0.9 and 1.7 ° C, respectively, and the amount of precipitation in the same period was 96.9 mm lower in the first and 12.2 mm higher in the second compared to the values of the multi-year average. October, November and December of the 2019/20 production year were marked by a higher amount of precipitation compared to the first production year, but also by a multi-year average, which had a positive effect on germination, germination and initial growth of plants and created soil moisture reserves. On the other hand, during the first production year, a significant amount of precipitation was observed during May-July (from the beginning of earing until the harvest) with almost daily rain in the ripening period.

Nitrogen mineral nutrition shows an extremely large influence on grain yield and protein content in winter triticale grain. The average values of grain yield of the tested triticale cultivars depending on the dose of applied nitrogen during the two - year research period are shown in Table 2.

Table.2. Grain yield of the tested triticale varieties depending on the dose of applied nitrogen (t ha^{-1})

Varieties (A)	N applications									
	2018/19					2019/20				
	0	N1	N2	N3	Average	0	N1	N2	N3	Average
Kg-20	3.12	3.98	4.43	4.71	4.06	3.95	4.52	4.86	4.93	4.56
Favorit	3.10	4.12	4.37	4.66	4.06	4.19	4.72	5.08	5.42	4.85
Triumph	3.43	4.54	4.79	5.24	4.50	4.42	4.86	5.30	5.85	5.11
Knjaz	3.24	4.26	4.51	4.83	4.21	4.28	4.81	5.14	5.61	4.96
Average	3.22	4.22	4.52	4.86	4.21	4.21	4.73	5.09	5.45	4.87
LSD	A	B	AxB			A	B	AxB		
0.05	0.326	0.326	0.652			0.319	0.319	0.637		
0.01	0.439	0.439	0.879			0.429	0.429	0.858		

ANOVA Table

Source of variation	Degrees of freed.	F cal.	F prob.	Fcal.	F prob.
Replication	2	21.589	0.000	41.800	0.000
Treatments	15	6.924	0.000	2.017	0.050
Factor A	3	2.770	0.059	3.253	0.035
Factor B	3	31.642	0.000	5.929	0.003
AxB	9	0.070	1.000	0.301	0.960
Error	30	-	-	-	-
Total	47	-	-	-	-

The average grain yield had the absolute lowest value–in the control (without nitrogen fertilization-and it was 3.22 t ha^{-1} in the first and 4.21 t ha^{-1} in the second year of testing. The use of fertilizers led to a very significant increase in grain yield in all cultivars included in the study, in both years compared to the grain yield achieved on the variant without fertilization (Table 2). The average values of grain yield for all cultivars were the highest in the variants where nitrogen was applied in the amount of 150 kg ha^{-1} and it ranged from 4.66 t ha^{-1} in the cultivar Favorit in the first year to 5.85 t ha^{-1} in the cultivar Triumph in the second year of research. The Triumph variety, in both years of research, stood out as the most productive. Observed by years of research, all varieties achieved higher grain yield in the second year. Meteorological conditions, i.e. air temperature and precipitation are the basic non-genetic factors that determine the success of the production of winter triticale and other small grains. The total amount of precipitation in the second year of research was 109.1 mm higher than in the first year and distribution, was more even during germination and initial growth of plants and also during February to June and this had a positive effect on plant development and grain yield as the end result of production. Our results agree with those reported by Gulmezoglu and Aytac (2010) who pointed out that precipitation amount after topdressing, their distribution and the amount of soil available N caused a difference in grain yield. Similar results were pointed out by Đurić et al. (2015) and Madić et al. (2018). Grain protein contents are the result of a complex interaction between N and water availability, grain yield and temperature. Differences in climatic conditions in the grain filling phase from year to year cause a difference in the protein content in the grain because the amount and distribution of precipitation in the mentioned period are key environmental factors that have a strong influence on the above (Garrido-Lestache et al., 2004; Gulmezoglu and Aytac, 2010).The protein content in the grain, in our research, varied depending on the weather conditions in the years of testing, the variety and the applied dose of nitrogen fertilizer (Table 3). All tested cultivars had significantly higher protein content in grain on fertilized variants compared to the control.

Table 3. Protein content of the tested triticale varieties depending on the dose of applied nitrogen (%)

Varieties (A)	N applications									
	2018/19					2019/20				
	0	N1	N2	N3	Average	0	N1	N2	N3	Average
Kg-20	12.72	15.02	14.23	14.14	14.03	12.23	14.12	13.62	12.65	13.15
Favorit	12.94	15.34	14.65	14.60	14.38	12.35	14.46	13.95	12.82	13.39
Triumph	12.33	14.76	14.20	13.97	13.81	11.74	13.96	13.31	12.34	12.84
Knjaz	12.47	15.11	14.98	14.51	14.27	11.94	14.32	13.74	12.55	13.14
Average	12.61	15.06	14.51	14.30	14.12	12.06	14.21	13.65	12.59	13.13
LSD	A	B	AxB			A	B	AxB		
0.05	0.266	0.266	0.531			0.172	0.172	0.344		
0.01	0.358	0.358	0.715			0.232	0.232	0.464		

ANOVA Table

Source of variation	Degrees of freed.	2018/19 F cal.	F prob.	2019/20 Fcal.	F prob.
Replication	2	4.602	0.018	16.254	0.000

Treatments	15	28.414	0.000	58.324	0.000
Factor A	3	7.579	0.001	14.827	0.000
Factor B	3	131.474	0.000	273.967	0.000
AxB	9	1.005	0.457	0.941	0.505
Error	30	-	-	-	-
Total	47	-	-	-	-

According to the data obtained during the present study, the protein content in the grain of the tested triticale varieties varied from 11.74% in Triumph variety in the variant without fertilization in the second year of the study, to 15.34% in Favorit variety in the first year, in the variant where nitrogen was used in the amount of 60 kg ha^{-1}. The highest protein content on average was obtained on the N_1 variant of fertilization. The achieved increase in protein content in this variant was statistically justified in relation to the control, but also to other variants of fertilization. The Favorit variety had the highest average grain protein content in both years of testing, while the lowest grain protein content was recorded in the Triumph variety and these differences were significant at $p<0.01$. Also, the average protein content in the grain of the Triumph variety in both years of research was significantly lower compared to the Favorit variety.

The results of the research also showed that the examined triticale cultivars had different protein content on the same fertilization variant, which indicates the existence of varietal specificity according to this quality indicator.

Milovanović et al. (2006), Đekić et al. (2010), Lalević et al. (2020) pointed out that protein content in the grain is a trait of the genotype and its inheritance is controlled by factors of a complex nature, and the content is largely dependent on enviromental conditions. These studies is confirmed by our results as the protein content of the grain varied by the years of the study.

Conclusions

From the presented results we can conclude the following:

- The use of fertilizers led to a significantly large increase in grain yield and grain protein content in all tested varieties in both years of research.
- All varieties had the highest grain yield in the variant where nitrogen was used in the amount of 150 kg ha^{-1}.
- The highest grain yield among all examined varieties, in both production years, was achieved by the variety Triumph (4.5 t ha^{-1} in the first year, and 5.11 t ha^{-1} in the second year).
- The lowest yield in the first year was recorded in the varieties Kg-20 and Favorit (4.06 t ha^{-1}), and in the second year in the variety Kg-20 (4.56 t ha^{-1}).
- The first year (2019/2020) was more favorable for the production of triticale, with more precipitation and higher average temperature during the growing season, so the average yield of 4.87 t ha^{-1} was achieved, while in the production year 2018/2019 the average yield was lower (4.2 t ha^{-1}).
- The highest content of crude protein was noticed in the variety Favorit and it amounted to 14.38% in the first and 13.39% in the second year of testing. The protein content of other varieties was slightly lower (from 13.81 to 14.27% in the first and from 12.84 to 13.15% in the second year).
- The highest protein content in the grain was recorded when using the least amount of nitrogen.

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CHARACTERISTICS OF EARLY-RIPENING BLACKBERRY CULTIVARS (*RUBUS FRUTICOSUS* L.)

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Abstract

The paper presents two-year results of investigation of the phenological, biological and production characteristics of early-ripening blackberry cultivars: Loch Ness, Black Satin and Čačanska Bestrna. The most cultivated blackberry cultivar in Serbia is Čačanska Bestrna. Agroecological conditions enable the successful cultivation of other cultivars whose purpose, apart from processing, can also be table consumption. According to the results, Loch Ness and Black Satin cultivars had an earlier beginning of the flowering phenophase in relation to Čačanska Bestrna. The earliest beginning of fruit ripening was achieved in the Loch Ness cultivar – on July 15. Two days later, the ripening of Black Satin fruits started, and two more days later, the Čačanska Bestrna fruits began to ripen. The length of the fruit ripening period in Čačanska Bestrna and Loch Ness was 25 days, while in the Black Satin cultivar it was shorter - 22 days. The largest fruit mass was in the cultivar Čačanska Bestrna - 7.82 g, the smaller was in the cultivar Loch Ness - 6.65 g, and the smallest in the cultivar Black Satin - 4.95 g. The content of total soluble solids ranged from 10.3 °Brix (Čačanska Bestrna) to 12.3 °Brix (Loch Ness). Čačanska Bestrna proved to be the cultivar with the highest yield - 22.75 t / ha, while the yield of the other two cultivars was significantly lower - 9.59 t/ha for Black Satin, and 8.35 t/ha for Loch Ness.

Keywords: *blackberry, fruit characteristics, yield, flowering, harvest.*

Introduction

Blackberry is a widespread berry fruit species. In terms of economic importance in Serbia, blackberries are in third place in the group of berry fruit species, right after raspberries and strawberries (Nikolić and Milivojević, 2015). Immediately after the USA and Mexico, Serbia ranks third in world blackberry production (Strik et al., 2006; FAOSTAT, 2018). Most of the fruit is exported as frozen, while the rest is used for various types of processing and fresh consumption. The fruit is of high nutritional value (Pantelidis et al., 2007; Ali et al., 2011; Sangiovanni et al., 2013; Ivanović et al., 2014). In order to increase the production of blackberries and maintain them at the level of about 25,000 tons, which is the optimum for Serbia, it is necessary to renew the orchards and change the current assortment of cultivars (Nikolić and Milivojević, 2015). The most cultivated cultivar of blackberry in Serbia is Čačanska Bestrna. In the last few years, the cultivars Loch Ness, Chester Thornless and Triple Crown have been increasingly planted in new orchards. Their fruits are suitable for fresh consumption (Jazić, 2019; Mikulic-Petkovsek et al., 2021). The aim of this research is to examine and analyze the biological and pomological characteristics of different cultivars of blackberries grown near Čačak, in Western Serbia. Some of the examined cultivars are newer and are recommended for the renewal of orchards and change of assortment.

Material and methods

The experiment was conducted in 2020 and 2021 in a blackberry orchard located in the village of Gornja Gorevnica, 9 km northwest of Čačak (43.958135 ° N; 20.322866 ° E; 390 m above sea level). The orchard was planted in the spring of 2016, and in the years of research, it was in the fifth and sixth growing season, respectively.

The orchard was planted on the land of the grove type. The planting distance is 3 m between rows and 1.2 m inside the row, which corresponds to a planting density of 2,777 plants per hectare. In the orchard, standard cultural practices were applied, except for irrigation.

The material for the research were blackberry cultivars of the earlier ripening time - Loch Ness and Black Satin, compared to the most cultivated blackberry cultivar in Serbia - Čačanska Bestrna, which served as a standard. The following properties of blackberries were examined: Basic phenological properties (beginning and end of flowering and ripening); Characteristics of fruiting branches (number of fruiting branches per shoot, length of fruiting branches per shoot and number of flowers per fruiting branch); Characteristics of blackberry fruit (length and width of fruit, fruit shape index, fruit weight and total soluble solids content (TSS) in fruit), as well as yield.

Results and discussion

The results of the work related to the phenophase of flowering and ripening of blackberries are shown in the Figure. 1.

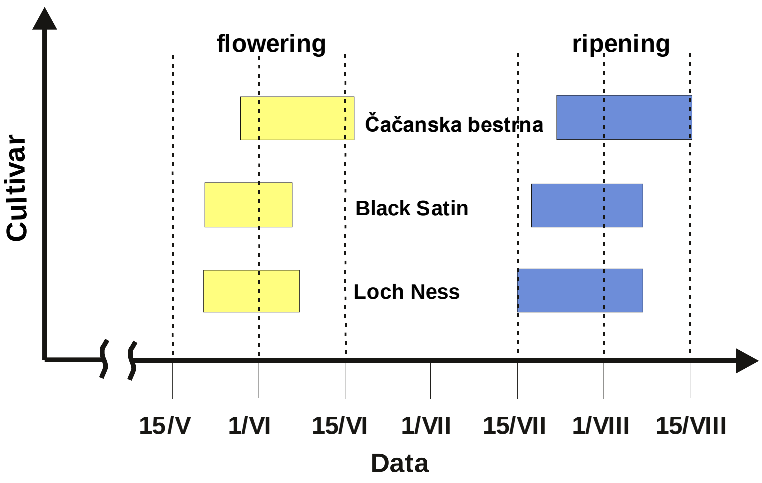

Figure. 1. Time and duration of flowering and ripening phenophases

In relation to Čačanska Bestrna, which started flowering on May 26, the cultivars Black Satin and Loch Ness bloomed earlier. The beginning of flowering in both cultivars was on May 20 (average for 2 years). When it comes to the duration of the flowering phenophase, Čačanska Bestrna had a significantly longer flowering period - 20 days, while this phenophase in the Loch Ness cultivar lasted 17 days, and in the Black Satin cultivar 15 days.

Fruit ripening first began with the Loch Ness cultivar - 15.07. and lasted 25 days. A couple of days later, ripening also occurred in the Black Satin cultivar, but the harvest lasted shorter - 22 days. Čačanska Bestrna started to ripen on July 21 and the harvest lasted as long as Loch Ness - 25 days.

Table 1. Properties of blackberry branches

Cultivar	Number of fruiting branches per shoot	Length of the fruiting branch (cm)	Number of flowers per fruiting branch
Loch Ness	15.7 ± 0.7 b	28.1 ± 1.9 b	9.6 ± 0.2 b
Black Satin	19.7 ± 1.1 a	24.9 ± 2.2 b	11.8 ± 0.6 b
Čačanska Bestrna	15.8 ± 0.9 b	47.1 ± 3.8 a	22.1 ± 0.9 a
ANOVA	*	*	*

Values in columns marked with different letters indicate statistically significant differences at the level of R ≤ 0.05 (LSD test)

Figure 2. The appearance of fruiting branches of blackberry cultivars

Regarding the number of fruiting branches per shoot, we can see in the table 1 that the Black Satin cultivar had the largest number (19.7). On the other hand, the number of fruiting branches in the other two cultivars was smaller - 15.7 in Loch Ness, and 15.8 in Čačanska Bestrna. Regarding the length of the fruiting branch, the Čačanska Bestrna cultivar has the largest length (47.1 cm), while the fruiting branches of the Loch Ness and Black Satin cultivars were significantly shorter (Figure 2). Čačanska Bestrna also had the largest number of flowers per fruiting branch (22.1), which is in line and directly proportional to the length of the fruiting branch (Glišić, 2004). The cultivars Black Satin (11.8) and Loch Ness (9.6) had a significantly smaller number of flowers per fruiting branch.

The results of the e characteristics of the blackberry fruit are shown in Table 2.

Table 2. Properties of blackberry fruit

Cultivar	Fruit height (mm)	Fruit width (mm)	Fruit shape index
Loch Ness	27.2 ± 1.1 ab	19.8 ± 0.4 ab	1.4
Black Satin	25.3 ± 1.2 b	18.2 ± 0.3 b	1.4
Čačanska Bestrna	29.3 ± 1.3 a	22.0 ± 0.5 a	1.3
ANOVA	*	*	*

Values in columns marked with different letters indicate statistically significant differences at the level of R ≤ 0.05 (LSD test)

Cultivars Čačanska Bestrna (29.3 mm) and Loch Nes (27.2 mm) had the highest fruit height, while the cultivar Black Satin had a lower fruit height (25.3 mm). A similar regularity was observed with the width of the fruit. Height, width, and fruit shape index were examined by Zia-UI-Haq et al. (2014) and obtained similar results regarding the dimensions of the fruit of Čačanska Bestrna. The fruit shape index is similar in all examined cultivars and in accordance with the results of Zia-UI-Haq et al. (2014). Milošević (1997) and Stanisavljević

(1999) state that the cultivar Čačanska Bestrna has an elongated fruit and therefore has a higher index of fruit shape compared to other cultivars.

Table 3. Fruit weight, total soluble solids content and yield in blackberry cultivars

Cultivar	Fruit weight (g)	Total soluble solids (°Brix)	Yield (kg/ha)
Loch Ness	6.65 ± 0.23 b	12.3 ± 0.5 a	8,353.07 b
Black Satin	4.95 ± 0.21 c	10.9 ± 0.5 b	9,589.74 b
Čačanska Bestrna	7.82 ± 0.28 a	10.3 ± 0.3 b	22,756.72 a
ANOVA			

Values in columns marked with different letters indicate statistically significant differences at the level of R ≤ 0.05 (LSD test)

The average value of fruit weight varied greatly depending on the cultivar, so the highest fruit weight was in the cultivar Čačanska Bestrna (7.82 g), while the cultivar Loch Ness had significantly smaller fruit (6.65 g). The Black Satin cultivar had the lowest fruit weight - 4.95 g. These results are in accordance with that of Milošević et al. (2016). Petrovic et al. (2020) state that Čačanska Bestrna is a cultivar with very large fruit, with an average weight of 9 g.

TSS content was the highest in the Loch Ness cultivar (12.3 °Brix). The cultivars Čačanska Bestrna (10.3 °Brix) and Blek Satin (10.9 °Brix) had a lower content of TSS. Vrhovsek et al. (2008) state that the cultivar Loch Ness also had a higher content of TSS in their research than the cultivar Čačanska Bestrna. Stanisavljević (1999) notes that the content of TSS in blackberry fruits was about 9%, while according to Mišić and Nikolić (2003) it was 8.47%. According to Zia-UI-Haq et al. (2014), this parameter depends on the cultivar and it range from 6.40 to 9.72 °Brix. Also, Pantelidis et al. (2007) point out that TSS in blackberry fruits varied from 9.80 to 11.50 °Brix.

The highest yield was found in the cultivar Čačanska Bestrna (22.7 t/ha), while the yield in the other two examined cultivars was significantly lower - 9.59 t/ha in the cultivar Black Satin, and 8.35 t/ha in the cultivar Loch Ness. Yields of blackberries vary widely. Maximum yields are about 20 tons per hectare (Milošević, 1997), and up to 35 tons per hectare. According to Mišić and Nikolić (2003) in the conditions of maximum agrotechnical practices and irrigation, in 2001 a record was achieved in the yield with the cultivar Čačanska Bestrna, which is considered to be an extremely yielding cultivar (Petrović et al., 2020). They noted that on 10 acres of orchard 4.6 tons of fruits were achieved, which corresponds to a yield of 46 tons per hectare.

Yield is the result of cultivar characteristics and applied cultural practices (Turemis et al., 2003). In our case, the absence of irrigation significantly reduced yields and confirmed that without irrigation, satisfactory results in blackberry cultivation cannot be achieved.

Conclusions

Black Satin and Loch Ness cultivars bloom earlier and have a somewhat shorter flowering period compared to the Čačanska Bestrna cultivar.

The earliest ripening was in the Loch Ness cultivar (July 15). It was followed by the cultivars Black Satin (2 days later), and Čačanska Bestrna (6 days later the beginning of ripening). The harvest period for the cultivars Čačanska Bestrna and Loch Ness was 25 days, while for the Black Satin cultivar it was 22 days.

Compared to the other two cultivars, the Čačanska Bestrna cultivar had the longest fruiting branches and the largest number of flowers per fruiting branch, which confirmed its high yield potential. Čačanska Bestrna proved to be the cultivar with the largest fruits. Loch Ness had the smaller fruit weight, while Black Satin was the smallest. Regarding the content of

TSS, the highest value was in the cultivar Loch Ness, while the lower content of TSS was found in the cultivars Čačanska Bestrna and Black Satin. The highest yield was achieved with the cultivar Čačanska Bestrna, while the remaining two cultivars had a lower yield. In general, it can be concluded that the early cultivars of blackberries - Loch Ness and Black Satin, although less profitable than Čačanska Bestrna, can occupy a significant place in blackberry orchards in Serbia. This is true especially for the cultivar Loch Ness, which is characterized by very large, attractive and high-quality fruits.

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PROPERTIES OF NEW SERBIAN GENOTYPES OF EUROPEAN PLUM GROWN IN THE REGION OF ČAČAK (SERBIA)

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Abstract

'Lana' and 'G' are the latest genotypes of European plum that were developed in the Republic of Serbia. 'Lana' was obtained at Fruit Research Institute, Čačak from the cross 'California Blue' × 'Ruth Gerstetter' and named and released in 2020. Genotype 'G' is spontaneous seedling selected at Faculty of Agriculture in Novi Sad and was recognized at the beginning of 2022, but has not yet been named. In order to examine performance of those cultivars in agro-ecological conditions of Čačak, this study was done during three consecutive years (2016–2018). The most relevant biological characteristics (flowering and ripening time; morphometric, chemical and organoleptic properties of fruit) and field resistance to causal agents of economically important viral (*Plum pox virus*) and fungal (red leaf spot, rust and fruit rot) diseases were evaluated. 'Čačanska Rana' was used as a standard for 'Lana', while 'Čačanska Lepotica' was control cultivar for 'G'. 'Lana' exhibited later flowering onset (one day) and ripening time (10 days), larger fruit and higher level of field resistance to causal agents of red leaf spot and rust than 'Čačanska Rana'. The fruit organoleptic properties and field resistance to *Plum pox virus* of 'Lana' were in line with standard, though similar or lower values of investigated fruit chemical properties were observed. The flowering and ripening time of genotype 'G' occurred two and 10 days later than in 'Čačanska Lepotica'. The morphometric and organoleptic fruit properties of genotype 'G' were similar to those of 'Čačanska Lepotica', although significantly higher values of the assessed fruit chemical properties were found. Also, genotype 'G' demonstrated the same level of field resistance to *Plum pox virus* as standard and better results regarding the field resistance to causal agents of fungal diseases.

Key words: *Plum, flowering and ripening time, fruit quality, field resistance*.

Introduction

Breeding of European plum (*Prunus domestica* L.) is mainly concentrated on the territory of Europe, while breeding of Japanese plum (*Prunus salicina* Lindl.) is being done in other parts of the world, especially in the USA (Milatović, 2019). During the last decades, a large number of new plum cultivars of better production characteristics have been released worldwide (Sottile *et al.*, 2022). However, sharka resistant cultivars with blue coloured, large and firm fruit are missing (Neumüller, 2011).

European plum is the most spread fruit crop in the Republic of Serbia (73,010 ha according to Food and Agricultural Organization, 2022) of large traditional and economic importance. Therefore, activities related to breeding of new plum cultivars have been carried out in several research institutions in the Republic of Serbia. In this sense, Fruit Research Institute, Čačak (FRI) has the longest tradition and the best achieved results. Using planned hybridization, 18 plum cultivars have been named and released at FRI (Milošević *et al.*, 2021). The last one was cultivar 'Lana', recognized in 2020 (Paunović *et al.*, 2021). In addition to the FRI, breeding of European plum is also perform at the Faculty of Agriculture in Čačak (Milošević and Milošević, 2011), as well as at the Faculty of Agriculture in Belgrade and at the Faculty of Agriculture in Novi Sad. The work on plum breeding at the aforementioned institutions resulted in a number of promising genotypes which are in different testing phases. Also, genotype 'G' represents spontaneous seedling which was selected at Faculty of Agriculture in Novi Sad. This genotype was recognized at the beginning of 2022, but has not been named so far.

The aim of this work was to investigate the most essential phenological and pomological properties of the newly released genotypes 'Lana' and 'G' and compare obtained results with those of the standard plum cultivars 'Čačanska Rana' and 'Čačanska Lepotica', respectively. In addition, reaction to causal agents of economically important plum diseases under natural infection pressures in the field was monitored. The obtained results would indicate whether new cultivars could be interesting for commercial growing in Čačak agro-ecological conditions. The findings are also expected to be useful in further breeding work on European plum.

Material and Methods

Plum trees of 'Lana', 'G', 'Čačanska Rana' and 'Čačanska Lepotica' on Myrobalan (*Prunus cerasifera* Ehrh.) seedling rootstock, grown in experimental orchard of FRI (Ljubić facility, 43°53'N, 20°20'E, 250 m a.s.l.) were used as a material for investigation. The orchard was established in 2011 with 5 × 3 m planting distance. The training system was central leader, and during the study period (2016–2018) standard cultural practices without irrigation were applied.

The most relevant biological characteristics such as flowering and ripening time; morphometric (weight, height, width and thickness of fruit, stone weight and flesh percentage), chemical (content of soluble solids, total and inverted sugars, sucrose and total acids, pH value of fruit and ratio between soluble solids and total acids content) and organoleptic (attractiveness, taste, aroma and consistency) properties of fruits, as well as field resistance to causal agents of economically important viral [*Plum pox virus* (PPV)] and fungal [red leaf spot (*Polystigma rubrum* Pers. DC), rust (*Tranzschelia pruni-spinosae* (Pers) Dietel, fruit rot (*Monilinia laxa* (Aderhold & Ruhland) Honey), pockets plum (*Taphrina pruni* Tul.)] diseases were evaluated.

The flowering phenophase was investigated in accordance with recommendations of the International Working Group for Pollination (Wertheim, 1996) and dates of flowering onset (10% open flowers) and end of flowering (90% of the petal fall) were recorded. Ripening time was determined as a date when the fruits were sufficiently coloured and soft to be eaten (Funt, 1998). The fruit and stone weight (g) and dimensions (mm) were determined on the Technical scale Adventurer Pro AV812M (Ohaus Corporation, Switzerland) and Digital calliper (0–150 mm, Kronen GmbH, Kehl am Rhein, Germany) using standard morphometric procedures. Flesh percentage (%) was calculated representing the share of fruit flesh in the total fruit weight. Binocular refractometer (Carl Zeiss, Germany) was used for determination of soluble solids content (%), while fruit pH by a CyberScan 510 pH-meter (Nijkerk, Netherlands) was measured. Luff-Schoorl method, described by Egan *et al*. (1981), was used for examination of total sugars (%), invert sugars (%) and sucrose (%) contents. Total acids content (%) was determined by titration with 0.1 N NaOH to end point of pH 8.1 with the presence of phenolphthalein as an indicator. Ratio between soluble solids and total acids content (SSC/TA) was calculated. Organoleptic properties of fruits were assessed in accordance with the guidelines for testing the values for cultivation and use of plum cultivars specified by the Regulations of the Ministry of Agriculture, Forestry and Water Management

of the Republic of Serbia and described by Glišić *et al*. (2016). The total of all individual points i.e. attractiveness (0–6), taste (0–8), aroma (0–2) and consistency (0–4) resulted in the overall organoleptic score (0–20). The degree of resistance of the studied cultivars to aforementioned causal agents of the most important viral and fungal disease of plum under natural infection pressures in the field was examined according to the methodology recommended by IBPGR (1984) and described by Glišić *et al*. (2016). The symptoms intensity was expressed on a scale from 1 to 9 (1 – no symptoms, 3 – minor symptoms, 5 – moderate symptoms, 7 – strong symptoms, 9 – very strong symptoms).
The Fisher model analysis of variance (ANOVA) and LSD test at 5% level of significance were used for statistical analysis of fruit morphometric properties.

Results and Discussion

During the period of investigation ‘Čačanska Rana’ had the earliest beginning of flowering (depending on year from 22 March to 6 April) followed by ‘Lana’ and ‘Čačanska Lepotica’ who started flowering one and three days later respectively (Table 1). The genotype ‘G’ was characterised by the latest flowering onset (between 28 March and 8 April). In cultivars ‘Lana’ and ‘Čačanska Lepotica’, somewhat shorter intervals of flowering in comparison with ‘Čačanska Rana’ and ‘G’ were observed.

Table 1. Characteristics of flowering and ripening phenophases of new and standard plum cultivars (2016–2018).

Cultivar	Year	Flowering phenophase		Ripening time
		Flowering onset	End of flowering	
Lana	2016	23 March	01 April	21 July
	2017	02 April	11 April	12 July
	2018	06 April	15 April	27 July
Čačanska Rana	2016	22 March	02 April	11 July
	2017	01 April	11 April	08 July
	2018	06 April	16 April	14 July
G	2016	28 March	05 April	28 July
	2017	03 April	13 April	25 July
	2018	08 April	20 April	04 August
Čačanska Lepotica	2016	25 March	03 April	20 July
	2017	03 April	12 April	15 July
	2018	08 April	18 April	22 July

On average, ripening season of ‘Čačanska Rana’ was at the end of the first and the beginning of the second decade of July, followed by the cultivars ‘Lana’ and ‘Čačanska Lepotica’, whose fruits ripened about ten days later. Ripening time of genotype ‘G’ was mid-early, i.e. from the third decade of July to the first decade of August. Having in mind the statements of Milatović (2019) and the results obtained in this study, ‘Lana’ and ‘Čačanska Rana’ are early-flowering cultivars, while ‘G’ and ‘Čačanska Lepotica’ belong to the group of medium-flowering genotypes. In terms of ripening time, the studied cultivars are connected to each other providing a continuous offer of fresh fruits on the market.
The obtained results related to the morphometric characteristics of fruits indicate significant differences among the new and reference cultivars (Table 2). The fruits of new cultivars were larger in size than the fruits of standard cultivars. The differences between cultivars ‘Lana’ and ‘Čačanska Rana’ were more pronounced than between genotypes ‘G’ and ‘Čačanska Lepotica’. Also, ‘Lana’ is characterized by larger stone and higher flesh percentage (2.42 g

and 97.14%) in comparison with ‘Čačanska Rana’ (2.09 g and 96.65%), whereas in genotype ‘G’ larger stone (1.90 g) and lower flesh percentage (95.42%) than in ‘Čačanska Lepotica’ (1.49 g and 96.34%) were found.

Table 2. Morphometric properties of fruits of new and standard plum cultivars (2016–2018).

Cultivar	Fruit weight (g)	Fruit height (mm)	Fruit width (mm)	Fruit thickness (mm)	Stone weight (g)	Flesh percentage (%)
Lana	85.04±6.07a	50.37±1.00a	49.34±1.6a	50.21±2.09a	2.42±0.35a	97.14±0.60a
Čačanska Rana	62.67±2.04b	46.83±2.32b	39.07±0.79b	36.42±0.45b	2.09±0.30b	96.65±0.52b
G	41.87±2.98a	47.97±1.01a	33.75±0.35b	39.34±1.73a	1.90±0.22a	95.42±0.51b
Čačanska Lepotica	38.59±4.03b	43.55±0.79b	39.67±0.98a	37.65±1.47b	1.49±0.15b	96.34±0.56a

*The various lowercase letters in respective columns indicate significant differences at P ≤ 0.05 according to the LSD test.

The values of investigated fruit chemical properties of new and standard plum cultivars are shown in Table 3. Higher average content of soluble solids (14.54%), sucrose (4.81%) and total acids (1.20%) was found in fruits of ‘Lana’, while the average values of total (10.44%) and inverted (5.72%) sugars, pH value (3.52) and ratio between soluble solids and total acids (17.17) were higher in the fruits of ‘Čačanska Rana’. Genotype ‘G’ outperformed standard cultivar ‘Čačanska Lepotica’ regarding soluble solids content (17.80%), content of total sugars (10.32%) and sucrose (3.95%), ratio between soluble solids and total acids content (17.49) and pH of fruit (3.45).

Table 3. Chemical properties of fruits of new and standard plum cultivars (2016–2018).

Cultivar	Soluble solids *(%)*	Sugar content *(%)*			Total acids *(%)*	SSC/TA	pH
		Total	Inverted	Sucrose			
Lana	14.54	9.86	4.79	4.81	1.20	12.55	3.39
Čačanska Rana	14.07	10.44	5.72	4.43	0.99	17.17	3.52
G	17.80	10.32	6.17	3.95	1.02	17.49	3.45
Čačanska Lepotica	15.63	9.46	7.33	2.02	1.13	13.91	3.37

*SSC/TA: Ratio between soluble solids and total acids content

The new plum cultivars showed the same score in evaluated fruit organoleptic properties as the cultivars used for comparison (Table 4). The fruits of all investigated cultivars received maximum score for attractiveness (6). The flesh of ‘Lana’ and ‘Čačanska Rana’ was scored as tastier (6) and more aromatic (2) than the flesh of ‘G’ and ‘Čačanska Lepotica’ (5 and 1, respectively). On the other hand, the excellent consistency of fruits (4) in ‘G’ and ‘Čačanska Lepotica’ was found, while consistency of ‘Lana’ and ‘Čačanska Rana’ was assessed as medium (2).
The most important parameters of European plum fruit quality are fruit size and shape, fruit skin and flesh colour, flesh firmness, content of sugars, organic acids and aroma components (Neümuller *et al*., 2012). The fruit appearance will determine the first buy of plum fruit by consumers, but return buy is conditioned by fruit taste (Kader, 2002). Attractiveness is usually based on fruit size and fruit skin colour, while taste represents combination of texture, flavour, aroma and sugar to acids ratio (Callahan, 2003). Crisosto *et al*. (2007) pointed out that soluble solids content and titratable acidity play a significant role in consumer acceptance. According to Milatović (2019) fruits of ‘Lana’ and ‘Čačanska Rana’ could be categorized as very large, while ‘G’ and ‘Čačanska Lepotica’ belong to the group cultivars of

large fruits, and outperformed set criterion for dessert cultivars. Also, new cultivars meet quality requirement regarding soluble solids content given by Crisosto *et al*. (2004, 2007) and Neümuller (2011). In addition, the content of soluble solids in fruits of genotype ‘G’ meets criteria given for the late ripening cultivars. In contrast to the fact that the fruits of the cultivars ‘Lana’ and ‘Čačanska Rana’ were characterized by a lower content of soluble solids, they had a better score of fruit taste. That is another confirmation that acceptability is a specific feature of each genotype and should be individually assessed (Crisosto *et al*., 2004; Glišić *et al*., 2021). As emphasized by previous studies, cultivars ‘Čačanska Rana’ and ‘Čačanska Lepotica’ exhibited the best organoleptic properties among several evaluated cultivars (Nenadović-Mratinić *et al*., 2007; Milatović *et al*., 2018), which could indicate the possible acceptability of ‘Lana’ and ‘G’ by consumers as well.

Table 4. Fruit organoleptic properties of new and standard plum cultivars (2016–2018).

Cultivar	Attractiveness (0–6)	Flavour (0–8)	Aroma (0–2)	Consistency (0–4)	Total (0–20)
Lana	6.00	6.00	2.00	2.00	16.00
Čačanska Rana	6.00	6.00	2.00	2.00	16.00
G	6.00	5.00	1.00	4.00	16.00
Čačanska Lepotica	6.00	5.00	1.00	4.00	16.00

The results of field resistance to causal agents of the economically most important viral and fungal diseases observed in new and standard plum cultivars are given in Figures 1 and 2. As for the manifestation of the pressure of red leaf spot (*Polystigma rubrum* Pers. DC), rust [*Tranzschelia pruni-spinosae* (Pers) Dietel] and pockets plum (*Taphrina pruni* Tul.), the newly developed genotypes had no displayed typical symptoms on the leaves and fruits, while standard cultivars displayed minor to medium symptoms intensity. Symptoms of fruit rot [*Monilinia laxa* (Aderhold & Ruhland) Honey] were observed only in ‘Lana’. Both new and standard cultivars showed symptoms of PPV on the leaves and not on the fruits, so it can be concluded that new cultivars are Sharka tolerant, same as the standard cultivars.

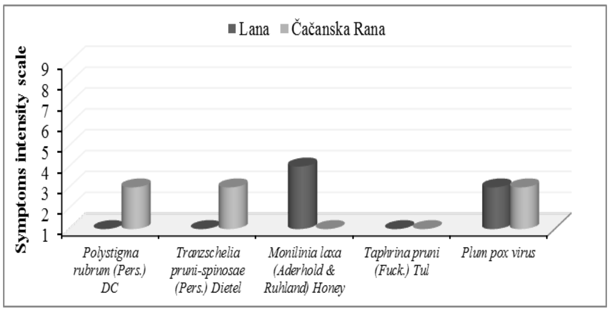

Figure 1. Field resistance of ‘Lana’ and ‘Čačanska Rana’ cultivars.

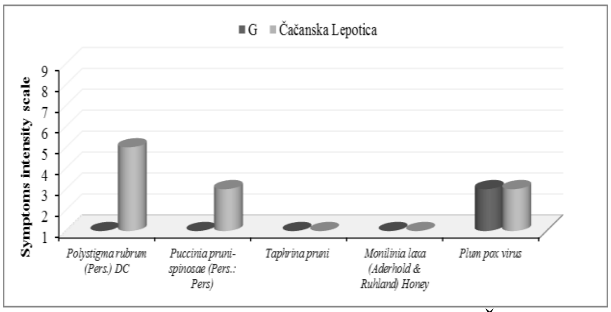

Figure 2. Field resistance of ‘G’ and ‘Čačanska Lepotica’ cultivars.

In addition to fruit quality and yield performance, PPV tolerance/resistance has top priority among the breeding objectives (Neumüller *et al*., 2021). Also, the great importance is given to resistance to causal agents of fungal diseases such as red leaf spot, rust and fruit rot (Milatović, 2019). Introduction of resistant or tolerant genotypes in commercial growing is a key measure for reduction of fungicide usage and achieving healthy crops (Rungjindamai *et al*., 2014). Also, it is well known that the only solution for dissolving the Sharka virus problem is growing resistant/tolerant cultivars (Ranković *et al*., 1994). Both new cultivars showed low degree of sensitivity to the causal agents of economically important fungal and viral diseases. Therefore, the low degree sensitivity of new cultivars is of great importance for plum growers and consumers, as well as for breeders.

Conclusions

Owing to the values of parameters determined in this study, ‘Lana’ and ‘G’ can be recommended for further promotion and commercial growing as PPV tolerant cultivars of very large and large fruits suitable for fresh consumption. In order to define additional processing purposes of genotype ‘G’, more detailed investigations should be carried out in future. New cultivars can take a significant place in further plum breeding programmes, as a source of large fruits, as well as resistance/tolerance to the PPV and the most important fungal diseases.

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EXAMINATION OF SEED QUALITY PARAMETERS OF THREE PEPPER VARIETIES IN A FIVE-YEAR PERIOD (*CAPSICUM ANNUUM* L.)

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Abstract

Sweet pepper (*Capsicum annum* L.) is considered one of the most popular vegetables in the world. During five consecutive years (2017-2021) the most important indicators of seed quality (seed germination, energy, seed purity and moisture) and health safety were analyzed in three varieties of peppers (*Capsicum annuum* L.): Palanačko čudo, Župska rana and Duga bela. During the research, very high values of all important parameters of seed quality were determined. The average determined seed purity was 98.6 % with a variation over the years of examination from 99.10% to 99.9%. Seed germination varied in the range of 73% to 94%, while the average germination was 86%. Germination energy was in the range of 61% to 88%. Seed moisture data showed that it was in the tolerance range of 6.3% to 7.4% per test year, with an average value of 6.9%. *Alternaria* spp and *Fusarium* spp were detected in minimal percent or no detection on variety of Palanačko čudo. Duga bela had the highest percentage of *Alternaria* spp. in 2020 and 2021, which were 2% and 3%, respectively. *Fusarium* spp. was presented in percentage more than in Župska rana and Palanačko čudo. The total germination of the Palanačko čudo, Župska rana and Duga bela during the observed five-year period was above the legal level of minimum and can be classified as quality seed.

Key words: *seed, energy, germination, moisture, purity.*

Introduction

Sweet pepper (*Capsicum annum* L.) belongs to the *Solananceae* family and is considered one of the most popular vegetables in the world. Sweet peppers can be consumed as fresh, processed and dehydrated spice. Sweet pepper is considered as a commercial vegetable crop, grown mainly in greenhouses or in fields (Cvetković et al., 2022). Also, in Serbia pepper is one of the leading commercial vegetables, which covers an area of 2 million hectares (FAOSTAT, 2018). Good quality pepper seed is one of the factors which can enhance the final yield and reduce unnecessary losses caused by defective or infected pepper seeds (Wang et al., 2018). Methods for selecting high-quality seeds are based on physical methods such as germination, energy, weight, purity, moisture and biochemistry (Yildirim & Demir, 2020). Infected seeds are an important route of introduction and spread of several plant pathogenic fungi that can affect the quality and sustainability of plants, thus causing economic losses in the field or greenhouse (da Silva Pereira et al., 2018, Gebeyehu, 2020). There are common types of fungi in the literature that are associated with a disease or defective pepper seeds *Alternaria* spp. and *Aspergillus* spp., while *Fusarium* spp. acts as a member of the damping off complex in *Capsicum* spp. Moreover, these pathogens can produce toxins that render agricultural produce unsuitable for consumption (Samarah et al., 2016; Fajardo-Rebollar et al., 2021; Ahmad et al., 2022). Various factors such as taking seeds from healthy plants, adequate storage, and different preparations can significantly improve the quality of seeds.

The lower moisture and temperature can extend the germination of the seed (Wang et al., 2018).

Material and Method

Seed samples of three varieties of sweet pepper (Palanačko čudo, Župska rana and Duga bela) were used as research material. Parameters of quality seeds were examined during 2017-2021 on experimental fields on the locality of Smederevska Palanka at the Institute for Vegetables Crops. During the five-year period, the following seed quality indicators were monitored: total germination, energy, purity, moisture content and seed health. The parameters of seed quality were tested following the Standard of seed quality of agricultural plants (47/87), which is harmonized with the ISTA rules (2020). For testing energy and germination, 4 x 100 seeds were placed in a petri dish on filter paper previously moistened with 0.2% KNO_3 solution were used. The analysis was performed after 7-14 days in a thermostat at 20-30 ºC and relative humidity of 95%. The estimated energy and total germination after 14 days were recorded for all three varieties of pepper. Seed purity is the ratio of the amount of pure seed of the tested species and the amount of seed from other agricultural plants, weeds and inert materials. The seed health of Duga bela, Palanačko čudo and Župska rana was tested on *Alternaria* spp. and *Fusarium* spp. Health testing of three varieties of peppers was performed with the standard method on filter paper. The allowed percentage of infected seeds is 5%. The procedure of moisture testing was performed with 5 g of a sample of three pepper varieties on an analytical balance. Moisture determination was performed at a temperature of 105 ºC ± 2 ºC for 17 h ± 1 h. Samples were statistically processed with SPSS software (version 23, IBM, USA) and compared with ANOVA and Tukey test. Statistical significance cut-off was $p < 0.05$.

Results and Discussion

The analyses of seed quality parameters (total germination, energy, purity, moisture and health) of Župska rana, Duga bela and Palanačko čudo were monitored from 2017 to 2021. The pepper varieties were compared with each other with a statistically significant difference between varieties and within one group. According to the Standard of seed quality of agricultural plants (47/87) for pepper (*Capsicum annuum* L.), the minimum purity is 97%, germination 65% and maximum moisture 12%. Results of quality parameters of three varieties pepper in five periods are shown in Table 1. The purity of all tested peppers was significantly above the defined limit (Table 1).

Table 1. Seed quality parameters of three varieties of pepper in a five-year period

Varity	**Characteristics**^{a}	**Years**				
	Percent (%)	2017	2018	2019	2020	2021
Palanačko čudo	Energy	79*	76	81	73	78
	Total germination	86	91^{*b}	94^{*b}	75*	87^{*b}
	Moisture	6.8	7.1	6.9	6.9	6.5^{*b}
	Purity	99.3	99.5	99.4	99.8	99.9
Duga bela	Energy	70	80	80	61	68
	Total germination	86	87	85*	73	77*
	Moisture^c	6.8	7.2	7.3	6.9	6.3
	Purity	99.5	99.6	99.2	99.9	99.9

Župska rana	Energy	81	73	86*	72	72
	Total germination	86	87	89^{*b}	74	77*
	Moisture	7.1^{*b}	7.3	6.6	6.6	7.4
	Purity	99.1	99.4	99.9	99.6	99.9

[a] Characteristics were compared on statistical significance ($p<0.05$) with years and between varieties of peppers but not with other characteristics.
* Statistical significance between years ($p<0.05$)
[b] Statistical significance between varieties

The moisture of Župska rana, Palanačko čudo and Duga bela was below the defined limit (< 12%). Statistical significance for moisture was obtained only for the last year between Palanačko čudo, Duga bela and Župska rana ($p<0.05$). There is no statistical significance between the Župska rana and Duga bela for moisture ($p>0.05$) (Table 1). Energy of Župska rana, Duga bela and Palanačko čudo was lowest in 2021 compared with other examined years. The highest percentage of energy was obtained in 2019 and amounted > 80% (Table 1).

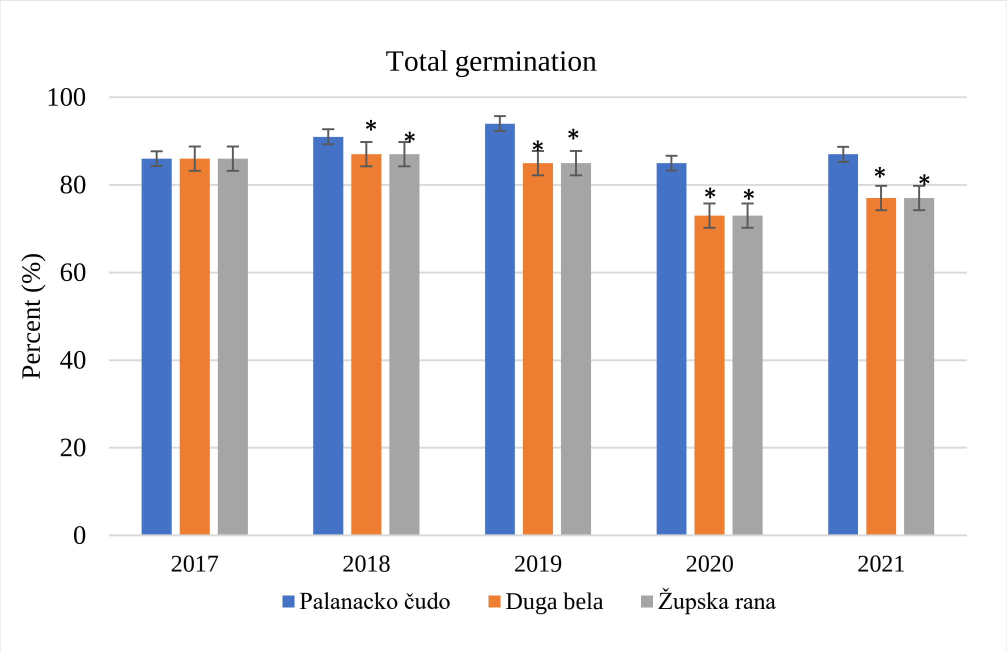

Figure 1. Total germination of Palanačko čudo, Duga bela and Župska rana in five years period with staistical significance ($p < 0.05$)

The total germination in the first observed year (2017) in all examined varieties of pepper was not statistically significant ($p>0.05$) and amounted to 86% (Figure 1, Table 1). The highest total germination was obtained for Palačko čudo (2018-2021) compared to Duga bela and Župska rana ($p<0.05$). There was no statistically significant difference between Duge bela and Župska rana in the observed five-year period for total germination ($p>0.05$). Total germination was the lowest in 2020 for all tested peppers compared to other observed years ($p<0.05$) (Figure 1). *Alternaria* spp and *Fusarium* spp were detected in minimal percent or no detection on variety of Palanačko čudo (Table 1). Duga bela had the highest percentage of *Alternaria* spp. in 2020 and 2021, which were 2% and 3%, respectively. *Fusarium* spp. it was detected in percentages more than in Župska rana and Palanačko čudo (Table 1).

Tabela 2. Detected phytopathogens on the seeds of three varieties of pepper over a period of five years.

	2017	2018	2019	2020	2021	2017	2018	2019	2020	2021
	Alternaria spp.					*Fusarium* spp.				
Palanačko čudo	1	0^{b*}	1	0^{b*}	1	0^{b*}	1	0	2	1
Duga bela	2^{*b}	1	1	2	3^{*b}	2	1	3^{b*}	2	1
Župska rana	0	2	1	3	1	2	2^{*b}	1	1	0^{b*}

* Statistical significance between years (p<0.05)
[b] Statistical significance between varietes.

Palanačko čudo had the lowest infection with *Alternaria* spp. and *Fusarium* spp. compared with Župska rana and Duga bela (p<0.05). In the observing period (2017-2021) Palanačko čudo had total germination > 85 % which can be related to low infection phytopathogens. *Alternaria* spp. and *Fusarium* spp. were detected in the highest percent compared to Župska rana and Palanačko čudo (p<0.05). Tufail et al., 2020 detected *Alternaria* spp. in all tested samples and an average infection by 16% that caused reduced seed germination. However, there are no varieties of *C. annuum* L. with total resistance for *Fusarium* spp. or *Alternaria* spp. Important fungi are *Fusarium oxysporum* which can infect roots, stems, and leaves, could persistence in seeds, and can cause yield losses of up to 100% (García-Rodríguez et al., 2010; Li et al., 2017). The lowest percentage of phytopathogenic fungi in the three years was detected in Palanačko čudo. Recent studies indicate that applying different treatments can significantly improve total germination or prolong seed life (White et. 2019; Castillo et al., 2009). Some studies were performed using a treatment with a mycorrhizal formulation that increased the total germination of pepper seeds by 3–4% (White et. 2019; Poštić, 2019). Likewise, these treatments have an antifungal effect and improve seed quality. The lowest germination was achieved in samples of seed varieties Duga bela with the highest percent isolation of pathogenic fungi. Recent studies used treatments with *Trichoderma* which has antifungal effects on phythopathogens and increased total germination (Konings-Dudin et al., 2014; Murphy., 2017). According to Debnath et al., 2012 the maximum germination was obtained in samples where the prevalence of pathogenic fungi was lowest. In our samples of seed, we obtained that the Palanačko čudo has the lowest prevalence of *Alternaria* spp. and *Fusarium* spp. The total germination of Župska rana, Palanačko čudo and Duga bela was obtained from natural seeds, which indicates the possibility of increasing germination by applying some organic treatments.

Conclusion

The parameters of pepper seed quality depend on the sum of various factors such as storage temperature and humidity, the quality of the pepper and percent infection of seeds or the plant, the varieties, and the interaction of these factors. This analysis of the population structure of the pathogen of three varieties of pepper seeds over five year period helps to define preventive measures for the control of phytopathogenic species as well as the possibility of increasing total germination for all tested sample seeds. The total germination of the Palanačko čudo, Župska rana and Duga bela during the observed five-year period was above the legal level of minimum and can be classified as quality seed.

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STATIC MAGNETIC FIELD IMPROVES EFFECTS OF BIOPRIMING BY *AZOTOBACTER CHROOCOCCUM* F8/2

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Abstract

Seed inoculation (biopriming) represents an agronomic practice directed towards improving germination, as well as fostering beneficial plant-microbe interaction from the very beginning of plants' life. Besides biopriming, static magnetic field (SMF) is studied as an abiotic factor affecting germination and plant growth. This paper is aimed to examine the combined effect of *Azotobacter chroococcum* F8/2 and SMF of 90 mT on germination. *A. chroococcum* F8/2 has been proven as a successful biopriming agent, with beneficial effect on cucumber, tomato, wheat, and soybean germination. This research starts from the hypothesis that the combined effect of *Azotobacter* inoculation and SMF could lead to synergistic improvement of germination parameters, compared to already shown effects of biopriming itself. The research was conducted with following cultivable plants: basil, cucumber, tomato, wheat, and soybean. Seed treatment was performed by 1h-immersion of surface-sterilized seeds into bacterial suspension (10^7CFU/ml), followed by exposure to SMF of 90 mT for 5 min and 15 min. The germination test was conducted with 100 seeds per treatment and lasted 7 days. The highest improvement of germination percentages was observed in cucumber and basil (an increase for 35-41% and 41-45%, respectively), compared to biopriming without SMF treatment. Tomato and wheat germination were not improved by addition of SMF treatment to biopriming. The obtained results indicate that the application of SMF can affect the germination parameters that are changed by biopriming. There is a need for further research in order to explain the differences between plant species' response.

Keywords: *Azotobacter chroococcum, biopriming, germination, static magnetic field.*

Introduction

Biopriming is a hydration of seeds with a saline/suspension of biological component that can be bioactive molecule (saliclic acid, giberellines) or Plant Growth Promoting Rhizobacteria, PGPR (Ashraf and Foolad, 2005; Hamayun et al., 2010). Microbial inoculation by PGPR represents a backbone of biopriming. Selected strains used as biocomponents characterize diverse Plant Growth Promoting (PGP) potential. Numerous studies highlight PGP properties of *Azotobacter* and affirm its representatives as biofertilisers, biostimulators, and biocontrol agents (Sumbul et al., 2020; Pirttila et al., 2021). In previous studies, the selected strain *A. chroococcum* F8/2 demonstrated a significant enhancing effect on the germination of cucumber, tomatoes, wheat, and soybeans (Kerecki et al., 2021). Since the germination is the most delicate stage of the plant's life cycle, the important question is how much abiotic factors can influence it. Rising temperatures, salinity, and changes in soil pH are all known to have a negative impact on seed fate and germination. On the other side, some abiotic environmental factors have been studied as promoters of germination and plant growth, and possibilities for their use are being studied. The static magnetic field (SMF) is known to be a ubiquitous and unavoidable abiotic factor that affects the living world. In the case of SMF, its strength and duration of exposure, plant species, and environmental factors all influence whether it has a positive or negative effect on plant growth (Zhang et al., 2017). These are the critical moments and data on the SMF impact is quite often contradictory. Nonetheless, the potential application of SMF and other forms of magnetic activity in sustainable plant production is supported by numerous scientific studies that indicate positive effects on germination (Bhardwaj et al., 2012), early-stage plant development (Souza et al., 2015), and final yield (Vashisth et al., 2013). Previously published data show that SMF can have a positive or negative effect on the growth and activity of microorganisms depending on the strength and timing of exposure, but it can also cause structural changes in nucleic acids and cell membrane characteristics (Belyavskaya 2004; Goodman et al., 1995). Unfortunately, there is a limitation of data on the influence of SMF on PGPR activity, as well as plant-microbe interaction. Studies have mainly been focused on either plants or microbes, without insight into SMF effect on overall plant-microbe community. The main aim of the present study was to determine the impact of 90mT SMF on the germination parameters (germination percentage, germination index, mean germination time, and vigor I) of different plant inoculated seeds and thus to characterize the relationship between the three priming actors: plants, inoculant, and magnetic fields.

Material and methods

Bacterial strain

Azotobacter chroococcum F8/2 belongs to the collection of The Department of Environmental Microbiology of the Faculty of Agriculture, University of Belgrade. The strain has been identified for the purposes of previous research, some of the PGP activities have been confirmed, as well as the strain potential to be used as a bioprimig agent (Kerečki et al., 2021).

Plant species and seed treatment

Seeds of plant cultures of basil (*Ocimum basilicum* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), wheat (*Triticum aestivum* L.), and soybeans (*Glycine max* L.) were homogenized and sterilized for 2 minutes with 70% alcohol (v/v) and 0.02 % of NaOCl (v/v), rinsed with sterile deionized water, and left to dry in sterile conditions. To verify sterilization success, ten seeds from each plant species were chosen, positioned on MPA medium, and incubated for 24 hours at 30 °C. The absence of bacterial growth indicated successful seed sterilization.

48h-old bacterial culture of *A. chroococcum* F8/2 was "scratched" from solid media, and resuspended in the sterile saline (0.9% NaCl) until the inoculum suspension of 10^7 CFU /ml was reached. Previously prepared seeds were immersed in the inoculum suspension and incubated in a rotary shaker (KS 260, IKA, Germany) for 1 hour at the temperature of 28±2 °C/130 rpm.

Following inoculation and drying, two portions of the seeds were subjected to 90 mT SMF for 5 and 15 minutes before being placed in Petri boxes for germination. The SMF exposure system was set up according to Jovičić-Petrović et al. (2021).

Germination assay

Three different treatments per plant species were tested: inoculated seeds, not subjected to SMF; inoculated seeds exposed to 90 mT for 5 min; and inoculated seeds exposed to 90 mT for 15 min. Using the modified filter paper method outlined by Kerečki et al. (2021) seeds germinated in Petri boxes at natural light and an average room temperature of 25°C. The periodical addition of sterile water supply ensured optimal humidity. For the next seven days, the number of germinated seeds was recorded daily. Germination parameters (final germination percentage - FGP, germination index - GI, mean germination time - MGT, and vigor I) were calculated using a germination measurement tool (Argon Info-Tech).

Statistical analysis

Tukey's test was used to compare the differences in means of the obtained results from inoculated seeds not treated in SMF versus inoculated seeds exposed to SMF, at a 5% level of probability.

Results and discussion

The results revealed that SMF of the given exposure-system had a beneficial impact on the germination of inoculated basil and cucumber seeds. Differences were not observed in soybean inoculated seeds, while some germination parameters of bioprimed wheat and tomato decreased, except in case of wheat exposed to SMF for 5 min (Table 1.).

Table 1. The effect of SMF on germination parameters achieved by *Azotobacter chroococcum* F8/2 biopriming

Plant	Treatment	FGP	GI	MGT	Vigor I
basil	T	58±8	3.00±0.31	5.01±0.27	294±48
	T5	82±5*	6.11±0.45	4.03±0.15*	363±32*
	T15	84±18*	5.56±0.77*	4.20±0.42*	336±72
wheat	T	89±7	14.38±1.53	1.97±0.13	1883±242
	T5	86±5	14.14±1.67	1.88±0.20	1445±212
	T15	80±3*	11.78±0.17*	2.16±0.16	1320±181
soybean	T	55±4	3.73±0.69	4.63±0.48	501±161
	T5	60±3	3.62±0.54	4.75±0.11	434±133
	T15	56±7	3.12±0.70	4.84±0.55	616±215
tomato	T	99±2	6.64±0.42	3.97±0.18	992±324
	T5	90±2*	6.66±0.33	3.60±0.14*	818±87
	T15	86±5*	6.40±0.87	3.87±0.43	729±104*
cucumber	T	71±2	7.39±0.72	2.81±0.15	1293±168
	T5	100±0*	14.87±1.12	2.09±0.38*	1688±96
	T15	96±5*	14.49±1.36	2.18±0.26*	1673±184

T-seeds bioprimed with *A. chroococcum* F 8/2; T5 – seeds bioprimed with *A. chroococcum* F 8/2 and subjected to 90mT SMF for 5 min; T15 – seeds bioprimed with *A. chroococcum* F 8/2 and subjected to 90mT SMF for 15 min; ± std. dev. * indicate statistically significant differences between the parameter in inoculated seeds (T), and combined biopriming-SMF treatments (T5 and T15) at $p \leq 0.05$

SMF application caused an increase of the basil and cucumber germination percentage by 35-41% and 41-45%, respectively. Basil also showed a 44% increase in GI and a 23% increase in vigor. This is in accordance to Jovičić-Petrović et al. (2021) who reported an increase in

germination percentage of white mustard by 53.2% as a result of the combined effects of biopriming and SMF of 90 mT / 15 min.
The five-minute treatment in SMF reduced MGT of basil by 24.3%, while SMF reduced MGT in both exposure durations in cucumbers by 34.5% and 28.9%. Although SMF decreased germination percentage of tomato, MGT was reduced by 10.3% when SMF was applied for 5 min. This is in accordance with Feizi et al. (2020) who reported a decrease of MGT of mustard seeds exposed to SMF. In basil, the positive SMF impact was reflected in the promotion of all observed germination parameters, whereas while in the case of wheat, SMF caused significant reduction of FGP and GI. The observed differences between tested plant species supported an earlier statement that the effect of SMF is determined by the plant`s genotype (Hernandez - Aquilar et al., 2009).
Furthermore, the result indicated that exposure time impacts the SMF effect, since differences in certain parameters were observed depending on the time of exposure, which is in the line with observation of Zhang et al. (2017). Similarly, Jovičić-Petrović et al. (2021) claimed that SMF effects on seed germination metrics are modulated by duration of seed exposure.
Although the metabolic events affected by SMF are not completely understood, we could take into account increased water assimilation and increased enzyme activity (peroxide, catalysis, super-oxide dysmutasis, glutathione-transferase, particularly amylase), to be critical moments in promoting germination parameters (Shine et al., 2011; Shine and Gurupasad, 2012; Maffei 2014). The fostered permeability of the cell wall of the seed, enabling greater absorption of water and energy molecules (Reina et al., 2001; Aladjadjiyan 2002), could result in richer metabolic activity (Iqbal et al., 2012). According to Kastenios et al. (2016), when SMF is applied to seeds, there is a significant increase α-amylase activity (Katsenios et al., 2016), that influences carbohydrate mobilization and degradation to monosaccharides necessary for seedlings development (Kataria et al., 2017).
It is important to note that the magnetic field can reduce or eliminate the negative effects of other abiotic factors (drought, increased salinity, and high heat) that cause germination, plant growth, and yield suppression (Kataria et al., 2017). In such cases, SMF can trigger the production of prolin, a biochemical indicator of stressful environmental conditions that stabilizes macromolecules, allowing plant cells to recover faster from oxidative stress (Matysik et al., 2002; Singh et al., 1973).
Previous research had highlighted the relevance of SMF on biopriming, probably caused by metabolic changes in both plant and biopriming agent (Jovičić-Petrović et al., 2021). This research showed that SMF can lead to the increase of bacterial indole-acetic acid production, as well as changes in abscisic acid production in plants. The overall effect on plant germination makes a good base to propose the use of new technique for seed revitalization and germination promotion, named biomnagnertic priming. In basil and cucumber, we can assume that similar metabolic changes contributed to the positive effect on germination. However, observed differences in response of different plant species indicate the need for further evaluation of specific interactions, and do not provide enough support general recommendations of the biomagnetic priming use in agriculture.

Conclusion

According to shown data carefully chosen plant, inoculant, and optimal exposure system of SMF (type of magnet, intensity, polarity, orientation, and exposure time) can improve germination and thus enhance later stages of growth and final yield. However, the effect of SMF combined with microbial inoculation is highly dependent on plant species and SMF exposure time. Further research is needed to provide sufficient data to define recommendations in terms of biomagnetic priming application in crop production.

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VARIABILITY AND HERITABILITY OF GRAIN YIELD AND HECTOLITER MASS IN WHEAT

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Abstract

In the two-year field experiment (2013/2014 and 2014/2015), the variability of yield and hectoliter grain mass of 14 winter wheat genotypes was examined. The research was conducted in three locations across the Republic of Serbia: Centre for Small Grains in Kragujevac, Institute for forage crops in Kruševac and Agroinstitute in Sombor. Significant differences in grain yield were found between varieties, locations, years and their interactions, while the influence of the location did not show significant differences in hectoliter mass. Grain yield of studied wheat genotypes in 2013/2014 varied from 3.91 t ha^{-1} (KG-244/4) to 5.55 t ha^{-1} (KG-60-3/3), and in 2014/2015 from 5.67 t ha^{-1} (KG-162/7) to 7.08 t ha^{-1} (KG-27/6). The hectoliter mass also varied, and most often ranged from 72.1 kg hl^{-1} (KG-28/6) to 77.2 kg hl^{-1} (KG-191/5-13) in the first experimental year and from 76.7 kg hl^{-1} (KG-27/6) to 81.9 kg hl^{-1} (KG-191/5-13) in the second year. Unfavorable weather conditions which prevailed in the first experimental year, conditioned the formation of smaller and shriveled grains. As a result, the examined KG-wheat genotypes gave lower values in grain yield and hectoliter mass in 2013/2014 compared to 2014/2015. In this study, higher values of heritability in a broad sense were obtained for hectoliter mass (73.66%), and lower values for grain yield (25.82%). This indicates that direct breeding for grain yield is less efficient and it is necessary to have knowledge about the nature of the inheritance of important yield components in order to improve this complex trait.

Keywords: *wheat, yield, hectoliter mass, variability,heritability.*

Introduction

Expression of the plant phenotype is under the control of genes that determine its development. Genetic control in quantitative traits is very complex, conditioned by a large number of minor genes (Joshi et al., 2002). However, the expression of these traits is greatly influenced by environmental factors, as well as their genotype × environmental interaction. In order to achieve success in breeding, it is very important to determine and assess the extent to which factors such as genotype, environment and their interaction contribute to the variation of wheat traits. The influence of genetic factors in determining a trait is different, and for an efficient breeding process, it is very important to know the share of the hereditary component that is passed on to the off spring. Therefore, it is necessary to determine heritability, because the value of heritability of a trait indicates whether it is more conditioned by genetic factors or environmental factors. Erkul et al., (2010) stated that the assessment of heritability is an important parameter in determining the genetic gain from selection.

In most wheat breeding programs, the main focus is on grain yield and yield components (number of ears per unit area, length of ears, number of ears, number of grains per ear, grain weight per ear and weight of 1000 grains) (Luković et al., 2020a). The most important components of spike fertility are considered to be: spike length, number of spikelets per spike, number of grains per spike and number of sterile spikelets per spike, from which the last three are also important indicators of spike fertility (Milovanović et al., 2019). Spikes with a greater number of grains per spikelet and a higher mass can be easily observed in the selection process from the generation of segregation, so these productivity indicators have an important role in the future increases of yield. Perišić et al. (2011) stated that work on improving the length of spikes and the number of spikelets per spike has greatly contributed to the increase in average wheat yields. The above authors stated that today's wheat varieties are capable of producing a greater number of grains per ear and unit area compared to formerly grown varieties. The aim of this study was to evaluate the variability and heritability of hectoliter mass and grain yield of divergent wheat genotypes grown in different agro-ecological conditions.

Materials and methods

Perspective lines of winter wheat (14 genotypes of wheat) created at the Center for Small Grains in Kragujevac and the standard variety Pobeda served as material in these studies. Two-year field trials (2013/2014 and 2014/2015) were performed at the experimental field of the Center for Small Grains in Kragujevac, the Agroinstitute in Sombor and the Institute for Forage Plants in Kruševac. The experiments were performed in three replications. Experimental plots of 4 m^2 were established according to a random block system. After harvest, the grain yield for each plot was measured in three replications and then converted to t ha^{-1}. Hectolitre mass was determined according to the standard method (JUS E.B1.200). Climatic conditions during the trial conducting were published (Luković, 2020b), so they are not presented in this paper.
To examine the influence of genotype, year and location on the analyzed traits of wheat, a model of three-factor ANOVA analysis using a completely randomized block design was used. Testing the significance of differences between wheat genotypes and locations was performed by the *Duncan* test. Lowercase and uppercase Latin letters were used to indicate significant differences between genotypes, and between locations, respectively. Components of phenotypic variance were calculated based on variance analysis data (Falconer, 1981; Jovanović et al., 1992). Heritability in a broader sense (h^2) represents the ratio of genetic and phenotypic variance and is calculated by the formula:

$$h^2 = \frac{\sigma_G}{\sigma_F} \times 100\ (\%)$$

where is σ_G -genetic variance, and σ_F- phenotypic variance.

Statistical analysis of the data was performed using the computer statistical program SPSS Statistics 22.

Results and discussion

Hectolitre mass is a genetically determined trait that varies greatly under the influence of environmental factors. At the Sombor site, in 2014, the highest average value of hectoliter grain weight was achieved by the genotype KG-47/21 (77.2 kg hl^{-1}), and in 2015 by the variety Pobeda (82.1 kg hl^{-1}). In Kruševac, in the first year of testing, the genotype KG-1/6 (77.7 kg hl^{-1}) was singled out for the observed trait, and in the second genotype KG-191/5-13 (83.6 kg hl^{-1}), whose the mean value was significantly higher than the mean value of all other genotypes.

Genotype KG-52/3 (76.6 kg hl^{-1}) achieved the highest average value of hectoliter grain mass in Kragujevac in the first year of testing, and in the second year the genotype KG-1/6 (82.2 kg hl^{-1}) performed best. The second year of testing, due to more favorable climatic conditions, was more convenientfor the comparison of hectoliter mass, so that five KG-lines showed higher values for this property compared to the standard (Tab. 1).

Table 1. Mean values per hectoliter of grain mass (kg hl^{-1}) of studied wheat genotypes

Genotype	2013/2014.				2014/2015.			
	SO	KŠ	KG	$\bar{x}$	SO	KŠ	KG	$\bar{x}$
KG-27/6	$73{,}8^{cA}$	$72{,}1^{abA}$	$72{,}1^{aA}$	72,7	$73{,}7^{aA}$	$78{,}5^{abcB}$	$77{,}8^{abcB}$	76,7
KG-244/4	$76{,}4^{eA}$	$75{,}9^{cdA}$	$76{,}0^{cdeA}$	76,1	$81{,}0^{eB}$	$79{,}5^{abcdeAB}$	$77{,}9^{abcA}$	79,5
KG-199/4	$76{,}7^{efA}$	$76{,}9^{cdA}$	$76{,}0^{cdeA}$	76,6	$79{,}3^{eA}$	$77{,}4^{abA}$	$77{,}4^{abA}$	78,0
KG-307/4	$74{,}2^{cdA}$	$74{,}6^{bcA}$	$74{,}8^{cA}$	74,5	$81{,}2^{efC}$	$79{,}0^{abcdB}$	$77{,}7^{abcA}$	79,3
KG-28/6	$71{,}6^{bA}$	$71{,}2^{aA}$	$73{,}6^{bB}$	72,1	$74{,}5^{bA}$	$77{,}4^{abB}$	$79{,}2^{bcdB}$	77,1
KG-162/7	$69{,}0^{aA}$	$74{,}6^{bcB}$	$75{,}0^{cdB}$	72,8	$80{,}8^{eB}$	$77{,}5^{abA}$	$79{,}6^{bcdeAB}$	79,3
KG-191/5-13	$78{,}8^{gB}$	$76{,}3^{cdA}$	$76{,}3^{eA}$	77,2	$81{,}7^{fgAB}$	$83{,}6^{fB}$	$80{,}5^{defA}$	81,9
KG-40-39/3	$74{,}4^{cdA}$	$74{,}4^{bcA}$	$75{,}7^{cdeA}$	74,9	$80{,}7^{eA}$	$81{,}8^{defB}$	$80{,}4^{defA}$	80,9
KG-52/23	$74{,}6^{dA}$	$74{,}4^{bcA}$	$76{,}0^{cdeA}$	75,0	$81{,}8^{fgC}$	$80{,}6^{cdeB}$	$79{,}7^{bcdeA}$	80,7
KG-60-3/3	$76{,}0^{eA}$	$75{,}9^{cdA}$	$75{,}4^{cdeA}$	76,0	$77{,}7^{cB}$	$77{,}2^{aAB}$	$76{,}4^{aA}$	77,1
KG-1/6	$76{,}5^{efA}$	$77{,}7^{dA}$	$76{,}1^{deA}$	76,8	$79{,}1^{dA}$	$82{,}3^{efB}$	$82{,}2^{fB}$	81,2
KG-52/3	$76{,}4^{efB}$	$74{,}4^{bcA}$	$76{,}6^{eB}$	75,8	$80{,}6^{eA}$	$81{,}2^{cdefA}$	$81{,}6^{efA}$	81,1
KG-47/21	$77{,}2^{fA}$	$75{,}8^{cdA}$	$75{,}6^{cdeA}$	76,2	$77{,}8^{cA}$	$80{,}2^{bcdeB}$	$79{,}7^{bcdeAB}$	79,3
Pobeda	$76{,}6^{efA}$	$75{,}7^{cdA}$	$75{,}5^{cdeA}$	76,0	$82{,}1^{gB}$	$81{,}0^{cdefAB}$	$79{,}9^{cdeA}$	81,0
Prosek	75,2	75,0	75,4	75,2	79,4	79,8	79,28	79,5

Legend: SO – Sombor, KŠ – Kruševac, KG – Kragujevac

Lowercase and uppercase Latin letters were used to indicate significant differences between genotypes, and between locations, respectively

The average grain yield of the studied wheat genotypes varied depending on the genotype, location and year (Tab. 2). In Sombor in 2014, the highest average grain yield was determined for genotype KG-60-3/3 (7.12 t ha^{-1}), which produced a significantly higher yield than all other genotypes in that location. In 2015, KG-307/4 (10.07 t ha^{-1}) and KG-244/4 (9.77 t ha^{-1}) stood out as the most productive genotypes. At this location, all studied wheat genotypes produced significantly higher grain yield in 2015 compared to 2014. In Kruševac, the highest grain yield, in the first year of testing, was recorded by the genotype KG-199/4 (5.40 t ha^{-1}), and in the second year, by the variety Pobeda (6.07 t ha^{-1}) and genotype KG-28/6 (5.87 t ha^{-1}) between which no significant difference in average values was found. Genotype KG-52/3 produced the highest average grain yield in Kragujevac in 2014 (4.61 t ha^{-1}), while in 2015, genotypes KG-60-3/3 (6.17 t ha^{-1}), KG-52/3 (5.77 t ha^{-1}) and KG-191/5-13 (5.76 t ha^{-1}) were the most productive.

Table 2. Mean values for grain yield (t ha^{-1}) in the studied wheat genotypes

Genotype	2013/2014.				2014/2015.			
	SO	KŠ	KG	$\bar{x}$	SO	KŠ	KG	$\bar{x}$
KG-27/6	$4,63^{cdA}$	$4,40d^{efA}$	$4,52^{eA}$	4,52	$9,57^{fB}$	$6,00^{eA}$	$5,68^{dA}$	7,08
KG-244/4	$4,97^{defC}$	$3,13^{aA}$	$3,63^{aB}$	3,91	$9,77^{fgC}$	$3,80^{aA}$	$5,70^{dB}$	6,42
KG-199/4	$5,17^{fgB}$	$5,40^{iB}$	$3,83^{abA}$	4,80	$8,53^{bcdC}$	$4,07^{abA}$	$4,72^{abB}$	5,77
KG-307/4	$5,43^{ghB}$	$5,33^{iB}$	$3,88^{abcA}$	4,88	$10,07^{gC}$	$4,27^{bcA}$	$4,83^{abB}$	6,39
KG-28/6	$4,70^{cdeB}$	$4,07^{cdA}$	$4,43^{deAB}$	4,40	$9,40^{efC}$	$5,87^{eB}$	$5,11^{bcA}$	6,79
KG-162/7	$4,10^{aA}$	$4,53^{fghA}$	$4,36^{cdeA}$	4,33	$8,47^{bcB}$	$4,07^{abA}$	$4,47^{aA}$	5,67
KG-191/5-13	$5,70^{hiB}$	$4,40^{defA}$	$4,58^{eA}$	4,89	$8,63^{cdC}$	$4,20^{bcA}$	$5,76^{deB}$	6,20
KG-40-39/3	$4,17^{abA}$	$4,00^{bcA}$	$4,34^{cdeA}$	4,17	$8,10^{bB}$	$4,60^{cA}$	$4,78^{abA}$	5,83
KG-52/23	$6,03^{iB}$	$4,87^{hA}$	$4,38^{deA}$	5,09	$8,53^{bcdB}$	$5,20^{dA}$	$5,50^{cdA}$	6,41
KG-60-3/3	$7,12^{jC}$	$5,33^{iB}$	$4,19^{bcdeA}$	5,55	$8,43^{bcC}$	$4,53^{cA}$	$6,17^{eB}$	6,38
KG-1/6	$5,13^{efgB}$	$4,47^{efgA}$	$4,39^{deA}$	4,66	$8,70^{cdC}$	$4,53^{cA}$	$5,53^{cdB}$	6,25
KG-52/3	$4,33^{abcA}$	$4,13^{cdeA}$	$4,61^{eA}$	4,36	$7,57^{aC}$	$4,20^{bcA}$	$5,57^{dB}$	5,78
KG-47/21	$4,60^{bcdB}$	$3,67^{bA}$	$4,00^{abcdA}$	4,09	$8,47^{bcC}$	$4,47^{bcA}$	$5,77^{deB}$	6,23
Pobeda	$5,87^{hiB}$	$4,80^{ghA}$	$4,25^{bcdeA}$	4,97	$9,03^{deC}$	$6,07^{eB}$	$4,75^{abA}$	6,62
Prosek	5,14	4,47	4,24	4,62	8,81	4,71	5,31	6,27

Legend: SO – Sombor, KŠ – Kruševac, KG – Kragujevac
Lowercase and uppercase Latin letters were used to indicate significant differences between genotypes, and between locations, respectively

The analysis of the variance of the three - factorial experiment determined a significant effect of genotype, year and location on the variation of wheat yield, while the location did not have a statistically significant influence on the expression of hectoliter mass. In addition to the individual influence, all forms of interaction had a statistically significant effect on hectoliter mass and grain yield (Table 3).

Table 3. Analysis of variance for hectoliter mass and grain yield

Source of variation	df	Hectoliter mass		Grain yield	
		MS	F	MS	F
Block	2	0.662	0.595^{ns}	0.153	2.652^{ns}
Genotypes G	13	38.956	35.024^{**}	1.946	33.733^{**}
Years Y	1	1177.460	1058.618^{**}	173.022	2999.422^{**}
Environmental E	2	0.203	0.183^{ns}	147.761	2561.504^{**}
G×Y	13	10.889	9.790^{**}	1.354	23.474^{**}
G×E	26	4.868	4.377^{**}	1.125	19.502^{**}
Y×E	2	4.130	3.713^{*}	67.138	1163.865^{**}
G×E×Y	26	7.260	6.528^{**}	1.032	17.894^{**}
Eror	166	1.112		0.058	
Total	251				

** Significant at P = 0.01 level;* Significant at P = 0.05level;^{ns} Non significant

Heritability in a broader sense was calculated based on the results of the analysis of variance after disassembly of the variance components of the examined traits.It is a relative indicator and is calculated from the ratio of genotypic and phenotypic variance. The calculated values of the components of variance indicated which factors and with what intensity influenced the manifestation of the analyzed properties. The value of heritability for hectoliter mass was 73.66% and indicated a significant influence of genotype × location × year, as well as genotype on the formation of this trait. Grain yield had low heritability (25.92%) and the expression of this trait depended mostly on the interaction of locality × year (Table 4).

Table 4. Components of variance and heritability in a broader sense for hectoliter mass and grain yield

Traits	**Componentsof variance and heritability for hectoliter mass and grain yield**									
	σ^2_G	σ^2_Y	σ^2_L	σ^2_{GL}	σ^2_{GY}	σ^2_{LY}	σ^2_{GLY}	σ^2_E	σ^2_F	h^2 (%)
HM	1.692	0.247	0.443	0	0.403	0	2.049	1.112	2.297	73.66
Y	0.028	0	0	0.015	0.036	1.574	0.325	0.058	0.108	25.92

Legend: Y-Yield; HM- hectoliter mass

Lower values of heritability in the broader sense for grain yield are in accordance with the results (31.1%) obtained by Akçura (2009). Ali and Shakor (2012) obtained a lower value of heritability for durum wheat grain yield (41.27%) in dry growing conditions and a high value of heritability for grain wheat grain yield (92.60%). Taneva et al. (2019) found moderately high heritability values for hectoliter weight (72%) and lower heritability values for grain yield (36%). The authors point out that a lower coefficient of heritability is associated with lower genetic gain for grain yield, which indicates non-additive gene action and reveals slower breeding progress in improving these traits. Therefore, efficient selection of genotypes by phenotype in early generations is not possible for these traits.

According to the results obtained in these studies, a strong influence of genotypes, locations, as well as years on grain yield and hectoliter mass can be observed and this indicates the reaction of genotypes to different environmental conditions during growth and development. The first experimental year is characterized by a mild winter with a small amount of water sediment. However, the stages of earing, fertilization and grain filling took place at a lower air temperature and an extremely large amount of precipitation. Such unfavorable weather conditions negatively affected the processes of filling and maturing wheat grains, causing the formation of smaller, poorly filled grains. As a result, the examined KG-genotypes of wheat achieved lower values of grain yield and hectoliter mass in 2013/2014 years compared to 2014/2015 cropping season. The obtained results are in accordance with the respective of Rajičić et al. (2019), who fount significantly lower values of hectoliter mass and grain yield in 2009/2010, which is characterized by extremely high rainfall in April-June.

Conclusion

The year 2014 is characterized by extremely large amounts of precipitation in the Republic of Serbia, especially during April and May, which caused catastrophic floods in some parts of Serbia. Only in this period, 356.1 mm of rain fell in Kragujevac, 315.4 mm in Krusevac and 187.8 mm in Sombor. As a result, the examined KG-genotypes of wheat achieved lower values of grain yield and hectoliter mass in 2013/2014 years compared to 2014/2015 year. Observed for all locations, in both years, in comparison with the standard for grain yield, the Kragujevac lines KG-52/23, KG-307/4, KG-60-3/3 and KG-28/6 stood out the most.

In this study, higher values of heritability in a broader sense were obtained for hectoliter mass (73.66%), and lower values for grain yield (25.82%). This indicates that direct breeding for grain yield is less efficient and it is necessary to have knowledge about the nature of the inheritance of important yield componentsin order to improve this complextrait.

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COMPARISON OF BIOCHEMICAL METHODS FOR β-CAROTENE EVALUATION OF MAIZE INBRED LINES IMPROVED THROUGH MARKER ASSISTED BREEDING

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Abstract

Marker assisted breeding program aimed at developing β-carotene (BC) rich maize for growing in temperate regions is being conducted at Maize Research Institute "Zemun Polje" (MRIZP). Molecular markers were used both for precise transfer of gene of interest (foreground selection) and the recovery of the recurrent parent's genome (background selection). Thus, the *crtRB1* recessive homozygotes with the highest recovery of recurrent parent's genome were identified and their progenies were screened for BC content to confirm their nutritional superiority. The results presented in this paper relate to biochemical analyses aimed to identify BC_2F_3 plants with increased BC in conversion of three MRIZP commercial inbred lines (RP_1-RP_3). Two methods were used to determine BC and results were compared. The average BC content obtained by HPLC ranged from 12.68 µg/g in RP_3 to 18.39 µg/g in RP_2. Similarly, spectrophotometry recorded BC from 12.81 µg/g in RP_3 to 20.23 µg/g in RP_2. Although few discrepancies between individual results derived from these methods, both methods were informative in our research. Spectrophotometry has proved to be simpler, faster and less expensive method that can be used to determine the increase in BC relative to the RP line. However, when more precise BC determination is required, HPLC is highly recommended. Out of 34 derivations from three lines, 24 were chosen for the highest increase in BC content. These lines will serve as an important breeding material for developing β-carotene rich maize hybrids adapted to temperate regions.

Key words: *maize, marker assisted selection, β-carotene, spectrophotometry, HPLC.*

Introduction

Vitamin A deficiency (VAD) is one of the most prevalent micronutrient deficiencies and poses a serious health problem with the consequences such as night blindness, reduced growth in children, and increased morbidity and mortality (Natesan et al., 2020). 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 (Edem, 2009). Maize (*Zea mays* L.) is one of the major sources of provitamin A for humans. Thus, the improvement of provitamin A carotenoids in maize varieties through breeding or biofortification is a promising strategy to alleviate VAD (WHO, 2009).
Two major genes associated with β-carotene accumulation in maize are lycopene epsilon cyclase (*lcyE*) located at chromosome 8 and β-carotene hydroxylase enzyme (*crtRB1*) at chromosome 10 (Harjes et al., 2008; Yan et al., 2010; Zunjare et al., 2018). Gene-specific molecular markers for both *crtRB1* and *lcyE* genes have been developed for their utilization in marker assisted selection. According to Babu et al. (2013), *crtRB1* gene has a much larger effect on provitamin A concentration than *lcyE*. It has been predominantly introgressed to

enrich maize with provitamin A (Muthusamy et al. 2014; Liu et al. 2015; Zunjare et al. 2017; Natesan et al. 2020).
Natesan et al. (2020) successfully increased the β-carotene concentration in parental maize lines UMI1200 and UMI1230 and generated the β-carotene enriched hybrids through marker assisted backcross breeding (MABB). The β-carotene concentration of six improved lines ranged from 7.056 to 9.232µg/g. Furthermore, ACM-M13-002 was a superior hybrid with a 7.3-fold increase in β-carotene concentration compared to original hybrid. Similarly, Muthusamy et al. (2014) introgressed the favourable allele in elite inbred parents of agronomically superior commercial maize hybrids through MABB. Concentration of β-carotene among the *crtRB1*-introgressed inbreds varied from 8.6 to 17.5 mg/g – a maximum increase up to 12.6-fold over recurrent parent. The reconstituted hybrids developed from improved parental inbreds also showed enhanced kernel β-carotene as high as 21.7 mg/g, compared to 2.6 mg/g in the original hybrid.
Marker assisted breeding program aimed at developing β-carotene rich maize for growing in temperate regions is being conducted at Maize Research Institute "Zemun Polje" (MRIZP). Molecular markers were used both for precise transfer of gene of interest (foreground selection) and the recovery of the recurrent parent's genome (background selection) (Kostadinović et al., 2020). Thus, the *crtRB1* recessive homozygotes with the highest recovery of recurrent parent's genome were identified and their progenies were screened for β-carotene content to confirm their nutritional superiority. The results presented in this paper relate to comparison of two biochemical methods aimed to identify BC_2F_3 plants with increased β-carotene in conversion of three MRIZP commercial inbred lines. Newly developed *crtRB1*-introgressed inbreds will be utilized as parents in the β-carotene rich maize hybrid breeding program.

Material and methods

Plant material. Three MRIZP commercial inbred lines in Serbia were chosen for marker assisted conversion to β-carotene rich maize. These lines, used as the recurrent parents (RP_1, RP_2 and RP_3), are components of the leading MRIZP hybrids. As a donor of the favourable allele of *crtRB1* gene, a line provided by the International Maize and Wheat Improvement Center (CIMMYT) was used.
The conversion process (Figure 1) was described in Kostadinovic *et al.,* 2020. The *crtRB1* recessive homozygotes with the highest recovery of recurrent parent's genome were identified and their progenies were screened for β-carotene (BC) content to confirm their nutritional superiority.

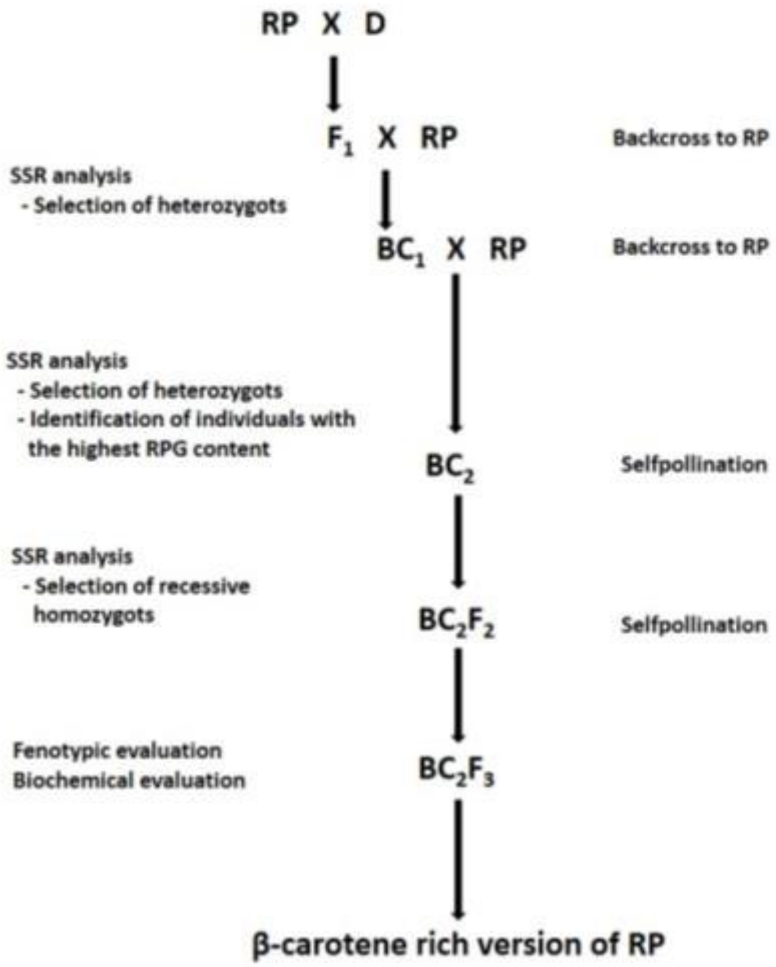

Figure 1. Schematic presentation of marker assisted conversion of standard maize to β-carotene rich maize line (RP-recurrent parent, D-donor line, RPG-recurrent parent's genome)

Biochemical analyses. Grain samples, consisting of 30 kernels each, were dried in a controlled oven at 65°C to constant dry weight and ground using a Perten 120 lab mill (Perten, Sweden) to obtain fine powder (<500 µm).

Spectrophotometry: Ground sample was extracted with water-saturated 1-butanol by means of homogenization for 30 min on horizontal shaker (Ika, Germany). The mixture was filtered and centrifuged at 10000 rpm for 5 min. The absorbance of supernatant was recorded on spectrophotometer (Shimadzu UV-1601) at 436 nm. β-carotene content was calculated using the conversion factor of 1.6632, expressed as µg per g of dry weight (DW) and reported as the mean value of two independent readings for two replicates.

High Performance Liquid Chromatography (HPLC): The extraction of β-carotene (BC) was performed with methanol. After the homogenization in ultrasound bath (Vims Elektrik, Serbia) for 5 min at 25 °C and on horizontal shaker (Ika, Germany) for 30 min at 25 °C, extracts were centrifuged and filtered (0.45 µm nylon syringe filter) directly into HPLC vials. Each extraction was replicated two times and both were injected two times into the Dionex Ultimate 3000 HPLC System (Thermo Scientific, USA). The mixture of methanol and acetonitrile (80:20, v/v) was used as the mobile phase for the separation of carotenoids. Samples were eluted through Hypersil GOLD® C18 column (150×4.6 mm, 3 µm) and detected with a photodiode array detector (at 450 nm and 470 nm). The concentration of BC was expressed as µg per g of dry weight and reported as the mean value of two independent injections for both replicates.

The t-test (Microsoft Excel) was used to determine how significant are differences between the results obtained with two methods.

Results and discussion

Biofortification of staple crops provides cost-effective and sustainable solutions to alleviate micronutrient deficiency, and is a viable approach over medical supplementation and dietary diversification (Tanumihardjo et al. 2007; Gupta et al. 2015). Maize is a reasonable choice of crops for biofortification with provitamin A carotenoids as it exhibits considerable natural variation in carotenoid content and profiles (Nkhata et al., 2020). Among all carotenoids in maize grain, β-carotene has the highest provitamin A activity and can be converted easily to vitamin A by the human metabolism (Haskell, 2012).

Marker assisted conversion of standard maize inbred lines to β-carotene rich genotypes adapted to temperate regions is being conducted at the Maize Research Institute "Zemun Polje" (Kostadinovic et al., 2020). After successful foreground and background selection, biochemical analyses were used to quantify and select for elevated β-carotene concentrations. Two methods were used to determine BC and results were compared (Table 1). As for HPLC, BC content ranged from 11.99 to 22.15 µg/g for RP_1 derivations, from 14.74 to 20.91 µg/g for RP_2 and from 9.37 to 16.44 µg/g for RP_3. When it comes to spectrophotometry, BC content ranged from 12.50 to 23.03 µg/g for RP_1, from 16.15 to 23.07 µg/g for RP_2 and from 9.63 to 15.81 µg/g for RP_3. Exept for a few results, it could be generally observed that values obtain from HPLC were somewhat lower compared to the ones from spectrophotometry. This also refers to their mean values, as well as to the values obtained for three RP lines. According to the t-test, the differences between the results from two methods were not significant at $p < .01$.

Table 1. β-carotene content of the three recurrent parents and their derivations

β-carotene content (µg/g)								
	HPLC	SPEC		HPLC	SPEC		HPLC	SPEC
RP_1	6.75±0.02	7.34±0.05	RP_2	3.63±0.13	4.23±0.15	RP_3	3.39±0.12	4.06±0.12
1	17.62±0.47	17.62±0.21	1	15.30±1.75	18.15±0.94	1	12.21±0.18	12.13±1.23
2	14.79±1.37	15.46±1.44	2	20.11±0.79	21.21±1.04	2	10.77±0.69	9.63±1.88
3	12.93±0.36	13.04±1.23	3	18.15±0.07	20.14±2.19	3	13.35±0.88	13.40±1.05
4	11.99±0.48	12.50±1.46	4	20.91±1.24	23.07±2.98	4	14.90±0.58	14.39±0.61
5	15.08±0.40	15.70±0.80	5	19.28±0.85	21.41±0.61	5	13.65±0.30	14.41±0.17
6	14.39±0.40	14.51±1.24	6	18.05±1.07	19.13±0.45	6	16.44±0.51	15.81±0.55
7	15.83±0.02	16.50±1.18	7	20.58±0.42	22.57±0.43	7	10.77±0.57	12.62±0.95
8	14.77±0.50	15.16±1.43	8	14.74±1.27	16.15±2.51	8	9.37±0.41	10.05±0.34
9	15.20±0.65	16.15±3.77						
10	18.54±0.21	18.44±2.27						
11	16.65±0.22	17.54±0.38						
12	17.07±0.16	17.71±1.35						
13	18.28±0.62	18.50±0.29						
14	19.56±0.60	20.18±0.24						
15	17.66±0.42	19.06±0.70						
16	22.15±0.51	23.03±1.45						
17	17.99±0.74	18.52±2.14						
18	20.70±1.00	21.97±0.55						

mean	16.73±0.51	17.31±1.23		18.39±0.93	20.23±1.40		12.68±0.52	12.81±0.85

* RP_1, RP_2 and RP_3 - recurrent parents, HPLC-high performance liquid chromatography, SPEC-spectrophotometry, mean: mean values for the derivations (RP values were excluded).

More importantly, the BC increase relative to RP lines should be discussed. These results are represented in Figure 2. The most noticeable discrepancies between two methods are within the RP_3 derivations, while this is not the case with the other two groups where the values are mostly in agreement. Compared to RP lines, BC increase was recorded in all three groups, considering both methods. As for HPLC, the highest BC increase was in RP_2 (4.06-5.76 times) and the lowest in RP_1 (1.78-3.28 times). This multifold BC increase among the BC_2F_3 progenies confirmed that introgressed favourable allele of the *crtRB1* gene had major effect on provitamin A concentration, due to reduced hydroxylation of β-carotene to further carotenoids in the pathway (Yan et al., 2010).

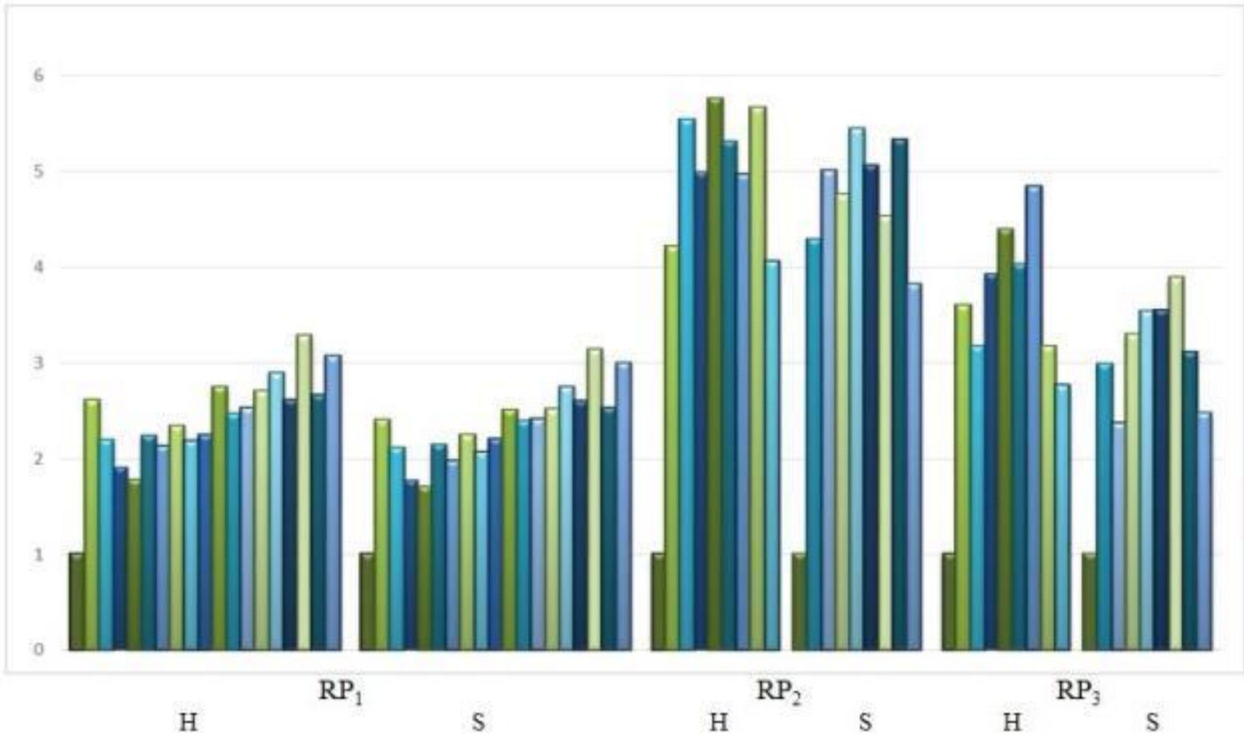

Figure 2. β-carotene content increase of the three recurrent parents′ derivations relative to their RP value (given as 1). RP_1-RP_3: recurrent parents, H: HPLC, S: spectrophotometry.

Similar trend was observed while validating the effect of *crtRB1* favourable allele in tropical maize. Babu et al. (2013) recorded a two-ten fold effect of *crtRB1-3´*TE on enhancing β-carotene and total provitamin A content. They concluded that MAS for the *crtRB1* locus alone appears to be a reliable strategy for rapidly achieving genetic gains for BC and total proA carotenoids in tropical maize breeding programs. As stated in Choudhary et al., (2016), research work at Indian Agricultural Research Institute’s (IARI) maize biofortification program has led to the introgression of *crtRB1* favourable allele into seven elite Indian inbreds using marker-assisted selection approach. Introgression of the target gene has resulted in increase of β-carotene by 6-14 folds over its original recurrent parent (Vignesh, 2012; Gupta et al., 2013). Introgressed inbreds possessed 5-7 folds more proA than their respective recipient parents in Zunjare et al. (2018), while the reconstituted hybrids had 4-5 folds higher proA over their original versions.

The present study thus analyzed concentration of kernel β-carotene among the MAS-derived inbreds, comparing the results obtained by two methods. Based on this biochemical validation, 24 lines were chosen for the highest BC increase compared to their parental line. Therefore, newly developed *crtRB1*-introgressed inbreds will be utilized as parents in the hybrid breeding program.

Conclusions

Both methods used to determine β-carotene were highly informative in our research. Spectrophotometry has proved to be simpler, faster and less expensive method that can be used to determine the increase in BC relative to the parental line. On the other hand, HPLC is strongly recommended when more precise determination is required. Out of 34 derivations from three lines, 24 were chosen for the highest increase in β-carotene content. The improved lines can be considered as the candidate parents for developing β-carotene hybrids adapted to temperate regions.

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GERMINATION OF ZANTHOXYLUM ARMATUM DC. SEED UNDER DIFFERENT TREATMENTS

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Abstract

Zanthoxylum armatum (rattan pepper, winged prickly ash) is a deciduous, spiny shrub originating from Asia. It has wide environmental tolerance and it can be used as an ornamental plant, suitable for living fences. Besides, rattan pepper is also used in folk medicine, essential oil production and as spice. It can grow in zone six and it is successfully planted in Bulgaria, indicating that this species can be cultivated in Serbia as well. For this reason, the seeds from healthy and vigorous plants growing in the Arboretum of the University of Forestry (Sofia, Bulgaria) were collected considering that mother plants were well adapted to climatic conditions in Balkans. The seeds were brought into the Laboratory for Seed Testing in Faculty of Forestry, Belgrade. The cold stratification (3-month or 4-month) and concentrated (96%) sulfuric acid (H_2SO_4) pretreatments were used. The highest germination rate was recorded after 3 months of cold stratification (22%), and other treatments resulted in lower germination rates. Obtained results may be affected by a quality of seeds from selected seed source, but also by testing time because *Z. armatum* usually has a low germination and the high germination percentage was reported 150 days after placing seed on germination. In our research seed testing was conducted during 28 days. However, vegetative propagation should also be considered for this species.

Keywords: *winged prickly ash, rattan pepper, dormancy, stratification, sulfuric acid.*

Introduction

Zanthoxylum armatum (rattan pepper, winged prickly ash, Timroo) is a large, spiny shrub or small tree with glossy green leaves which become reddish in autumn, and numerous yellow flowers followed by attractive red fruits containing black seeds. It is native to Asia, mostly distributed in subtropical and temperate zones of the Himalaya. Rattan pepper has wide environmental tolerance, it is an easy-to-grow, drought tolerant, ornamental plant, suitable for living fences, but it can also be planted in the large pots and it can be grown as a bonsai. Besides, rattan pepper is a medicinally important plant used in both traditional and modern medicine, for the treatment of gastrointestinal, respiratory, dental and cardiovascular disorders. It has antioxidative, anti-inflammatory, insecticidal, larvicidal, anthelmintic, antimicrobial, hepato-protective properties. Besides, rattan pepper is also used in essential oil production, its bark is used as traditional dye yielding resource and dried fruits are used as spice (Khare, 2007; Singh and Shikha, 2017; Khan, 2017; Kashyap *et al*., 2021).
Z. armatum can be propagated vegetatively, by cuttings or air layering (Purohit *et al*., 2016; Daudi *et al*., 2016), but generative propagation is a more common way for propagation of this species (Purohit *et al*., 2015; Datt *et al*., 2017). However, seed germination in *Z. armatum* can be low due to the presence of hard seed coat and scarification combined with cold stratification is recommended for successful germination (Purohit *et al*., 2015).

Rattan pepper is successfully planted in Bulgaria, growing in hardiness zone six, indicating that this species can also be successfully cultivated in Serbia. For this reason, the aim of this study was to propagate *Z. armatum* growing in the Arboretum of the University of Forestry (Sofia, Bulgaria) to obtain plants that can be grown successfully in Serbia, considering that mother plants were well adapted to climatic conditions in Balkans.

Material and Methods

Ripe fruits *were collected* from healthy and vigorous plants growing in the Arboretum of the University of Forestry (Sofia, Bulgaria) in October and they were brought into the Laboratory for Seed Testing in the Faculty of Forestry, Belgrade. They were kept in polyethylene bags at the temperature of 3-5°C for 4 weeks, and after that weight of fruits was measured as well as weight of seeds. Obtained data were used for calculation of an *extraction factor (the weight of cleaned seeds per given weight of* fruits*, expressed in percent),* number of seeds in 1 kg and *absolute weight of seeds (weight of 1000 seeds).*
The chemical scarification treatment was used for seed coat dormancy breaking and *cold stratification was* used for embryo dormancy breaking.
The experiment consisted of the following treatments.
- *cold stratification*
The seeds were disinfected by 0.6% solution of the fungicide *Captan* and *stratified in perlite at the temperature* 2 - 5ºC, for 3 or 4 months.
- **sulfuric acid scarification treatment**
The seeds were soaked in a concentrated (96%) sulfuric acid (H_2SO_4) for 120 minutes, then carefully rinsed using a running water, than stirred in water for additional 24 h using electromagnetic stirrer and disinfected by 0.6% solution of the fungicide Captan.
- **combination of sulfuric acid scarification and** *cold stratification* **treatment**
The seeds were treated with concentrated sulfuric acid as described above and *stratified in perlite* for 3 or 4 months before testing germination.
- control
The seeds were imbibed for 24 hours in distilled water, before they were placed on germination.
Germination testing was conducted by placing seeds *on the top of two layers of filter paper in the petri dishes, at the temperature 18-21°C, in long day conditions (light/dark period 16/8h). The number of germinated seeds was recorded daily, and germination energy was noted on 7th day. After 28 days, remaining seeds were cut in order to determine the percentage of viable seeds which is necessary to calculate the* real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić *et al*., 2010). *Each treatment consisted of four replicates with 50 seeds each.* Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

The extraction factor of collected seeds was 44.7%, 1 kg contains 50633 seeds and absolute weight of seeds is 19.75 g. Obtained germination rate was low, not exceeding 22% for seeds subjected to 3 months of cold stratification. Low germination rate of seeds treated with sulfuric acid followed by 3 months of cold stratification (3.5%) indicated that sulfuric acid treatment inhibited germination in our research. It can be explained by the long exposure of seeds to sulfuric acid treatment as well as with a high concentration of sulfuric acid. For example, Purohit *et al*. (2015) obtained very high real germination rate (93.3%) of *Z. armatum* after treatment with diluted sulfuric acid (50%) for 15 minutes, while longer (25 minutes) and shorter exposure (5 minutes) to diluted sulfuric acid resulted in lower real germination (less than 40%). However, Purohit *et al*. (2015) monitored germination up to 190 days after placing seeds on germination and there is a possibility that germination rate could be higher in our research after a longer period. Also, Purohit *et al*. (2015) conducted sulfuric acid treatment after cold stratification, while in our research sulfuric acid treatment was conducted before cold stratification.

Table 1 Germination of collected seeds

Treatment	Germination rate	Germination energy	Real germination rate
Control	4.50^{c}	0.00^{a}	6.44^{dc}
H_2SO_4	2.50^{c}	0.00^{a}	2.50^{d}
3 months stratification	22.00^{a}	0.00^{a}	22.00^{a}
4 months stratification	10.50^{b}	0.00^{a}	10.50^{cb}
H_2SO_4 + 3 months stratification	3.50^{c}	0.00^{a}	3.50^{d}
H_2SO_4 + 4 months stratification	14.50^{b}	0.00^{a}	14.50^{b}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Datt *et al*. (2017) reported a real germination rate of 72.5% of *Z. armatum* seeds treated with a combination of 200 ppm gibberellic acid and 100 ppm kinetin without prior cold stratification. However, treatments with only 200 ppm gibberellic acid or only 100 ppm kinetin resulted in considerably lower germination (35% and 40%, respectively). Also, they obtained a relatively high germination rate of 44.5% of seeds soaked in the water for 4 days without any plant hormone treatment. Similarly, Daudi *et al*. (2016) reported up to 33% germination rate of seeds soaked in the water for 12 hours before they were placed on germination, without additional pretreatments or cold stratification. However, in our research, the seeds were soaked in the water for 24 hours resulting in germination of only 6.5%.

Based on all this, we can assume that sulfuric acid treatment can enhance germination rate, but it is important to use low concentrations for a shorter period of time. It is possible that longer treatment with more diluted sulfuric acid (e.g. 10% or less) can result in better germination rate. Although Purohit *et al*. (2015) obtained germination rate of 93.3%, Datt *et al*. (2017) showed that effect of interaction of seed sources and pretreatments is statistically significant, which means that seeds originating from different sources will not have the same response to the same germination pretreatment. For this reason, it is important to conduct additional research, with more different treatments, in order to obtain a high germination rate of seeds collected from chosen plants.

Vegetative propagation can also be considered for propagation of the desired genotypes, but additional research is also necessary, because according to available data, the rooting percentage of green cuttings of *Z. armatum* is relatively low, not exceeding 50% (Daudi *et al*., 2016).

Conclusions

Z. armatum is an easy-to-grow, drought tolerant, ornamental, medicinal and edible plant which should be cultivated in Serbia. Seed collected from plants growing in Bulgaria had a low germination rate, not exceeding 22%. Although the best result was obtained after 3 months of cold stratification without other pretreatments, it is expected that treatment with low concentrations of sulfuric acid, soaking of seeds in water, or treatment with hormones can promote germination rate. However, obtained results may be affected by the quality of seeds from the selected seed source, and further research is necessary in order to obtain a higher germination rate of seeds.

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GERMINATION OF TETRADIUM DANIELLII (BENN.) T. G. HARTLEY. SEED EXPOSED TO DIFFERENT TREATMENTS

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Abstract

Tetradium daniellii (*syn. Evodia hupehensis* Dode), bee-bee tree or Korean evodia is a fast growing deciduous tree, with fragrant flowers blooming during summer at a time when few other trees are in flower. This melliferous species is native to Korea and China, but it is not recorded as an invasive species in Europe. It can be propagated by seed or vegetatively by root cuttings. There is no available data regarding generative propagation of this species and expected germination rate. For this reason, the seed pods were collected from healthy, vigorous elite tree growing in Temerin, in November. The following treatments were used: cold stratification in sand (30 or 60 days), immersion of *seeds* in *hot water* at 90°C for 90 seconds, alternate *freezing* and thawing (24 h at -18°C, 24h at the room temperature). The highest germination rate was obtained after four alternate *freezing* and thawing treatments (27%). Lower germination of 19.5% was obtained after single freezing and thawing treatment, while cold stratification resulted in very low germination, not exceeding 5%. There were no germinated seeds after hot water treatment and only 2.7% seeds germinated in the control, suggesting that appropriate pretreatments are required for T. daniellii germination, but additional research should be conducted in order to improve germination rate.

Keywords: *bee-bee tree, Korean evodia, cold stratification, alternate freezing and thawing.*

Introduction

Korean evodia or bee-bee tree (*Tetradium daniellii* (Benn.) T. G. Hartley, *syn. Evodia hupehensis* Dode, *Evodia daniellii* (Benn.) F.B.Forbes & Hemsl.) is a fast growing, small deciduous tree, with height up to 15 m, native to North and South Korea and southwestern China. It is cultivated as an ornamental tree, but it is not recorded as an invasive species in Europe (Roloff *et al*., 2018). Korean evodia is a medicinal and important melliferous species which blooms in July and August, at a time when few other tree species are in flower. The honey obtained from bee-bee trees is aromatic and it has high quality (Umeljić, 1999). The flowers are small, fragrant, white, sometimes tinted with purple, numerous in terminal panicles, followed by reddish-purple two-seeded pods which ripen in October and November (Bojnanský and Fargašová, 2007; Vukićević, 2006; Idžojtić 2019).

Korean evodia is a low demanding species which prefers moderately fertile, well-drained soils in full sun, but it can tolerate light shade, dry and sandy soils and it is resistant to urban air pollution (Roloff *et al*., 2018; Vukićević, 2006). For this reason *T. daniellii* should be grown more often in green spaces in Serbia. There are Korean evodia trees which are well adapted to the local conditions in Temerin (Serbia), and they can be used as a source for obtaining plants suitable for growing in urban conditions in Serbia. *T. daniellii* can be propagated by seed or vegetatively by root cuttings (Милев *et al*., 2004), but there is no much available data in literature regarding generative or vegetative propagation of this species, therefore the aim of this study was to test the effect of some pretreatments on germination of seed collected from elite plants growing in Temerin.

Material and Methods

Fruit clusters were collected from healthy, vigorous elite tree growing in Temerin, near school Kalaj Imre, in November. After bringing into the Laboratory for Seed Testing in the Faculty of Forestry, Belgrade, the following parameters were measured: length of fruit clusters including peduncle, width of fruit clusters without peduncle, thickness of fruit clusters, the weight of fruit clusters containing seeds and the weight of seeds obtained from single fruit clusters. These data were used for calculating an *extraction factor (the weight of cleaned seeds per given weight of* fruits, *expressed in percent). The* number of seeds in 1 kg and *absolute weight of seeds (weight of 1000 seeds) were determined by measuring 4 groups of 100 seeds and calculating mean value.*
After collection, part of collected seeds were placed on germination without any pretreatment (control). Part of the seeds were *stratified in sand at the temperature* 2 - 5ºC, for 30 or 60 days and germination was tested after stratification. Remaining seeds were kept at the room temperature for 2 months and following treatments were used: immersion of *seeds* in *hot water* at 90°C for 90 seconds, *freezing* and thawing (24 h at -18°C, 24h at the room temperature), 4 times alternate *freezing* and thawing (24 h at -18°C, 24h at the room temperature).
Germination testing was conducted by placing seeds *on the top of two layers of filter paper in the petri dishes, at the temperature 18-21°C, in dark conditions. The germination of seeds stratified for 60 days was tested both on the filter paper and on sand. The number of germinated seeds was recorded daily, and germination energy was noted on 7th day. After 21 days, remaining seeds were cut in order to determine the percentage of viable seeds which is necessary to calculate the* real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić *et al*., 2010). *Each treatment consisted of four replicates with 100 seeds each.* Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.
Part of the seeds collected in November from the same tree, were sown in cold frames, without any pretreatment, according to the recommendation by Krüsmann (1981). The seeds were covered with a 2-3 cm thick layer of mixture of garden soil and sand (1: 1), and they were monitored during the next 2 years.

Results and Discussion

Measured morphometric parameters are shown in Table 1. The extraction factor of collected seeds was 48.15%. The seeds were dimorphic, each capsule contained one smaller and one larger seed. The mixture of large and small seeds was measured and absolute weight of seeds was 6.38 g, which corresponds with data given by Милев *et al*. (2004) where absolute weight of seeds is 6g. According to that, 1 kg contains 156740 seeds. Also, we separated small and large seeds and calculated their absolute weight, which was 4.72 g for smaller seeds and 8.25 g for larger seeds.

Table 1. Morphometric parameters of collected seeds

Length of fruit clusters (cm)	Width of fruit clusters (cm)	Thickness of fruit clusters (cm)	Weight of fruit clusters (g)	Weight of seeds per fruit clusters (g)
11.0	8.04	5.23	17.18	8.27

The highest germination rate (27%) was obtained after four alternate *freezing* and thawing treatments (Table 2). Lower germination of 19.5% was obtained after single *freezing* and thawing treatment, while cold stratification *resulted in very low germination, not exceeding 5%. There were no germinated seeds after hot water treatment*, which suggests that this treatment probably damaged seeds. Freeze-thaw treatment is mainly used to overcome coat-imposed seed dormancy producing scars on seed surface (Tiryaki and Topu, 2014; Geneve, 2003; *Kimura and Islam, 2012*). Similarly, hot water soaking can also be used efficiently for breaking seed coat dormancy for some species (São José *et al.*, 2019; Giasson *et al.*, 2019). However, hot water treatment also can be used for seed disinfection, destroying pathogens outside and even inside the seed testa, sometimes more effective than chemical treatments, but its use should be evaluated carefully because it can be damaging for some species (Singh *et al.*, 2020).

Table 2. Germination of collected seeds

Treatment	Germination rate	Germination energy	Real germination rate
Control, filter paper	2.75^{dc}	0.25^{b}	2.77^{dc}
30 days stratification, filter paper	3.50^{dc}	0.00^{b}	3.52^{dc}
60 days stratification, filter paper	5.25^{c}	2.50^{a}	5.78^{c}
60 days stratification, sand	4.00^{c}	2.00^{a}	4.04^{c}
freezing and thawing, 4 times	27.00^{a}	0.75^{b}	27.28^{a}
freezing and thawing, single time	19.50^{b}	0.25^{b}	19.69^{b}
hot water treatment	0.00^{d}	0.00^{b}	0.00^{d}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

The seeds sown in cold frames germinated 16 months after sowing, overwintering twice in the cold frames. The seeds did not germinate in the spring after the first winter, but germination percentage after the second winter reached 80%.
Obtained results suggest that T. daniellii has endogenous dormancy and a hard seed coat and *appropriate scarification treatments should be combined with stratification in order to achieve uniform and rapid germination.*

Conclusions

Korean evodia is a low demanding ornamental, medicinal and melliferous species, resistant to air pollution and suitable for planting in urban areas in Serbia. It can be propagated by seed, and germination can reach 80% two years after sowing in cold frames in nursery. Freeze-thaw treatment can promote germination but obtained germination is low, not exceeding 27%. For this reason, additional research investigating the effect of combined scarification and stratification treatments is necessary *in order to improve the germination rate of this species.*

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EFFECTS OF RED AND FAR-RED LIGHT ON SEED GERMINATION OF CASUARINA CUNNINGHAMIANA MIQ.

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Abstract

Casuarina cunninghamiana is an ornamental medium or large tree originating from Australia but today it is widely cultivated worldwide, including European countries (Spain, Portugal, United Kingdom). It is fast-growing tree suitable for planting near rivers or lakes, growing even in saline soils, and it is used in erosion control or wind protection. The wood is used for panelling, furniture or as a fuelwood. *C. cunninghamiana* can be propagated by seed or vegetatively by cuttings, but generative propagation is the most commonly used for propagation of this species. The seeds are small and possibly photoblastic and the aim of this research was to determine the effects of red and far-red light on seed germination. The collected seeds imbibed for 72 hours, followed by exposure to red light (R), far-red light (FR), their combinations before placing seeds on germination. The seeds in the control treatment were kept in dark. Obtained results showed that River oak seeds are photoblastic, and the best germination rate (90.0%) was obtained after red light treatment, followed by germination in white light conditions (80.0%), and combination R-FR-R (87.8%). The germination in a control treatment was low (30.9%). However, germination energy was significantly higher in white light conditions (80.0%), than after red light treatment (11.1%).

Keywords: *River oak, germination, photoblastic seed, photosensitivity, far-red light.*

Introduction

Casuarina cunninghamiana (river she-oak, river oak) is a long-lived, fast-growing, medium or large tree, 15-35 m tall, originating from Australia. River she-oak usually grows on well drained, light-textured sandy or gravelly soils, with acidic to neutral pH value, it tolerates slightly alkaline soils, becoming chlorotic on highly calcareous soils. River oak also tolerates clay soils, moderate droughts, periodic waterlogging, and moderate salinity. *C. cunninghamiana* is suitable as a windbreak and shade tree, also for planting near rivers or lakes, along stream banks to protect them from erosion (riverbank stabilization). The timber is durable and can be used for flooring, firewood, poles, panelling or furniture, the bark can be used as tanbark, the foliage can be eaten by livestock, and the flowers are an important source of pollen for bees. Besides, river oak is an ornamental tree species with distinctive needle-like, green-gray, pendulous foliage (Whistler and Elevitch, 2006; Castle *et al.*, 2008; Orwa *et al*., 2009; National Research Council, 1984; Sun and Dickinson, 1995).
It is grown worldwide in Africa, Asia, Central, South, and North America, and in some European countries (Spain, Portugal, United Kingdom). Although *C. cunninghamiana* became naturalized and potentially invasive in some countries, this species is still recommended as a suitable alternative for invasive species *Casuarina equisetifolia* in many environments (Potgieter *et al.*, 2014; Whistler and Elevitch, 2006; Castle *et al.*, 2008; https://www.cabi.org).

C. cunninghamiana can be propagated vegetatively using softwood, hardwood or root cuttings, there are some reports regarding micropropagation of this species (Jiang *et al*., 2012; Shen *et al.,* 2009), but generative propagation is the easiest and the most commonly used method for propagation of this species. The expected germination rate ranges mostly 30-70% (sometimes up to 90%) depending on genotype, season of harvest and the collection site (Castle *et al*., 2008; Whistler and Elevitch, 2006; Lundquist and Torrey, 1984; Hartmann *et al*., 2014; Bonner and Karrfalt, 2008). The seeds are small and possibly photoblastic and the aim of this research was to determine the effects of red and far-red light on seed germination.

Material and Methods

River oak seeds were collected in the Parc de la Ciutadella, in Barcelona, Spain, in September. The seeds were kept in dry and dark conditions, in plastic bags, at room temperature, before setting the experiment in the Laboratory for seed testing at the Faculty of Forestry, University of Belgrade (Serbia). Before light treatments, the seeds were imbibed for 72 hours in distilled water, at a temperature of 20˚C. After that, the seeds were exposed for 5 minutes to red light (R) or far-red light (FR) (Table 1) using two types of filters and two types of lamps. Far-red light was obtained using a dark red plastic filter which was 4 mm thick and an incandescent lamp (Tesla 100w). Red light was obtained using a red plastic filter which was 3 mm thick and a fluorescent lamp (Philips 100w). Seeds in the control treatment were kept in dark. Seeds exposed to natural white light conditions were kept in the long day conditions (16h light and 8 h dark).

Table 1. Germination treatments

	Treatment
D	24 h dark - control
L	white light
R	red light
R-FR	red light + far-red light
R-FR-R	red light + far-red light + red light

The seeds were placed on germination on the top *of two layers of filter paper in the Petri dishes. The filter paper was previously moistened with 0.3%* (v/v) Previcur N fungicide solution (Bayer AG Germany, active ingredient Propamocarb hydrochloride).
The seeds that were exposed to red or far-red light and seeds that were kept in the dark were placed on germination in dark conditions, at 25˚C. Seeds exposed to natural white light conditions were germinated in the same long day conditions, also at 25˚C.
Each treatment consisted of *three replicates with 30 seeds (total 90 seeds per treatment). The number of germinated seeds was recorded daily during the period of 14 days, and* the germination energy was recorded on *5th day. After that, the number of remaining viable seeds that did not germinate was determined in order to calculate the* real germination rate, as a percentage of sound (viable) seeds that germinate (Grbić *et al.,* 2010). Obtained data were statistically analysed using the program Statgraphics Plus, Ver 2.1.

Results and Discussion

The highest germination rate (90%) was obtained after red light treatment, slightly lower germination was after combination R-FR-R (87.8%) and in white light conditions (80%), while germination in dark was very low, only 30.9% (Table 2). Obtained results showed that River oak seeds are photoblastic, and that red light treatments can be used to obtain high germination rate, even in dark conditions. Also, germination energy was significantly higher in white light conditions (80.0%), than after red light treatment (11.1%) or R-FR-R treatment (26.6%). However, real germination rate showed that there is no difference among white light, red light or R-FR-R treatments (Table 2).

Table 2. Germination of River oak seed

Treatment	Germination rate (%)	Germination energy (%)	Real germination rate (%)
D	30.9^{c}	4.4^{d}	37.2^{b}
L	80.0^{a}	80.0^{a}	93.3^{a}
R	90.0^{a}	11.1^{c}	96.4^{a}
R-FR	48.9^{b}	0.0^{d}	57.2^{b}
R-FR-R	87.8^{a}	26.6^{b}	96.3^{a}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Germination rate obtained in our research was high, because expected germination rate is mostly lower 30-70%, rarely reaching 90% (Castle *et al*., 2008; Lundquist and Torrey, 1984; Whistler and Elevitch, 2006; Bonner and Karrfalt, 2008; Hartmann *et al*., 2014). However, Hartmann *et al*. (2014) states that low germination results from shriveled, empty, and insect damaged seeds. Also, El-Lakany and Shepherd (1983) reported that germination temperature can influence the germination rate of *C. cunninghamiana*, and that germination was better at 30°C than at 22°C.

Conclusions

C. cunninghamiana is a fast-growing, ornamental tree, suitable for as a windbreak and shade tree and for riverbank stabilization, which tolerates moderate droughts, periodic waterlogging, and moderate salinity. It is propagated mostly by seeds, and our results showed that river oak seeds are photoblastic because high germination rate (80-90%) was obtained after red light treatments or in natural light conditions, compared to control and far-red light treatments where germination was significantly lower.

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THE IMPACT OF THE COVID-19 PANDEMIC ON GREENERY TRADE IN SERBIA

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Abstract

Besides cut flowers, greenery (cut greens or cut foliage) also represents an important part of the floricultural industry. The Covid-19 pandemic crisis led to the worldwide reduction of the production due to workers absence from work or working reduced hours, resulting in the drop in consumption and income. For this reason, the aim of this study was to compare greenery trade in Serbia during a five-year period before the pandemic (2015-2019) with the period during the pandemic (2020 and 2021). The total quantity (tons) of exported greenery decreased by 23.2% in 2020 compared to five-year period before the pandemic. However, the total value (in USD) of exported greenery rose by 57.6%. The quantity of exported cut foliage continued to decrease in 2021 by 7.1% compared to 2020, and the value of exported greenery rose by 6.9%. The main export markets are the European Union (EU), Switzerland and the countries of the Central European Free Trade Agreement (CEFTA). Despite expectations, the quantity of imported greenery doubled in 2021 compared to 2015-2019 period, and the value of imports in 2021 increased by 276.4% in comparison with five-year period before the pandemic. The greenery was imported mainly from EU and Costa Rica. The obtained data indicate that there is a rising demand on cut foliage markets and that cut greenery production should be increased in Serbia. The main amount of greenery for export is wild harvested and there is room for expanding existing nursery production.

Keywords: *cut greens production, cut greens trade, cut foliage market.*

Introduction

Cut foliages (greenery, cut greens) are decorative branches cut from trees, shrubs and perennials for use in bouquets and other flower arrangements. During the past decades, the worldwide demand for greenery is increasing, leading to increased production, and today cut foliage represents an important part of the floricultural industry (Hanks 2018; Whiriskey and Carthy, 2006; Centre for the Promotion of Imports from developing countries – CBI, 2022).

During the past three years, the Covid-19 pandemic had a great impact not only on human health (WHO 2022), but it greatly influenced economy at local, regional and international levels, with the reduction of the production system, drop in consumption, reducing income-generating activities, commercial establishments were temporary closed and the unemployment occurred in some sectors. The floriculture was also influenced by the pandemic, including production, trade and consumption of cut greenery (Anacleto *et al.*. 2021; Clair *et al.*, 2021; Lamm *et al.*, 2021; Lioutas and Charatsari, 2021). For this reason, the aim of this study was to compare greenery trade in Serbia during the five year period before the pandemic (2015-2019) with the period during the pandemic (2020 and 2021), and to assess if the pandemic influenced greenery trade in Serbia.

Material and Methods

The data on the export and import of cut greenery (given in tons and USD) were obtained from the databases of the Republic Bureau of Statistics for the period 2015-2021 (https://data.stat.gov.rs/?caller=SDDB). In order to obtain more accurate data, the cut greenery trade in Serbia during the pandemic years (2020 and 2021) was compared to the average data for the five-year period before the pandemic (2015-2019). Also, the trend analysis was used to calculate trends for the years 2020 and 2021 based on five years data (2015-2019) in order to determine expected change in export and imports according to data collected before the pandemic. Data obtained by trend analysis were compared with real data collected for the pandemic years 2020 and 2021, to see the difference between expected value of import and export and the real value recorded during the pandemic. Microsoft Excel 2007 was used for trend analysis.

Results and Discussion

The total quantity (tons) of exported greenery decreased by 23.2% in 2020 compared to the average export quantity during the five-year period before the pandemic, but the total value (in USD) of exported greenery rose by 57.6% (Table 1). The quantity of exported cut foliage continued to decrease in 2021 by 7.1% compared to 2020, and the value of exported greenery rose by 6.9%. However, the growth of greenery price in 2020 and 2021 was expected according to the values estimated by trend analysis, because estimated values were 0.55M and 0.62M and recorded export values were 0.57M and 0.61M in 2020 and 2021 respectively. Similarly, the estimated quantity of exports for 2020 and 2021 was not significantly different than the real amount of export that was recorded for these years, indicating that the pandemic did not influence the export of greenery from Serbia. Also, these data indicate that there is a trend of constant growth of export price of greenery during the past 7 years. The main export markets are the European Union (EU), Switzerland and CEFTA (Central European Free Trade Agreement). However, the highest average price per ton is recorded for exports to EU and the lowest average price per ton is recorded for exports to CEFTA countries (Table 1).

Table 1. Export of cut foliage from Serbia during 2015-2021.

Year	European union		CEFTA		Switzerland		World		World	
	t	USD*	t	USD*	t	USD*	t	USD*	t	USD*
2015	80.6	129.3	30.9	11.7	38.4	77.8	149.9	218.7	Estimated values based on 2015-2019 data	
2016	83.5	188.8	131.9	6.8	36.5	76.7	251.9	272.3		
2017	68.9	323.8	57.6	3.9	35.7	74.1	162.3	401.9		
2018	99.1	402.4	0.0	0.2	36.6	75.0	135.8	477.6		
2019	103.8	362.2	14.3	1.8	35.4	78.0	153.5	442.1		
2015-19*	87.2	281.3	46.9	4.9	36.5	76.3	170.7	362.5		
2020	79.2	513.4	29.7	3.8	22.2	54.3	131.1	571.6	138.0	558.1
2021	90.6	549.8	2.0	2.9	30.9	58.3	121.7	611.0	127.1	623.4

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period

Despite expectations, the quantity of imported greenery doubled in 2021 compared to the average value for the 2015-2019 period, and the value of imports in 2021 increased by 276.4% in comparison with the average value for the five year period before the pandemic (Table 2). The amount of export (in tons) was lower in 2020 and higher in 2021 than it was

estimated by trend analysis, indicating that the pandemic may have influenced the exports in 2020 and 2021, although these differences could be the result of some other influences and changes on global greenery trade market.
The greenery was imported mainly from the EU and Costa Rica (Table 2), but there are many other countries with a small share on import market during the observed period, including Guatemala, India, Mexico, United States etc.

Table 2. Import of cut foliage to Serbia during 2015-2021.

<table>
<tr><th rowspan="2">Year</th><th colspan="2">European union</th><th colspan="2">CEFTA</th><th colspan="2">China</th><th colspan="2">Costa Rica</th><th colspan="2">World</th><th colspan="2">World</th></tr>
<tr><th>t</th><th>USD*</th><th>t</th><th>USD*</th><th>t</th><th>USD*</th><th>t</th><th>USD*</th><th>t</th><th>USD*</th><th>t</th><th>USD*</th></tr>
<tr><td>2015</td><td>40.3</td><td>178.3</td><td>0</td><td>0</td><td>1.4</td><td>8.7</td><td>15.7</td><td>41.8</td><td>75</td><td>267.1</td><td colspan="2" rowspan="6">Estimated values based on 2015-2019 data</td></tr>
<tr><td>2016</td><td>55.9</td><td>147.8</td><td>0</td><td>0</td><td>1.9</td><td>14.1</td><td>13.7</td><td>47.8</td><td>81.2</td><td>254</td></tr>
<tr><td>2017</td><td>46.4</td><td>135.3</td><td>20.3</td><td>1.4</td><td>3.0</td><td>19.7</td><td>18.6</td><td>67.2</td><td>102</td><td>288.7</td></tr>
<tr><td>2018</td><td>60.3</td><td>144.7</td><td>6.5</td><td>19.4</td><td>3.9</td><td>24.9</td><td>28.2</td><td>59.0</td><td>113.9</td><td>311.6</td></tr>
<tr><td>2019</td><td>53.5</td><td>202.3</td><td>4.6</td><td>10.3</td><td>1.6</td><td>10.2</td><td>36.3</td><td>65.6</td><td>115.7</td><td>356.4</td></tr>
<tr><td>2015-19*</td><td>51.3</td><td>161.7</td><td>6.3</td><td>6.2</td><td>2.4</td><td>15.5</td><td>22.5</td><td>56.3</td><td>97.6</td><td>295.6</td></tr>
<tr><td>2020</td><td>50.8</td><td>223.5</td><td>0.6</td><td>0.5</td><td>2.8</td><td>20.5</td><td>23.1</td><td>78.9</td><td>109.0</td><td>445.6</td><td>131.8</td><td>366.4</td></tr>
<tr><td>2021</td><td>87.6</td><td>317.3</td><td>1.4</td><td>2</td><td>10.1</td><td>68.4</td><td>28.9</td><td>151.9</td><td>184.1</td><td>817.1</td><td>143.2</td><td>390.0</td></tr>
</table>

*Note: The value is given in thousands of USD; 2015-19 presents average value for five years period

Imported cut foliage mostly includes non-hardy plants, which in Serbian climatic conditions can only be produced in the greenhouses. However, the majority of the exported cut greens are obtained by collecting in nature, mostly branches from evergreen trees. Also, some native shrub species are suitable for obtaining cut greens, such as *Ruscus hypoglossum* L. or *R. aculeatus* L. which can be collected in accordance with the Decree on putting under control the use and trade of wild flora and fauna (2005). Although there are more than 300 registered nurseries in Serbia, the production of cut foliage is still underrepresented. Cut foliage production is considered as a new, innovative market-led sector of commercial horticulture in the world (Hanks 2018; Whiriskey and Carthy, 2006), and CBI (2022) consider EU, especially the Netherlands (as the major importer of foliage in Europe), as a very promising market for cut greens originating from developing countries. Therefore, all necessary actions should be taken in order to develop the outdoor production of cut greenery in Serbia. Also, the necessary measures should be taken for the sustainable use of forest resources, and for example, branches after de-limbing should be collected and transported from distant forest areas to be sold on the florist market, thus decreasing the need to overcollect them from living trees growing near areas with accessible transportation.

Conclusions

Cut foliage is used in a similar way as cut flowers, occupying an important position in the market, representing an important part of the floricultural industry, having a trend of constant growth of its price during the past years. The obtained data indicate that the pandemic did not influence greenery trade in Serbia and that there is a rising demand on cut foliage markets and that greenery production should be increased in Serbia. Currently, the main amount of greenery for export is wild harvested in Serbia and the necessary steps need to be taken to develop the outdoor production of cut greenery in Serbia.

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CONJUNCTIVE EFFECT OF ENVIRONMENT AND GENOTYPE IN MAIZE SEED PRODUCTION

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Abstract

Significant sources of normal plant development are the amount of available water, light, temperature and nutrients. This study aimed to examine to what extent the relationship between plant genetic structure and environmental conditions affects habitus and plant yield. In the two-year research, 2019 (Y1), and 2020 (Y2), with three maize lines (L1, L2, L3) produced at the Maize Research Institute, experiments were performed to assess the impact of genotype and environmental conditions on plant height to tassel (PHT), plant height to ear (PHE), ear weight (EW), cob weight (CW) and grain yield (GY). Seeds of different sizes were used in three sowings: large (S1), small (S2), and undivided (S3). The results of the PHE trial in the first year indicated a dominant genotype effect. L1 for all three sowings by seed size had the lowest cob position, S3G1 (69 cm), while L3 had the highest, (86.72 cm) for S3G1. In the second year of the study, environmental conditions and seed size significantly affected ($p \leq 0.05$) the PHE, as well as the PHT. The significance of the year effect was not confirmed by the weight of the cob. The highest yield was achieved for L1Y1S1 at 9.01 t ha^{-1} and the lowest for L1Y2S3 at 2.18 t ha^{-1}. Significant mutual effects of factors on the variability of traits are Y × L, Y × S, and Y × L × S. Differences in environmental conditions significantly affect the variability of maize corn seed properties. These effects can be reduced by proper genotype selection and the sowing of uniform size seeds.

Keywords: *environmental conditions, maize, cob position*.

Introduction

Each crop production is unique in its characteristic features. The reason for this is various factors that affect the expression of traits. The characteristics of the produced hybrid maize seed depend on many factors, such as genotypic combinations of parental lines, matching in the flowering of parental lines, duration of the fertilization time, duration of the grain-filling time, and abiotic and biotic factors. During the developmental phases of plants, agroecological conditions change, as a result of which they act differently, primarily temperature, radiation level, and humidity. Variability of traits and mode of expression is conditioned by time and space (García-Martínez et al., 2020), and changes in the growth and development of maize depend on these factors (Asare et al., 2011; Baoyuan et al.; 2016). Loss of phenotypic expression under the influence of lack of water is obvious for all cereals. Manifestations of suppression of traits in maize are expressed by reducing the number of seeds per cob, seed weight, cob length, plant height, and cob weight (Nabizadeh et al., 2012). The reduction of dry matter accumulation, which results in lower seed weight, especially in the period from silking to full maturity, is mainly caused by drought stress and insufficient radiation (Sah et al., 2020). Many phenotypic traits act as absorbers of stressful environmental conditions, so today there is maize breeding and selection of inbred lines for

specific living conditions that use these maize traits (Pandit et al., 2018). Due to climate change caused by anthropogenic factors, a relative decline in maize yield is predicted (Bolaños et al., 2019). Therefore, timely and objective predictions of the effect of factors on future crops are of great importance for production planning and providing sufficient food for a constantly growing population (Li et al., 2007).
This study aimed to identify the variability of morphological traits and yields of maize in production fields, which are the potential for proper crop management.

Materials and Methods

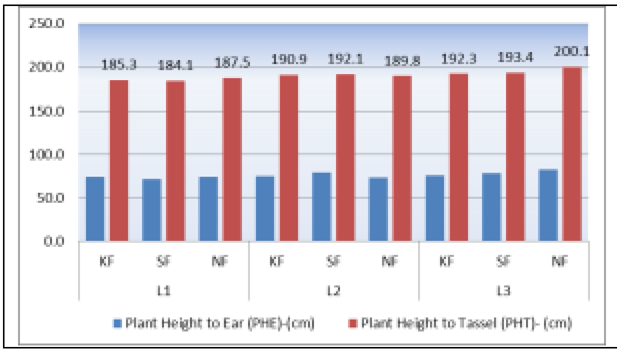
The material used in the experiment was three maize inbred lines: L1, L2, and L3 produced at the Maize Institute, maturity group FAO 400. The experiment was set up according to a completely random design in four repetitions, at the location of Zemun Polje, for a period of two years (2019-2020). The sowing in the experiment was manual, the seeds used for sowing were divided by size into a large fraction KF (8.5-11), a small fraction SF (6.5-8.5) and an undivided fraction NF (6.5-11). The elementary plot was 5 × 5.6, with nine rows of maize (3 × 3 rows of line x seed size). During the vegetation, standard agrotechnical measures of fertilization and protection against pests and diseases were applied. In the fertilization phase, the height of the plant to the tassel (PHT) and the height of the plant to the ear (PHE) was determined. Three plants from the middle row were randomly selected to measure the height using a wooden meter. Harvesting was done after determining the grain moisture of 25 %. The cobs were harvested manually and dried to 14 %. After harvesting and drying, samples of five cobs were formed. Ears from the centre row were selected to determine ear weight (EW) and cob weight (CW). The ears were measured first and then shelled to measure the cob weight (CW). The yield was determined based on the weight of all mid-row ears.
Meteorological data for the duration of the experiment were obtained from the measuring station at the Maize Research Institute. The average monthly temperature in the vegetation period in 2019 was 20 °C, which was 1.14 °C higher than in 2020, with the average temperature in the April-October period being 18.86 °C. Compared to the official standard period of 61-90, this was 2.51 °C higher for Y1 and 1.37 °C higher for Y2. As for precipitation in 2020, the amount of precipitation was 401.70 mm in the period from April to October, and in 2019 it was 373.7 mm. Compared to the official standard period of 61-90 of 433.0 mm, this was 59.80 mm less for Y1 and 31.30 mm less for Y2. The year 2019 was characterized by large amounts of precipitation in May-June (129.6-113.7 mm), and 2020 with 125.9 mm of precipitation in June.
All data were processed with the SPSS statistical package. The analysis was performed based on the mean values of the obtained results and the analysis of variance (ANOVA) to determine the significance of the differences.

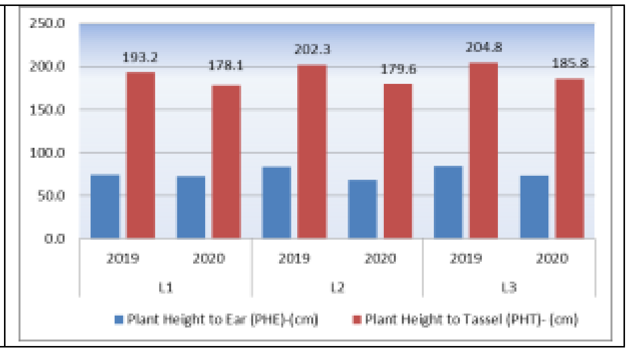
Results and discussion

Based on the mean values of morphological traits of PHT and PHE, all three genotypes differ, as well as for cob traits CW, EW, and GY. The L3 line stood out, which has the highest results for all observed traits.
Seed sizes, in all fractions in genotype L3, had the highest results for PHT (KF-192.32, SF-193, NF-36,200.11), and for PHE, (SF-78.33 and NF-82.2) (Graph 1). The seed size factor affected variation in mean values also for EW, CW and GY. In inbred lines, L3 and L2, seeds of fraction KF had the highest GY, 8.08 t ha^{-1}, and 6.9 t ha^{-1}, respectively (Graph 3). The inbred line L1 had the highest yield of 6.6 t ha^{-1} with seeds of SF. For EW and CW traits in L1 and L2 lines, LF had the best effect for exhibiting these traits, while for L3 it was NF.

Maize seeds are one of the most diverse in their shape and size. The role of seed shape and size from the point of view of evolution and ecology is presented as a theory of survival and reproduction (Westoby et al., 1992). From the agronomic aspect, this phenomenon in nature still does not have a clear perspective.

According to the environmental conditions, the first year of production was more favourable for the manifestation of morphological traits. Thus, all three lines in 2019 had a higher plant up to the ear (PHE) and a higher one up to the tassel (PHT). The highest average for PHT is for the L3Y1, 204.78 cm (Graph 2).



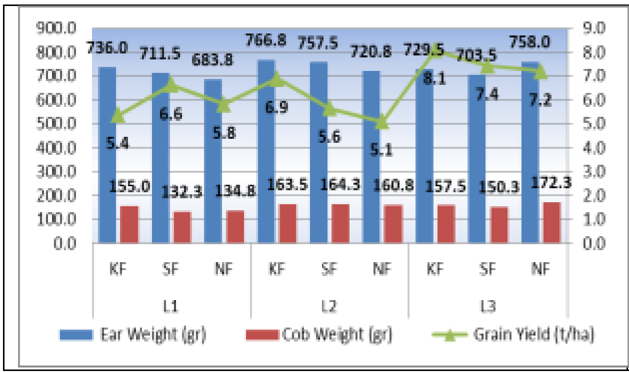


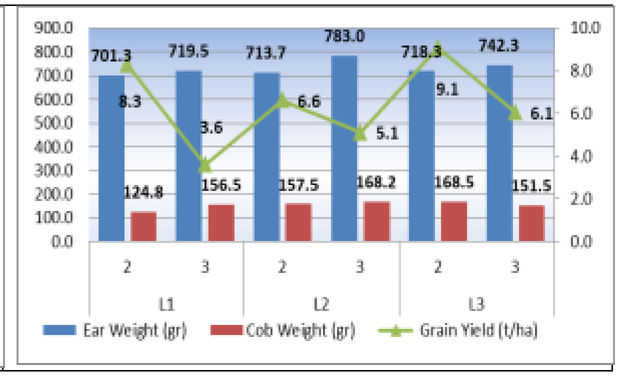
Graph 1. *Mean values for plant height to tassel (PHT) and plant height to ear (PHE) for inbred maize lines and seed size. L(1,2,3)-inbred lines, KF-large fraction, SF-small fraction, NF-undivided fraction.*

Graph 2. *Mean values for plant height to tassel (PHT) and plant height to ear (PHE) for inbred maize lines in different production years. L(1,2,3)-inbred lines.*

The year of production at GY in 2019 had a greater effect compared to 2020. All three lines had higher yields in Y1. The highest yield was 9.1 t /ha for L3Y1, and the lowest for L1Y2, 3.1 t ha^{-1} (Graph 4). Maize grain yield is a product of yield components that are interconnected. During plant development, different agro-ecological conditions are defined that contribute to differences in GY (Milander et al., 2016; Madić et al., 2021).

The heaviest EW was for L2Y2 (156.6 gr), and the heaviest CW was for L3Y1 (33.7 gr). In favourable environmental conditions, the weight of the cob is positive interdependence with other characteristics of the cob, such as the length and number of rows of grains (Milander et al., 2017).





Graph 3. *Mean values for ear weight (EW), cob weight (CW) and grain yield (GY) for inbred maize lines and seed size. L(1,2,3)-inbred lines, KF-large fraction, SF-small fraction, NF-undivided fraction.*

Graph 4*. Mean values for ear weight (EW), cob weight (CW) and grain yield (GY) for inbred maize lines in different production years. L(1,2,3)-inbred lines.*

The effects of different sources of variation were assessed by analysis of variance (ANOVA). As expected, the genotype is a factor that significantly influenced the differences between the properties of inbred lines ($p \leq 0.05$). The conditions for different year production are also significant for all differences resulting from the comparison of morphological trait's mean values ($p \leq 0.05$). The factor seed size was the least important. PHE is a stable trait, which was not affected by seed size. Further, regardless of the seed size factor, there were EW variations. Interactions are a significant source of variation in the manifestation of traits. In the experiment, all interactions, double and triple, had great effects. The double G×Y interaction had the greatest effect (Table 1).

Table 1. Estimation of factorial variance for phenotypes and morphological characteristics of maize lines

	F-value				
	PHE	PHT	EW	CW	GY
L	5.594*	6.889**	1.47ns	6.548**	0.978ns
FR	0.425ns	0.777 *	0.685ns	1.142*	0.745 *
Y	43.40 **	79,863 **	4.126 *	2.369ns	66,987 **
L * FR	2.108 *	0.706ns	1,050ns	1,628ns	0.258ns
L * Y	7.489 **	1,043ns	0.835ns	6,734 **	8.417 **
FR * Y	2.405ns	1,869ns	5.857 **	3.592 *	0.882ns
L * FR * Y	1.965ns	2.102 *	0.346ns	0.877ns	0.180ns

*- Significant at the 0.05 level, **- Significant at the 0.01 level, L- inbred line, KF- large seed fraction, NF- undivided seed, SF-small fraction, PHE- plant height to ear, PHT - plant height to tassel, EW-ear weight, CB- cob weight, GY grain yield, L-inbred lines, FR-seed fractions, Y-years.

Testing the significance of differences between the level of factors and the interaction at the level of significance $p \leq 0.05$, it was found that the differences between the two sets of seeds produced in different years are relevant for almost all traits except for CW. Less significant differences were also influenced by genotype factors. There are significant differences between L1 and L3 in PHT and CW, between L1 and L3 for PHE, and for L1 and L3 there were no significant differences.

Production years affected the differences in PHT, PHE, EW and GY ($p \geq 0.05$); only CW was equal in both years. Under the influence of different seed fractions, GY changed significantly, while other traits did not show variability under the influence of seed size (Table 2).

Table 2. Mean difference of a dependent variable

Dependent variable		Mean difference (1-2)				
1	2	PHE	PHT	EW	CW	GY
L1	L2	-2.5133	-5.2796 *	-7.5833	-4.4333 *	0.0642
L1	L3	-5.7858 *	-9.6262 *	-3.9833	-3.8667 *	-1.6338 *
L2	L3	-3.2725	-4.3467	3.60	0.57	-1.6979 *
KF	NF	-1.5388	-2.9708	4.65	0.55	0.7321 *
KF	SF	-1.1517	-.3688	3.98	1.95	0.2096
NF	SF	0.3871	2.6021	-0.67	1.40	-0.5225
Y1	Y2	9.331 *	18.952 *	-0.036 *	-0.008	35.053 *

*- Significant at the 0.05 level, L- inbred lines, KF- large seed fraction, NF-undivided seed, SF-small fraction, Y1- the production year 2019, Y2- the production year 2020, PHE- plant height to ear, PHT - plant height to tassel, EW-ear weight, CB-cob weight, GY grain yield.

Conclusion

Variations in the morphological characteristics of maize lines depended on all observed factors (genotype, location, seed size). Favourable amounts and precipitation schedule in 2019 are the result of the better expression of PHT and PHE, as well as GY. When establishing production, in addition to genotype and location, seed size also indicates the importance, first of all, of plant height characteristics and ear position on the plant, and then of the other characteristics, GY, EW, and CW. Identification of morphological traits of inbred lines that reduce stress effects is one of the indicators for proper genotype selection and production management.

Acknowledgement

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ANALYSIS FOR GRAIN YIELD OF MAIZE HYBRIDS IN WESTERN SERBIA USING EBERHART AND RUSSELL MODEL

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Abstract

The main goal of breeding in the past period was mainly aimed at increasing the yield potential, but in the last 40 years, attention was paid to the stability of yields in different environmental conditions. The distribution of hybrids, ie its value depends not only on its yield potential, but also on the ability to maintain such a property at a high level in different environmental conditions: different years, location, crop density, cultivation method, fertilizer levels, sowing date, type of land, etc. An objective evaluation of maize hybrids in intensive cropping systems requires identification not only of yield components and other agronomically important traits, but also of stability parameters. This study aimed to analyze grain yield and thousand-kernel weight in 11 maize hybrids having different lengths of the growing season (FAO maturity groups 300–700) at three locations in the Republic of Serbia over a period of two years (2017–2018) using the Eberhart and Russell Model. There were significant differences between individual hybrids and localities in terms of grain yield and 1,000-kernel weight. Also, both analyzed traits showed a significant interaction with the environment. A comparative review of average yields and stability parameters showed that, regardless of the maturation group, hybrids with higher yields showed generally less favorable values of stability parameters, ie better adaptation to more favorable conditions and specific reaction. The latter hybrids had low yields and high yield stability compared to the other hybrids.

Keywords: *maize, hybrids, stability parameters, grain yield, 1,000-kernel weight.*

Introduction

Despite the accelerated development of biotechnology and its increasing application in breeding, yield tests are still indispensable in the selection process. Therefore, the selection of appropriate genotypes and sites for testing is key to the success of a plant breeding program. Different years (climate change) and locality significantly affect yield (Lee and Tollenaar, 2007; Popovic, 2010; Changizi, et al. 2014). Changes in the environment affect both crop growth and grain yield due to significant interactions of genotype × environment (GEI) (Djurović et al., 2014; Stojaković et al., 2015; Oyekunle et al., 2017 and Faria et al., 2020). The stability and adaptability of genotypes is best assessed by assessing varieties in different environments and ecological regions. A genotype is considered stable if its performance is relatively constant under different environmental conditions. According to Becker and Leon (1988), the concept of biological or static stability implies that a stable genotype has minimal variance (say, yield) in different external environments. Several, stability analyzes have been proposed that could use the GE interaction to identify genotypes that show good performance or high yield in different agronomic conditions. Stability measures are based on either regression analysis or principal components analysis (Bernardo 2002). Some of the most commonly used stability parameters are the Finalii and Wilkinson regression coefficient

(1963), the Eberhart and Russell deviation regression (1966), the stability variance (Shukla (1972), the Kang yield stability parameter (1993) and the AMMI model, Gauch (1992). Multi-environment trials are being increasingly used for analysing GEI, assessing the stability of quantitative traits and finding associations among molecular markers and quantitative trait variation based on association analysis (Federer and Crossa, 2012). The model proposed by Eberhart and Russell (1966) has been widely used in recent decades mainly for classifying variations in genotype performance into predictable (regression) and unpredictable (regression deviations) components. Hypothetically, Eberhart and Russell’s method evaluates both yield (regression) and stability (deviation from regression), with regression being predictable and controlled to a certain extent through the selection of genotypes for specific locations. In this model, a regression coefficient greater than 1.0 indicates superior performance compared to the overall average of all genotypes. However, Alwala et al. (2010) reported that the regression coefficient indicates the “suitability“ of the model rather than genotype performance per se. Moreover, if the regression coefficient does not show significant differences across genotypes, regression lines can be grouped, with only deviation from regression being used as a stability parameter for genotype classification. Tollenaar and Lee (2002) reported that high-yielding maize hybrids can differ in yield stability and that yield stability and high grain yield are not mutually exclusive. The present study was therefore aimed to evaluate maize hybrids for their stability of performance for yield and yield components across different environments by using Eberhart and Russell (1966) stability model.

Material and methods

The research included 11 two-line maize hybrids of different maturity groups (300-700), namely: medium-early FAO hybrids 300-400 (ZP 330M, ZP 404, ZP 42A and ZP 480) medium-season hybrids FAO 500 (ZP 539, ZP 599 and ZP 580), and medium-late hybrids FAO 600-700 (ZP 677, ZP 633, ZP 704 and ZP 753).

The hybrids were tested in comparative field trials during 2018 and 2019 at three localities in Western Serbia, without irrigation, on lands of different production abilities: Parmenac (alluvium soil type), Mojsinje (vertisol soil type) and Tavnik (pseudogley soil type). The experiments were laid out in a randomised block design with four replications. Each hybrid was sown in four rows per replication with 17 hills (each with two plants) per row at a spacing of 0.44 m making up a plant density of 65,000 plants ha^{-1}. Two middle rows for measurements and analyzes were taken in the samples, and the end rows were excluded from the sampling (60 plants in total). Sowing was conducted in the first half of April in both years at all locations. Into the land with basic cultivation was introduced 20t ha^{-1} of manure i; in pre-sowing preparation 400 kg N: P: K ha^{-1} (16:16:16) and in top-dressing (phase 5-6 leaves) 250 kg ha^{-1} KAN. After harvest, in each plot, ear yield (60 plants) was measured and yield per ha was calculated at 14% moisture content. Laboratory analysis involved determination of 1,000-kernel weight (g) for all hybrids per replication by counting out four random samples of 500 kernels and calculating their weight. The results obtained were subjected to the factorial analysis of variance using F and LSD tests, and stability parameters were evaluated by regression analysis (Eberhart and Russell, 1966):

$Yij = m + biIj + dij$ ($i = 1, 2,..t$, $j = 1,2,..s$),

Where Yij – average of i-th hybrid at j-th location, m - overall average, bi - regression coefficient for cultivar “i” in relation to the environmental index, Ij – environmental index (difference between the hybrid average at “j-th location and overall average), dij – deviation from the regression line of hybrid “i” at location “j”. Standard t-test was used to test the significance of differences between regression coefficients and the average value (bi = 1).

Results and discussion

Combined analysis of variance ispresented in Table 1. Results of common analysis of variance for grain yield and 1,000-kernel weight showed significant differences among environments, hybrids, and environments × hybrid interactions that advocate adequacy stability analysis. Similarly, in their research Faria et al. (2017), Božović et al. (2018), Arunkumar et al. (2020) and Madić et al. (2021) pointed out significant differences among environments, hybrids and their interactions.

Table 1. Joint analysis of variance for grain yield and 1,000-kernel weight

		Grain yield (t ha^{-1})		1,000-kernel weight (g)	
Years		2017	2018	2017	2018
	Df	MS	MS	MS	MS
Sources of variation	10	16.92*	10.5**	5528.8**	3747.1**
Hybrids (H)	2	975.95**	618.46**	99915.6**	186896.5**
Locations (L)	20	7.71**	1.42**	1091.9**	419.9**

The highest yield on average in the first year had a hybrid ZP 599, while in the second ZP 677 (Table 2). Hybrids ZP 753, ZP 704 and ZP 536 in both years in terms of yield were also better than other hybrids, while the lowest yield, significantly lower than others had a hybrid ZP 330M. Significantly higher yields at all localities were in 2018. Hybrids of lower maturation groups have a higher potential for adaptation and also show a low reaction with the external environment and often cannot take advantage of favorable conditions (Troyer 1995). Genotype adaptability and stability are useful parameters for recommending hybrids for known cultivation conditions.

Table 2. Grain yield (t ha^{-1}) and stability parameters of maize hybrids over two years

Years	2017			2018		
Hybrid	Yield	$b_i \pm SE$	S^2d_i	Yield	$b_i \pm SE$	S^2d_i
ZP 330M	8.69	0.703 ± 0.1214	0.547**	7.03	0.967 ± 0.071	0.093*
ZP 404	10.28	0.569** ± 0.0106	-0.102	7.68	0.899 ± 0.181	0.876**
ZP 42A	10.78	0.866 ± 0.0349	-0.053	8.64	0.886 ± 0.164	0.711**
ZP 480	11.14	0.822 ± 0.0448	-0.018	8.57	0.863 ± 0.006	-0.046
ZP 536	12.09	1.175 ± 0.0898	0.252*	8.63	0.925 ± 0.095	0.207**
ZP 599	12.68	1.205 ± 0.1118	0.447**	9.55	1.148 ± 0.081	0.058
ZP 580	10.78	0.813 ± 0.0763	0.151	8.54	0.972 ± 0.052	0.028
ZP 677	12.39	1.285 ± 0.1463	0.842**	10.32	1.135 ± 0.091	0.186**
ZP 633	11.27	1.097 ± 0.3613	5.685**	9.57	1.103 ± 0.029	-0.024
ZP 704	12.10	1.234 ± 0.2023	1.708**	9.38	1.178 ± 0.021	-0.034
ZP 753	12.46	1.231 ± 0.0386	-0.040	8.94	0.925 ± 0.158	0.975
Average	11.33	1.000		8.81	1.000	
LSD 0.05	0.753			0.501		
0.01	0.999			0.664		

Eberhart and Rassell (1966) proposed an estimate of the variety's response to changed environmental conditions using the coefficient of linear regression and variance of deviation from regression. Cultivars are classified according to the value of their linear regression coefficients into those having bi less than, equal to, or greater than unity, as well as according to the value of the variance of regression deviations (equal to or different from zero).

Namely, cultivars that have regression coefficients greater than unity would be more adapted to favourable growing conditions, those with regression coefficients less than unity would be adapted to unfavourable environmental conditions, and those with regression coefficients equal to unity would have an average adaptation to all environments.
Also, genotypes with a variance deviation variance equal to zero would have very predictable behavior, while those with a non-zero regression deviation would have quite unpredictable behavior. The regression coefficient (*bi*) by year was not in agreement. The highest value of the regression coefficient (1.285) in the first year was (ZP 677), while the lowest, also significantly different from the average value was recorded in the hybrid ZP 404 (0.569). The regression coefficient of ZP 563 was closest to the mean value (1.097), with the exception of hybrid ZP 580 whose deviation from regression was not significant (0.151), most of the other hybrids had significant, ie highly significant deviations from regression (Table 2). The *bi* values in the second year did not differ significantly from the mean. The highest regression coefficient (1.178) was determined in ZP 704 and the lowest in ZP 480 (0.863). The regression coefficient of ZP 580 and ZP 330M (0.972 and 0.967) was closest to the mean. In the second year, the lowest and non-significant deviation from regression was exhibited by ZP 580 and ZP 599. The value of the standard error of the average regression coefficients in the first year indicates a greater heterogeneity of the values of this parameter compared to the second (Table 2). Alwala et al. (2010) reported that genotypes with high regression coefficients are considered high-yielding, as opposed to low-yielding genotypes (hybrids) with low values of the regression coefficient. Likewise, genotypes having a high value of $S^2 d_i$ are considered highly unstable, whereas those with low values as highly stable. The same authors underlined that regression analysis, although widely used in the previous period, provides useful information on genotype stability. A broader picture of grain yield cannot be completely predicted by this model. Although deviations from regression can be used as a stability parameter, the regression coefficient cannot be taken as a yield substituent due to the absence of significant correlation between regression coefficients and yield.

Table 3. Thousand-kernel weight (g) and stability parameters of maize hybrids over two years

Year	2017			2018		
Hybrid	1,000-kernel weight	$b_i \pm SE$	S^2d_i	1,000-kernel weight	$b_i \pm SE$	S^2d_i
ZP 330M	316	0.841 ± 0.1594	44.992	305	0.948 ± 0.003	-46.208
ZP 404	370	0.700 ± 0.185	85.198	317	1.101 ± 0.053	-21.994
ZP 42A	381	0.434** ± 0.119	-6.655*	341	0.847 ± 0.021	-42.692
ZP 480	371	0.877 ± 0.079	-43.05	338	0.997 ± 0.087	18.614
ZP 536	347	1.024 ± 0.203	116.282	322	1.145* ± 0.029	-38.880
ZP 599	367	1.205 ± 0.119	-7.275	332	1.165 ± 0.073	-1.307
ZP 580	381	0.811 ± 0.189	92.560	344	1.009 ± 0.022	-42.278
ZP 677	380	1.381* ± 0.070	-48.575	368	0.809 ± 0.081	9.551
ZP 633	347	1.074 ± 0.069	-49.203	318	1.032 ± 0.057	-18.546
ZP 704	333	1.513* ± 0.124	-0.858	315	1.180 ± 0.044	-30158
ZP 753	356	1.067 ± 0.147	27.101	338	0.768 ± 0.099	37.031
Average	359.02	1.000		330.8	1.000	
LSD 0.05	19.375			15.64		
0.01	25.703			20.66		

Thousand-kernel weight was highest hybrids ZP 580, ZP 42A and ZP 677, and lowest in ZP 330M. Significant differences in the trait were observed between hybrids as well as between locations. The highest and highly significant regression coefficient in the first year, relative to the average, was found in medium-late hybrids ZP 704 and ZP 677, the lowest hybrid ZP 42A, and the closest to the average value of hybrids ZP 539, ZP 753 and ZP 633. Positive values of regression deviation were insignificant (Table 3).

In the second year, hybrids ZP 704 (1.180) and ZP 539 (1.145) was significantly higher values of the regression coefficient compared to the average, and significantly lower hybrids ZP 42A (0.847) and ZP 677 (0.809). The closest mean value is the value of *bi* hybrid ZP 580 (1.009). According to the values of deviation from regression from the previous year in a small number of hybrids, positive deviation values were insignificant. A significantly higher value of the standard error (SE) of the average regression coefficients in the first year indicates much greater heterogeneity compared to the second (Table 3). Hybrids ZP 42A (high value 1,000-kernel weight) ZP 330M (minimum 1,000-kernel weight) and ZP 480 showed greater stability in relation to other hybrids. The significance of the hybrid / locality interaction for thousand-kernel weight is indicated Djurović et al. (2014), while Guanendera et al. (1996) indicates that this property does not interact with the external environment. The behavior of most hybrids in terms of this parameter is quite uneven, which is reflected in the discrepancy of values, especially by age. The hybrid ZP 704 with a low 1,000-kernel weight showed below-average stability, while unlike it, ZP 42A showed above-average stability with a high value.

Conclusion

There were significant differences between individual hybrids and localities in terms of yield and thousand-kernel weight. Yield and thousand-kernel weight also showed significant interaction with the external environment.
Hybrids with higher mean values of the analyzed traits, regardless of belonging to the maturation group, generally showed sensitivity, ie. adaptability to favorable environmental conditions in relation to hybrids with lower mean values.
From the group of hybrids of medium vegetation length, the ZP 599 hybrid achieved higher yields and had less favorable values of stability parameters compared to hybrids of longer vegetation.
Hybrids of FAO maturing groups 600 and 700 mainly had unfavorable values of stability parameters for all tested traits, ie showed specific response and better adaptation to favorable environmental conditions, but their yield level could rarely be endangered by the yield of an earlier hybrid.

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STABILITY PERFORMANCES OF DIFFERENT WHEAT GENOTYPES GROWN UNDER FAVORABLE AND SALINITY STRESS CONDITIONS

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Abstract

The present study was carried out to investigate the phenotypic variability and genotype × environment interaction (G×E) for spike weight of different wheat genotypes. The experiment included 27 wheat genotypes, grown under favorable conditions (Rimski Šančevi locality, Chernozem soil type) and salinity stress conditions (Kumane locality, Solonetz soil type), during two growing seasons. Using the AMMI analysis of variance we found a statistically significant ($p<0.01$) influence of additive and non-additive sources of variation on the phenotypic variation of spike weight. Additive sources of variation (genotype and environment) had a share of 62.29% in the total sum of square. The environmental factors (growing season and soil type) contributed to the variation of spike weight with a share of 53.75% in the total variation of the experiment, while the factor of genotype had a significantly smaller share (8.54%). The G×E participated to the total variation of spike weight with 20.84%, where the first two principal interaction components (PCA_1 and PCA_2) explained 91.74% of the interaction. The genotypes Harmonija, KG-58, Orašanka, Renesansa, Morava, Perfekta and Bankut 1205 were characterized by high values of spike weight and high stability. Genotypes Bankut 1205, Banatka, Grbljanka and Morava were in positive interaction with the vector of environment Rimski Šančevi 2015/2016 (favorable conditions), while the genotypes Harmonija, Gružanka, Oplenka, Šumadija and Premija reacted well to salinity stress conditions of environment Kumane.

Key words: *AMMI analysis, Chernozem, G×E, Solonetz, stability, wheat.*

Introduction

Wheat (*Triticum* spp.) accounts for 30% of world grain production and for 45% of cereal nutrition, thus representing a major food crop species (Charmet, 2011). Due to its primary presence in human nutrition, wheat is ranked first, as the most important among cereals (Iqbal *et al.*, 2021). In 2020, the total global production of wheat was 760 million tons, with global trade greater than all other crops combined (http://www.faostat.fao.org). Due to the ever-growing human population, the need to increase the area under wheat is growing, both in favorable and unfavorable environmental conditions (Shewry and Hey, 2015). The Global Map of Salt-Affected Soils (GSASmap) shows that more than 424 million hectares of topsoil (0-30 cm) and 833 million hectares of subsoil (30-100 cm) in the world are salt-affected, where 85% of salt-affected topsoils are saline, 10% are sodic and 5% are saline-sodic (https://www.fao.org/soils-portal/en/). In addition to drought, increased sodium content in the soil is one of the most common causes of abiotic stress which greatly reduces crop growth and productivity (Wang and Huang, 2019). Soil salinity causes stress in plants in two ways -

high concentrations of salt in the soil reduce the uptake of water and essential minerals; and high concentrations of salt in the plant lead to ionic toxicity (Hassanuzzaman *et al.*, 2021). Khokhar *et al.* (2017) and Mansour *et al.* (2020) found a significant decrease in the value of the grain yield and grain yield components of wheat under conditions of increased soil salinity. In order to select useful genetic variability for successful wheat production under stress conditions, it is important to study genotypes under real environmental conditions (Moustafa *et al*., 2021). However, grain yield and its components are not only under the influnce of genotype and environmental factors, but also of their interaction (Mohammadi *et al.*, 2018; Verma and Singh, 2021; Sime and Tesafaye, 2021). A widely used multivariate method for studying genotype and environment interactions (G×E) is AMMI (Additive Main Effects and Multiplicative Interaction). This method is effective because it considers a large part of the G×E sum of the squares, clearly separates the interaction effects and provides a good interpretation of the genotypes stability. The AMMI method uses analysis of variance to separate additive from multiplicative variance (G×E) and then uses a multiplicative procedure - PCA analysis, to explain G×E in detail (Zobel *et al*., 1988). This study aimed to: (i) establish the influence of additive and non-additive effects of variation on the phenotypic expression of spike weight; (ii) determine the stability performances of genotypes in different agro-ecological conditions; as well as (iii) single out highly stable genotypes with high values of spike weight.

Material and Methods

The experiment included 27 wheat genotypes, such as: two local landraces (Banatka, and Grbljanka); old Hungarian variety (Bankut 1205); twenty genotypes (Gružanka, Zastava, Aleksandra, Srbijanka, Kosmajka, Orašanka, Rujna, Šumadija, Harmonija, Ljubičevka, Perfekta, Premija, KG-56, KG-75, KG-58, KG-78, Morava, Lepenica, Šumadinka, and Oplenka) created at the Centre for Small Grains in Kragujevac; and four genotypes (Renesansa, NSR-5, Jugoslavija, and Pesma) released by the Institute of Field and Vegetable Crops in Novi Sad.

A field trial was conducted at two localities (Kumane, Vojvodina Province, 45.522°N 20.195°E, on Solonetz soil type; and Rimski Šančevi, Vojvodina Province, 45.322° N 19.836° E, on Chernozem soil type), according to a randomized complete block design with three replications. Solonetz is considered a soil of unfavorable physical and chemical properties, due to the high content of clay and Na ions in argiluvian (Bt) horizon. Also, with the increase in the sum of exchangeable cations in the Bt horizon, the content of exchangeable Na increases, as well as the alkalinity (pH > 9) (Belić *et al*., 2012). Chernozem soil type, chosen as a control treatment, is characterized by a good crumbly structure, stable aggregates, and good water permeability. Also, this soil is well provided with humus (3-4%) and plant nutrients (Hadžić *et al*., 2002).

During the study, the usual agro-technical practices for wheat production were applied. The size of the basic plot was 2 m^2. In both soil types, the examined genotypes were sown by continuous sowing, where the row spacing was 10 cm, and the distance between plots was 25 cm. In both growing seasons, the harvest was performed at the optimal time (last week of June), when the grain moisture was below 14%. The spike weight was measured in 30 plants for each analyzed genotype. The weather conditions of the 2015/2016 growing season were more favorable for wheat production compared with the weather conditions of the 2017/2018 season, in both localities. The amount of precipitation was higher in Rimski Šančevi locality compared to the amount of precipitation recorded in Kumane locality, while the mean monthly temperatures did not differ significantly in the analyzed localities, in both seasons. The warmer weather and lack of precipitation affected the earlier maturation of plants in 2017/2018 season (Figure 1).

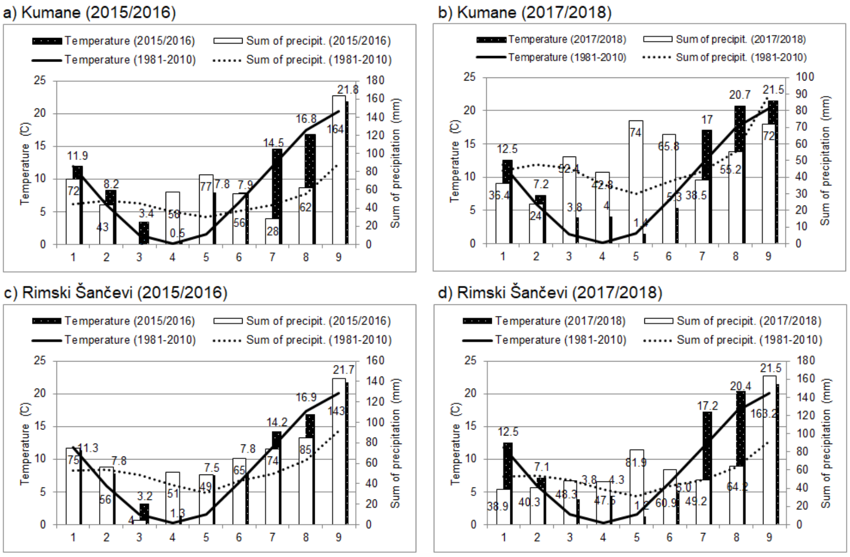

Figure 1. Mean monthly temperatures and sum of precipitation in Kumane locality (a, b) and Rimski Šančevi (c, d), during examined growing seasons

Additive main effects and multiplicative interaction (AMMI) analysis was applied in order to determine the stability of genotypes in different ecological environments. This analysis was performed using the program GenStat, Trial Version 18.1.0.17005 (https://www.vsni.co.uk/).

Results and Discussion

Using AMMI analysis of variance, a statistically highly significant ($p<0.01$) influence of additive and non-additive sources of variation on the variation of spike weight was found (Table 1).

Table 1. AMMI analysis of variance for spike weight in 27 wheat genotypes grown in different agro-ecological conditions

Source of variation	Df	Sum of squares (SS)	Mean of squares (MS)	F-value	Probability (p)	Share in variation (%)[1]
Total	323	108.13	0.335	-	-	100
Treatmants	107	89.89	0.840	11.90^{**}	0.000	83.13
Genotype (G)	26	9.24	0.355	5.03^{**}	0.000	8.54
Environment (E)	3	58.13	19.376	43.59^{**}	0.000	53.75
Block	8	3.56	0.445	6.30^{**}	0.000	3.29
G×E interaction	78	22.53	0.289	4.09^{**}	0.000	20.84
IPCA$_1$	28	13.35	6.76	6.76^{**}	0.000	59.25
IPCA$_2$	26	7.32	3.99	3.99^{**}	0.000	32.49
IPCA$_3$	24	1.86	1.10	1.10	0.349	8.25
Residue	0	0.00	-	-	-	-
Error	208	14.68	0.071	-	-	-

[1] The share of the sum of the squares of the main factors (genotype and environment), as well as the G×E interaction is expressed in relation to the sum of the squares of the total, while the share of the sum of the squares of the principal interaction components is expressed in relation to the sum of the squares of the G×E interaction (100%).

The environmental factor (growing season and locality) mostly contributed to the variation of spike weight, with a share of 53.75%, while the factor of genotype had a significantly smaller share (8.54%) in the total variation of the abovementioned trait. Similar results were established by Banjac (2015), in the study of wheat variability on ameliorated Solonetz. The share of G×E interaction in the total phenotypic variation of spike weight was 20.84%, where the first two main interaction components had a share of 91.74% in the G×E interaction ($IPCA_1$=59.25% and $IPCA_2$=32.49%), as shown in Table 1.
Assessment of genotype tolerance to salinity in real environmental conditions is of great importance in breeding wheat for increased tolerance to salinity (Moustafa *et al.*, 2021). In contrast to controlled laboratory conditions, in real environmental conditions the plants are exposed to all other abiotic factors, together with the factor of increased soil salinity. Therefore, an $AMMI_1$ biplot was created to assess the stability of wheat genotypes, in terms of spike weight, grown in favorable and unfavorable environmental conditions – in situ (Figure 2a). The genotypes Harmonija, KG-58, Orašanka, Renesansa, Morava, Perfekta, and Bankut 1205 showed high stability and high values of spike weight. Based on the expressed values of spike weight and stability, these genotypes are desirable for cultivation in the studied agro-ecological environments and for use in breeding programs. Also, these results confirmed that old genotypes, such as: Bankut, KG-58, Orašanka, and Morava, could be appropriate parental material in wheat breeding programs. Also, Gharib *et al.* (2020) emphasized that wheat landraces and old varieties can be considered valuable genetic resources for diversity and adaptation to salinity stress.

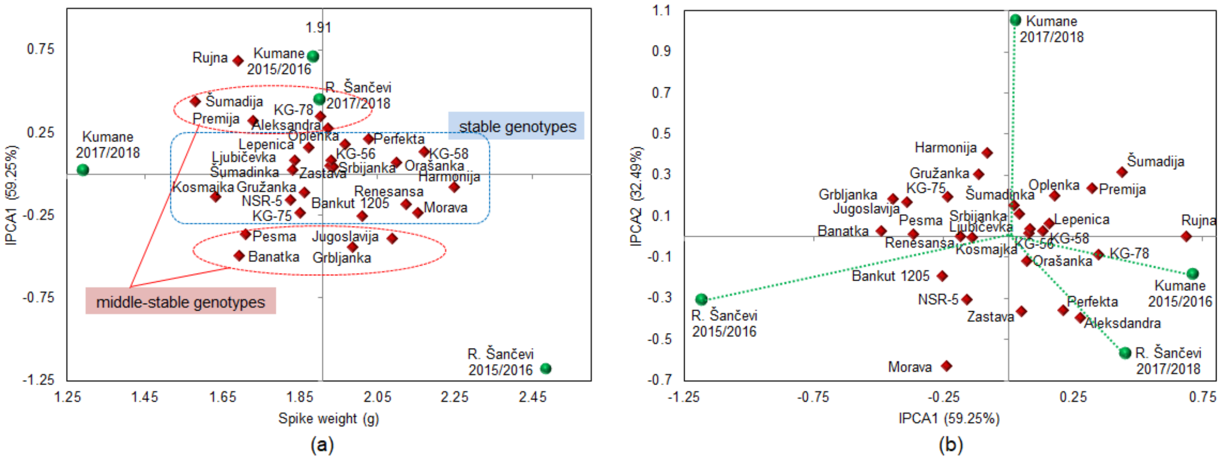

Figure 2. $AMMI_1$ (mean value of spike weight vs. $IPCA_1$) (a) and $AMMI_2$ ($IPCA_1$ vs. $IPCA_2$) (b) biplot for assessing the stability of 27 wheat genotypes grown on different environments

The genotypes Šumadinka, Ljubičevka, NSR-5, and Kosmajka had high stability, as well as values of spike weight that were lower than the average value for the trial. The genotypes Gružanka, KG-75, Lepenica, Oplenka, KG-56, Srbijanka, and Zastava were characterized by high stability and values of the spike weight close to the average for the experiment. Due to moderate stability and below-average values of analysed trait, the genotypes Šumadija, Premija, Pesma, and Banatka are considered undesirable for cultivation in different environmental conditions. The arrangement of the points of agro-ecological environments on the $AMMI_1$ biplot shows that genotypes reacted differently in various environmental conditions in terms of phenotypic expression of spike weight. The agro-ecological environment of Kumane 2017/2018 was characterized by the highest stability and the lowest average value of the abovementioned trait. On the other hand, the agro-ecological environment Rimski Šančevi 2015/2016 showed the highest instability and the highest average value of spike weight. Agro-ecological environments Kumane 2015/2016 and

Rimski Šančevi 2017/2018, with almost equal values of the spike weight, differed in the multivariate part of the variation, where environment Rimski Šančevi 2017/2018 had higher stability. Conditions of the increased soil salinity affected the decrease in the spike weight by 27% in relation to the value achieved in favorable soil conditions, on Chernozem soil type. Petrovic *et al*. (2016) found that spike weight on Solonetz was twice as small as spike weight achieved on Chernozem. Therefore, spike weight could be considered valuable phenotypic marker for selecting wheat genotypes with high salinity tolerance. Also, Khokhar *et al*. (2017) and Mansour *et al*. (2020) found a decrease in the value of wheat yield components under conditions of increased salinity.

By creating the $AMMI_2$ biplot, an additional 32.49% of the G×E interaction was explained (Figure 2b). The genotypes Ljubičevka, KG-56, KG-58, Lepenica, Srbijanka, Orašanka, Kosmajka, and Šumadinka showed low values of interaction with all agro-ecological environments. The genotypes Rujna, Šumadija, Morava, Aleksandra, Perfekta, Zastava, Harmonija, Grbljanka and Banatka showed the highest values of interaction components. However, the mentioned genotypes achieved positive correlations with the vectors of certain environments. The genotype Rujna achieved a positive correlation with the vector of environment Kumane 2015/2016. Unstable genotypes, such as: Aleksandra, Perfekta, and Zastava, with their interaction vectors, were close to the vector of agro-ecological environment Rimski Šančevi 2017/2018. Therefore, these genotypes reacted well to the favorable conditions of Chernozem soil in 2017/2018 growing season, which was characterized by a lack of precipitation. Genotypes Bankut 1205, Banatka, Grbljanka, and Morava, with moderately high and high values of $IPCA_1$ and $IPCA_2$, were positively correlated with the vector of agro-ecological environment Rimski Šančevi 2015/2016. The genotypes Harmonija, Gružanka, Oplenka, Šumadija, and Premija, were well adapted to the unfavorable conditions of environment Kumane 2017/2018.

Conclusion

A significant influence of additive and non-additive effects on the variation of spike weight has been established, where environmental factors had the largest share. Conditions of increased soil salinity reduced the value of spike weight by 27%, due to this trait can be an appropriate phenotypic marker in the selection of salt-tolerant wheat genotypes. The genotypes Harmonija, Orašanka, KG-58, Renesansa, and Morava were characterized by high stability and high values of spike weight. The highest stability and the lowest value of the analyzed trait was manifested in the agro-ecological environment Kumane 2017/2018, while the highest value of spike weight and highest instability were established in the environment Rimski Šančevi 2015/2016. The genotypes Harmonija, Gružanka, Oplenka, Šumadija, and Premija were well adapted to unfavorable conditions of Solonetz spil in conditions of reduced rainfall, while the genotypes Bankut 1205, Banatka, Grbljanka, and Morava reacted well on favorable environmental conditions of Rimski Šančevi locality in 2015/2016 growing season.

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IDENTIFICATION OF *ETR1* ALLELES IN SOME *MALUS* MILLER SPECIES

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Abstract

Wild *Malus* Miller species offer diversity essential for future breeding programmes that are not available within domestic and foreign apple cultivars. These species may provide novel alleles not just for disease resistance, but also for various desirable traits. Predicting and optimising the quality of stored apples and maximising their shelf life are becoming a major challenge worldwide. Ethylene regulates a broad spectrum of developmental and physiological processes in apple (including fruit ripening) and being perceived through the family of receptors, among which Ethylene Receptor 1 (ETR1) is one of the Subfamily I receptors, encoded by *ETR1* gene. This study aimed to identify the *ETR1* alleles in nine *Malus* Miller species from the *ex situ* collection of the Fruit Research Institute, Čačak, Republic of Serbia. The *ETR1* alleles were revealed by polymerase chain reaction (PCR) and additional restriction analysis of amplified PCR product (~5,000 bp) with *Rsa*I, *Alu*I and *Hinf*I enzymes. Upon comparison of the observed polymorphisms, six alleles of *ETR1* gene (*a*, *b*, *c*, *d*, *f* and *g*) were identified, among which *f* and *g* alleles were revealed in this study for the first time. Further characterisation of the novel alleles will be performed through cloning and sequencing of the corresponding DNA fragments. The polymorphisms observed upon PCR amplification and subsequent digestion with aforementioned restriction enzymes were generated in six allelic constitutions for *ETR1* gene – *bb* (one species), *bd* (one species), *bf* (one species), *b,a/c* (three species), *ff* (two species) and *g,a/c/d/g* (one species).

Keywords: *Apple, wild species, ethylene receptor, allelic constitution, fruit storage quality.*

Introduction

Ethylene is a gaseous phytohormone involved in various aspects of developmental and physiological processes throughout the plant life cycle, including fruit ripening (Abeles *et al*., 1992). This hormone is essential for fruit ripening, although its high production negatively affects shelf life and quality of apple fruits. Ethylene production commences from S-adenosyl-L-methionine via the intermediate 1-aminocyclopropane-1-carboxylic acid (ACC) in an ACC synthase (EC: 4.4.1.14; *ACS* gene) catalysed reaction and completes in an oxidation reaction catalysed by ACC oxidase (EC: 1.14.17.4; the *ACO* gene) (Yang, 1985). These two genes are encoded by the multigene families (Li *et al*., 2013; Binnie and McManus, 2009), whereas *ACS1* (Harada *et al*., 1997) and *ACO1* (Wakasa *et al*., 2006) are predominantly expressed in the ripening apple fruit tissue. Responses to ethylene are conducted by signalling through endoplasmic reticulum-localised receptors, which exist as a multimember family and act as negative regulators of the ethylene-signalling pathway (Gallie, 2015). Up to date, five receptor gene homologs have been reported in apple fruit (Yang *et al*., 2013). Among these receptors, Ethylene Receptor 1 (*ETR1* gene) homologue was isolated by Lee *et al*. (1998). The study of polymorphisms of genes involved in biosynthesis and perception of ethylene can help investigate the possibility that allelic forms of these genes might correlate with a wide range of its production during apple fruit ripening and, in addition, could be important in cultivar genotyping. Therefore, two alleles of *ACS1* gene (*ACS1-1* and *ACS1-2*; Sunako *et al*., 1999], several alleles of *ACO1* gene [alleles *a, b c, d* and *n* (alleles *a* and *b* correlate with alleles *A* and *B,* as well as *ACO1-1* and *ACO1-2*); Castiglione *et al*., 1999; Costa *et al*., 2005; Marić *et al*., 2005, 2019, 2021a, 2021b; Marić and Lukić, 2014, Marić, 2016] and five alleles of *ETR1* gene (*a*, *b*, *c*, *d* and *e*; Marić *et al*., 2007, 2009, 2021b) have been detected so far. In the latest studies, Marić (2016) and Marić *et al*. (2019) reported that allele *d* of the *ACO1* gene has been detected only in the *Malus* wild species so far, which supports the fact that wild species may provide novel alleles that could be very important for future breeding programmes. Predicting the quality of stored apples and maximising their shelf life are becoming major objectives of many breeding programmes, aiming to provide year-round high-quality apples to consumers (Zhu and Barritt, 2008). In order to achieve these, Marić and Lukić (2014) stated that the integration of marker-assisted selection into conventional breeding should consequently increase the breeding efficiency in apple, as an outcrossing fruit species whose genome is highly heterozygous. Therefore, the study of allelic forms of genes involved in the ethylene metabolic and signalling pathways is very important, since this hormone plays a major role in the ripening of climacteric apple fruit. This work aimed to study the allelic polymorphism of gene involved in ethylene perception (*ETR1* gene) in nine *Malus* species from the *ex situ* apple collection of the Fruit Research Institute, Čačak (FRI), Republic of Serbia.

Material and Methods

The following nine *Malus* species were sampled from the *ex situ* apple collection of FRI: *Malus* × *atrosanguinea* (Spaeth) Schneid., *Malus floribunda* Siebold ex Van Houtte, *Malus hupehensis* (Pamp.) Rehder, *Malus* × *micromalus* Makino, *Malus niedzwetzkyana* Dieck, *Malus prunifolia* (Willd.) Borkh. var. *microcarpa*, *Malus prunifolia* (Willd.) Borkh. var. *xanthocarpa*, *Malus sargentii* Rehder and *Malus sieboldii* (Regel) Rehder, that was established in 1986 at 'Zdravljak' facility near Čačak, with three trees per genotype, grafted on 'M26' rootstock, at a spacing of 4 × 1.5 m. Young leaf material of the assessed species was picked, frozen in liquid nitrogen and stored at -80°C. Frozen leaf sample was ground with four ball-bearings in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) and isolation of genomic DNA was based on the CTAB method of Doyle and Doyle (1987), with the extraction buffer modified with 0.5% β-mercaptoethanol and 2% polyvinylpyrrolidone. Isolated DNA was dissolved in TE buffer (10 mM Tris, pH8.0 and 1 mM EDTA), incubated with RNase A (10 μg ml^{-1}; Invitrogen, Groningen, the Netherlands) and kept at -20°C until used for PCR reaction. PCR reaction [50 µl volume with 100 ng of genomic DNA, 1× PCR buffer, 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0.2 µM of ETR1-F/R primers and 2.5 U of Taq DNA polymerase (Qiagen GmbH, Hilden, Germany)], amplification conditions (94°C for 1 min, followed by 10 cycles of 94°C for 10 sec, 63°C for 1 min and 68°C for 4 min and 25 cycles of 94°C for 10 sec, 63°C for 1 min and 68°C for 4 min + 10 sec per cycle, with a final 10 min at 68°C) and allele identification by restriction analysis with *Rsa*I, *Alu*I and *Hinf*I enzymes [26.8 µl PCR product was incubated for 12 h at 37°C with a solution of 3.2 µl containing restriction enzyme (10 U $µl^{-1}$) and buffer (10× dissolved)] were based on the methods of Marić *et al*. (2007, 2021b). PCR products of *ETR1* gene were separated by electrophoresis in a 1.5% agarose gel (70 V cm^{-1} for 3 h), whereas restriction fragments were analysed by electrophoresis in 2% agarose gel (70 V cm^{-1} for 4 h). Fragments were visualized by ethidium bromide (0.5 µg ml^{-1}) staining, photographed in BIO-PRINT-1500/26M imaging system (Vilber Lourmat, Marne-la-Vallée, France) and sized by comparison with a 1 Kb plus DNA ladder (Invitrogen, Groningen, the Netherlands).

Results and Discussion

Upon amplification of the *ETR1* gene, using ETR1-F and ETR1-R primers (Marić *et al*., 2007), PCR product of approximately 5,000 bp was obtained. The fragment size was consistent with the size of *ETR1* PCR product reported for autochthonous and foreign apple cultivars (Marić *et al*., 2007, 2009, 2021b) and seedlings from the cross 'Fiesta' × 'Totem' (Fernández-Fernández *et al*., 2008). Restriction analysis of the *ETR1* PCR product was performed with *Rsa*I [five segregating fragments of 800 bp (f_1), 890 bp (f_2), 1,050 bp (f_3), 1,130 bp (f_4) and 1,300 bp (f_5)], *Alu*I [a polymorphic fragment of 850 bp (f_1)] and *Hinf*I [a segregating fragment of 1,130 bp (f_2); additionally, Marić *et al*., 2009 reported another polymorphic fragment of 800 bp (f_1) in apple cultivar 'McIntosh' which possesses *e* allele] enzymes. The interpretation of the polymorphism obtained was based on the results reported by Marić *et al*. (2007, 2009, 2021b) and additional polymorphic fragments [three segregating fragments of 1,050 bp (f_3), 1,130 bp (f_4) and 1,300 bp (f_5)] revealed in this study upon digestion with *Rsa*I restriction enzyme. The relationship between restriction fragments and the alleles of *ETR1* gene is presented in Table 1, and an example of banding patterns obtained upon digestion with *Rsa*I is shown in Figure 1. Therefore, six alleles of *ETR1* gene were deduced, i.e. *a*, *b*, *c*, *d*, *f* and *g*, among which *f* and *g* alleles were revealed in this study for the first time. Cloning and sequencing of the DNA fragments corresponding to novel alleles (*f* and *g*) will provide their further characterisation. The most frequent allele among six identified was allele *b*, with a high frequency of occurrence (38.89%). *ETR1*-genotyping of *Malus* species revealed six allelic constitutions (Table 2). Of the nine evaluated species, one species each was scored as *bb*, *bd* and *bf*, while the phenotype *ff* was identified in two species; the allele *b* was identified in three species and the second allele might be *a* or *c*; the allele *g* was identified in one species and according to the phenotype the second allele might be *a, c, d* or *g*.

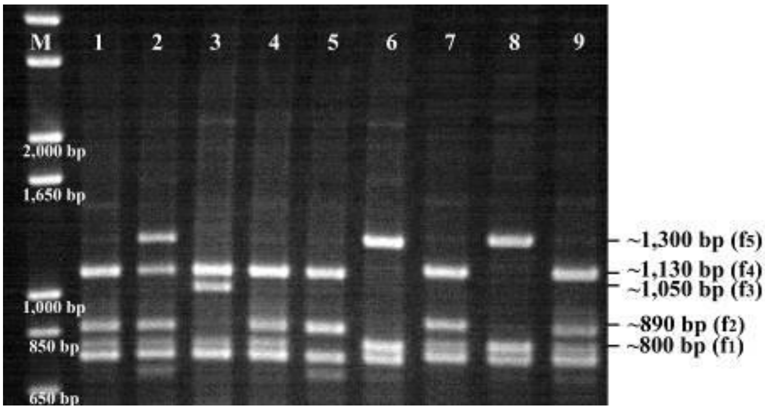

Figure 1. DNA fragments obtained upon digestion of the *ETR1* PCR product with *Rsa*I in nine *Malus* species, separated on a 2% agarose gel and stained with ethidium bromide: *Malus × atrosanguinea* (lane 1); *Malus sargentii* (lane 2); *Malus × micromalus* (lane 3); *Malus prunifolia* var. *xanthocarpa* (lane 4); *Malus floribunda* (lane 5); *Malus sieboldii* (lane 6); *Malus niedzwetzkyana* (lane 7); *Malus hupehensis* (lane 8); *Malus prunifolia* var. *microcarpa* (lane 9); 1Kb plus DNA ladder (M).

Table 1. Relationship between restriction fragments, obtained upon digestion of PCR product with *Rsa*I, *Alu*I and *Hinf*I enzymes, and the deduced *ETR1* alleles

*Rsa*I					*Alu*I	*Hinf*I		*ETR1* allele
800 bp (f_1)	890 bp (f_2)	1,050 bp (f_3)	1,130 bp (f_4)	1,300 bp (f_5)	850 bp (f_1)	800 bp (f_1)	1,130 bp (f_2)	
+	–	–	+	–	–	–	–	*a*
–	+	–	+	–	+	–	–	*b*
+	–	–	+	–	+	–	–	*c*
+	–	–	+	–	+	–	+	*d*
+	+	–	–	–	+	+	–	*e*
+	–	–	–	+	+	–	–	*f*
+	–	+	+	–	+	–	+	*g*

Table 2. Restriction analysis and *ETR1* genotypes in the assessed *Malus* species.

Species	*Rsa*I					*Alu*I	*Hinf*I		*ETR1* genotype
	800 bp (f_1)	890 bp (f_2)	1,050 bp (f_3)	1,130 bp (f_4)	1,300 bp (f_5)	850 bp (f_1)	800 bp (f_1)	1,130 bp (f_2)	
Malus × atrosanguinea	+	+	–	+	–	+	–	–	*b,a/c*
Malus floribunda	–	+	–	+	–	+	–	–	*bb*
Malus hupehensis	+	–	–	–	+	+	–	–	*ff*
Malus × micromalus	+	–	+	+	–	+	–	+	*g,a/c/d/g*
Malus niedzwetzkyana	+	+	–	+	–	+	–	–	*b,a/c*
Malus prunifolia var. *microcarpa*	+	+	–	+	–	+	–	–	*b,a/c*
Malus prunifolia var. *xanthocarpa*	+	+	–	+	–	+	–	+	*bd*
Malus sargentii	+	+	–	+	+	+	–	–	*bf*
Malus sieboldii	+	–	–	–	+	+	–	–	*ff*

In general, the literature provides little information concerning the allelic polymorphisms of genes involved in ethylene perception in different plant species, including apple. Discrimination of *ETR1* alleles in this study was performed on the amplification of the entire gene (comprised the entire coding region – from the first to the sixth exons, with introns – from the second to the sixth) and subsequent digestion with *Rsa*I, *Alu*I and *Hinf*I enzymes. The *ETR1* alleles – *a, b, c, d* and *e*, and six allelic constitutions (*aa*; *ab*; *b,a/c*; *c,a/c*; *d,a/d*; *e,a/b/c*) were reported in 29 autochthonous apple genotypes (Marić *et al*., 2007; 2009, 2021b) and 27 foreign cultivars (Marić *et al*., 2009). Fernández-Fernández *et al*. (2008) also reported that digestion with *Rsa*I was sufficient for discrimination of *ETR1* alleles *a* and *b* in the Y progeny (85 seedlings – segregated in the 45 *aa*, 28 *ab* and 12 *bb*), which was derived from the cross ‘Fiesta’ (*ab*) × ‘Totem’ (*ab*). Additionally, this study confirmed that *ETR1* is inherited in a Mendelian fashion, as well as linkage between this gene and *ACS1* gene (LG15) encoding the rate-limiting step enzyme (ACC synthase) in ethylene biosynthesis. So far, the allele *d* of *ETR1* gene has been found only in autochthonous apple material (Marić *et al*., 2007, 2009, 2021b). However, our study revealed the presence of this allele in *Malus* species. Therefore, this study described the first report on *ETR1*-genotyping of wild apple species. It is also interesting to point out that allele *d* of the *ACO1* gene has been detected only in the *Malus* wild species so far (Marić, 2016; Marić *et al*., 2019). However, the further work needs to be focused on the analysis of segregating progenies in order to elucidate the impact of the polymorphism of genes involved in ethylene biosynthesis and perception on the quality of apple fruit during storage period.

Conclusion

This study presents the additional genotyping survey of *Malus* species for the gene involved in perception of ethylene. Furthermore, this molecular survey represents continuity in the assessment of wild apple species at FRI in order to provide increased number of apple genotypes with a potential to be used as parents in breeding of low-ethylene producing cultivars with good storage ability within modern programmes.

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THE RESPONSE OF DIFFERENT CULTIVARS BIRDSFOOT TREFOIL (*LOTUS CORNICULATUS L.)* ON PRE-SOWING INOCULATION

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Abstract

Birdsfoot trefoil (*Lotus corniculatus* L.) is a widespread plant species, which is important in providing sufficient quantities of quality fodder on soils of low production potential. Like many other forage legumes, this plant species has a well-developed symbiosis with rhizobia. The aim of this study was to evaluate the effect of pre-sowing inoculation on height and green mass of birdsfoot trefoil. The experiment was a two-factorial design, where the growth of plants influenced by three variants of microbial inoculation (individual cultures of *Rhizobium loti*, azotobacter (*Azotobacter chroococcum*) and actinomycetes (*Streptomyces* spp.)) was compared with the growth of noninoculated controls for three cultivars (K-37, Rocco and Bokor). For this study, plants were sown on acid soil with pH 4.91. Measurements were performed three times during two years of research: one cut in the first year and two cuts in the second year. For tested parameters, interaction between cultivar and inoculation resulted in a different effect compared to the control. Highest plant height was achieved in cv. Rocco: in the first year using actinomycetes and in two cuts of the second year using azotobacter. In this study minimum height was recorded in cv. Bokor. By using actinomycetes, the highest value for green mass was achieved in cv. K-37 in the first year and in the first cut of the second year. In this cultivar, using azotobacter the highest green mass in the second cut of the second year was achieved.

Keywords: *Birdsfoot trefoil, Green mass, Height, Microbial inoculation.*

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.) is a widely distributed legume with high nutritive value (Escaray *et al.,* 2012) .and good adaptability to different soil and climatic conditions (Papadopoulos and Kelman, 1999; Churkova, 2011; Giagourta *et al.,* 2015). This plant specieshas a significant role in providing a protein component in animal feed on poor quality soil that is not suitable for growing alfalfa and red clover (Radovic *et al*, 2007). Thanks to high crude protein content and lot of B-carotene and vitamin C its forage quality has excellent (Sareen, 2004). According to Djukić *et al.* (2007) in Serbia birdsfoot trefoil among the perennial legumes takes the third place in importance, after alfalfa and red clover. This legume plant can be grown in pure crop or mixture with perennial grasses (Ćupina *et al.,* 2017), even on very acid soils (Tomić *et al.,* 2007). Soil acidity is one of the most important chemical properties and affects nutrient uptake and community function of rhizosphere bacteria (Liu and Hanlon, 2012; Wan *et al.,* 2020). Like other legumes, birdsfoot trefoil has a well-developed symbiosis with appropriate rhizobia, and the rhizospheric soil is rich in various microorganisms (Jarak and Čolo, 2007). The basic importance of microorganisms is to provide the plant with nutrients, and many of them

produce growth substances. Microbial inoculation aim to promote sustainable agricultural practices which are favorable to the environment and also improve the nutritional quality of plants products (Seman et *al.*, 2021) and they are one of the important components of integrated nutrient management in the near future (Elavarasi *et al.*, 2020). Establishing a symbiotic relationship and performing symbiotic nitrogen fixation rhizobia improve the nitrogen content in plant (Stajković-Srbinović *et al.*, 2021) which is an inexpensive way to enhance soil fertility and agricultural productivity (Meena *et al.*, 2014). Rhizobia is found in the soil, but the seed inoculation of selected microorganisms are achieved better results in legume production (Jarak *et al.*, 2007; Andjelković et *al.*, 2014). The bacteria have properties of biocontrol agents and may be applied to promote the growth of plants (Girija *et al.*, 2020).
Genus *Azotobacter* was highlighted important free-living N_2-fixing bacteria with proven efficacy for plant nutrition and biological soil fertility (Aasfar *et al.*, 2021). Azotobacter is able to influence directly plant growth by synthesizing biologically active substances: auxin, gibberellins, pyridoxine, biotin, nicotinic acid (Hayat *et al.*, 2010), antifungal compounds, antibiotics (Arora *et al.*, 2018). Actinomycetes represent the main component of microbial population in soil and about 90 % of population of actinomycetes are species from the genus *Streptomyces* (Poopal and Laxman, 2009). These microorganisms are known to be very prolific producers of specialized metabolites (Abdelrahman *et al.*, 2022), so have a major importance for agriculture, biotechnology, medicine (Barka *et al.*, 2016). Actinomycetes produce hydrolytic enzymes so that have role in the decomposition of organic matter, degrade the cellulose, pectin, chitin (Priaydharsini and Dhanasekaran, 2015). Also, actinomycetes produce different type of growth factors which act favourably on the physiological processes and inhibit the growth of several fungal and bacterial pathogens (Djebaili *et al.*, 2020), so have benefaction role in plants health (Kumar *et al.*, 2010; Bhatti *et al.*, 2017).
The aim of this study was to examine the influence of individual cultures rhizobium, azotobacter and actinomycetes on yield parameters (height and green mass) three cultivars birdsfoot trefoil (K-37, Bokor and Rocco) on acidic soil.

Materials and methods

The field experiment was carried out in the completely randomized block design in three replications, with the basic plot of 1 m^2 (1x1m) on soil of the eutritic brown type. This soil was acidic reaction (pH_{KCl} – 4.91), it has a medium rich in nitrogen (0.163 %) and humus (2.48 %), poor content accessible phosphorus (6.50 mg/100 g), and is very rich in potassium (37.89 mg /100 g). Geographic coordinates of location field experiment are 43°34'14''N and 21°25'36''E.
Research was conducted on three birdsfoot trefoil cultivars: K-37, Bokor and Rocco. For pre-sowing inoculation of seeds and soil, three variants were used: 1. Mesorhizobium loti strain Z (collection of Institute of Soil Science Belgarde); 2. *Azotobacter chroococcum;* 3. *Streptomyces* sp. *A. chroococcum* cultures were grown on the liquid substrate by Feodorov and *Streptomyces* sp. were grown on the substrate by Krasiljnikov (Jarak and Đurić, 2006). These microorganisms were obtained from the collections of the Department of Microbiology of the Faculty of Agriculture in Novi Sad. Birdsfoot trefoil seed was sterilized with 0.2 % solution of HgC_{l2} and 70 % ethanol, rinsed several times with sterile tap water, treated with an appropriate inoculum and then seeded.
The analysis of plant traits were done in the first cut in first year and two cuts in second year of cultivation at the beginning of flowering. The following traits were measured: plant height (cm)

and green mass (kg). Suppression weeding was done mechanically on several occasions. The crop was grown without irrigation.
The results were processed using the statistical package STATISTICA 8.0. The significance of the difference between the investigated treatments was determined by analysis of variance and LSD tests.

Results and Discussion

In the present study the interaction between cultivar and inoculation resulted in a different effect compared to the control.

Table 1. The effect pre-sowing inoculation on the height and green mass birdsfoot trefoil cultivars

Cultivar	Variant of inoculation	Plant height (cm)			Green mass (kg/m^2)		
		I year	II year		I year	II year	
		I cut	I cut	II cut	I cut	I cut	II cut
K-37	Control	26.33 ^{e}	90.00 ^{b,c}	32.00 ^{c,d}	0.36 ^{g}	4.00 ^{g}	1.15 ^{h}
K-37	Rhizobia	26.44 ^{d,e}	85.00 $^{e, d}$	31.22 ^{c,cd,e}	0.43 ^{d}	4.90 ^{c}	1.37 ^{e}
K-37	Azotobacter	29.22 ^{b,c}	86.33 ^{d}	32.78 ^{b,c}	0.58 ^{b}	5.00 ^{b}	1.93 ^{a}
K-37	Actinomycetes	30.00 ^{a,b}	88.00 $^{c, d}$	31.00 ^{d,e}	0.61 ^{a}	5.25^{a}	1.51 ^{d}
Rocco	Control	28.00 ^{c,d}	92.00 ^{a,b}	33.78 ^{b}	0.35 ^{g}	4.50 ^{e}	**1.03 ^{k}**
Rocco	Rhizobia	26.78 ^{d,e}	87.00 ^{c,d}	32.89 ^{b,c}	0.36 ^{f}	3.95 ^{h}	114 ^{h}
Rocco	Azotobacter	28.78 ^{b,c}	95.00 ^{a}	36.44 ^{a}	0.35 ^{g}	4.10 ^{f}	1.12 ^{i}
Rocco	Actinomycetes	30.88 ^{a}	83.00 ^{e,f}	31.56 ^{c,d}	0.44 ^{c}	4.55 ^{d}	1.21 ^{g}
Bokor	Control	25.67 $^{e, f}$	82.00 ^{e,f}	29.46 ^{e}	0.31 ^{h}	3.83 ^{i}	158 ^{c}
Bokor	Rhizobia	24.16 ^{f}	78.00 ^{g,h}	35.56 ^{a}	0.23 ^{j}	3.38 ^{l}	1.27 ^{f}
Bokor	Azotobacter	24.44 ^{f}	75.00 ^{h}	32.89 ^{b,c}	0.38 ^{e}	3.82 ^{j}	1.65 ^{b}
Bokor	Actinomycetes	25.22 $^{e, f}$	80.00 ^{f,g}	30.33 ^{d,e}	0.29 ^{i}	3.71 ^{k}	1.04 ^{j}

Note: Mean values with the same superscript(s) are not significantly different according to Fisher’s LSD test ($p<0.05$)

The highest plant height was achieved in cv. Rocco: in the first year using actinomycetes (30.88 cm) and in two cuts of the second year using azotobacter - 95.00 cm and 36.44 cm (Table 1). Also, microbial inoculation by azotobacter and actinomycetes positively influenced on plant height in cv. K-37 in the first year of cultivation. The using rhizobia in this cultivar in first year and azotobacter in the second cut in the second year of study the height was increased, but not statistically significant. Applied inocula decreased height plants in cultivar Bokor, only the application azotobacter in the second cut in the second year of research positive statistically significant results was achieved. Andjelković *et al.* (2014) reported the greatest effect on height plants of alfalfa in the treatment with combined cultures of rhizobia, azotobacter and actinomycetes. By using a mixed inoculum of these microorganisms, Jarak *et al.* (2007) reported maximum the emergence and growth of the plants birdsfoot treifoil was during the first 10 days. Data in Table 1 show that the application of actinomycetes, the highest value for green mass was achieved in cv. K-37 in the first year (0.61 kg/m^2) and in the first cut of the second year (5.25 kg/m^2), as well as using azotobacter in the second cut in the second year of study (1.93 kg/m^2). Positive effects of microbial inoculation on biomas were recorded in all the treatments on cv. K-37. In this cultivar, using azotobacter the highest green mass in the second cut of the second year was achieved. Similar to the results in the present study Stevović *et al*. (2017) have noted that two strains of *M. loti* have had a positive impact on yield components of the birdsfoot trefoil cultivars- K-37, Zora and Rocco. For most treatments applied inocula increased green mass in cv. Rocco. The exception were the application azotobacter in the first cut in first year and rhizobia and azotobacter in the first cut of the second year of research showed negative effects compared to control. Andjelkovic *et al*. (2010) reported that using microbial inoculation with these microorganisms increased green mass per plant of alfalfa. During the present study it was found that the response of cultivar Bokor to microbial inoculation was mostly negative. The higher biomass value compared to the control was recorded only using azotobacter in the first year and in the second cut in the second year of study. A microbial inoculum for seed treatment is an important prerequisite for profitable crop production (Doolotkeldieva et al., 2015), but in this way a large number of microorganisms is introduced into the soil and these can change the number and composition of the indigenous microbial population (Song et al., 2004). In what number will the introduced microorganisms survive in the new conditions depends largely on the properties of soil. Thanks to those microorganisms that adapted, microbiological processes are intensified and can increase available nutrients for plants (Miličić and Jarak, 2008). Positive plant-microbe interactions enable environmentally friendly strategies for conventional and organic agriculture to increase soil fertility (Bileva, 2016).

Conclusions

The apllication individual cultures rhizobia, azotobacter and actinomycetes on yield parameters examined cultivars birdsfoot trefoil on high soil acidity had differnt effect. There was no clear trend the response of cultivar to microbial inoculation on the tested parameters. The results suggest that artificial inoculation could be ecologically feasible for improving the production of this plant species.

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FORAGE QUALITY OF DIFFERENT FESTULOLIUM CULTIVARS

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Abstract

Hybrids created by crossing between genera *Festuca* and *Lolium* species are *Festulolium.* Crossing species of these genera aim to combine their positive characteristics, such as *Festuca* tolerance to abiotic stresses and edaphic and climatic conditions with the high quality and digestibility of *Lolium perenne* and *Lolium multiflorum* species. Forage quality of 15 different Lolioid *Festulolium* cultivars and six cultivars of *Festuca* and *Lolium* pure species were investigated in a moderate continental climate in Kruševac, Serbia. Plots in the trial (7,5 m^2) were completely randomized, each in three replications. In the year of establishment, the plots were cut without weighing and taking samples. In the next two years, two cuts were taken. The first cut was done at the beginning of the heading (the first half of May) and the second in the first half of July. On dry samples, from two cuts, the content of crude protein, crude cellulose, crude fat, ash, ADF, NDF and ADL was determined by standard laboratory methods. Results were presented as two-year average values. The best dry matter quality of *Festulolium* cultivars was determined for cultivars AberNiche and Felopa. The highest crude protein content in the first cut was determined for AberNiche (187.1 gkg^{-1}) and Felopa (169.6 gkg^{-1}). Also, the same cultivars noted the lowest values for ADF (296.7 and 303.5 gkg^{-1}) and for AberNiche was determined the lowest ADL contet (35.4 gkg^{-1}) in the first cut. These cultivars are in the group of cultivars with the lowest NDF value. Based on the obtained results, it can be concluded that the analyzed *Festulolium* cultivars have a higher crude protein content compared to the cultivars of *Festuca* pure species, but lower than *Lolium* species.

Keywords: *Festuca, Lolium, Festulolium cultivars, dry matter quality, crude protein content*.

Introduction

Festuloliums are inter-generic hybrids created by crossing between species of the genera *Festuca* and *Lolium*. These hybrids can occur naturally, but most synthetic hybrid cultivars are created by conventional plant breeding technologies. *Lolium* and *Festuca* species have different positive agronomic traits, such as high yield, persistency and tolerance to abiotic stresses and edaphic and climatic adaptations of *Festuca* and quick establishment, high yielding potential, high nutritional value, palatability and digestibility of *Lolium* (Černoch and Groenbaek, 2015). High forage quality as animal feed positively affects the profitability of milk and meat production and is a prerequisite for its improvement and reduction of production costs. For that reason, *Festulolium* hybrids become more interesting as sources of reliable, productive, and nutritionally valuable animal feed, especially because *Festulolium* has a higher tolerance to stresses caused by drought or cold than *Lolium* species. In recent years, climate change is one of the main global problems in agriculture and therefore also in the production of sufficient amounts of animal feed. Prolonged drought periods are becoming more common and particularly spring and summer droughts were increased, and due to the better tolerance of *Festulolium* to stressful environmental conditions compared to *Lolium*, which are carriers of quality, they can be a source of sufficient amounts of quality animal feed. The characteristics of the species of both genera have been transferred to the obtained *Festulolium* cultivars, but hybrids are dominated by the genome of one of them. *Festulolium* cultivars are divided into ryegrass dominated (Loloid) and fescue dominated (Festucoid) types. All *Festulolium* cultivars of the predominant contribution of *Lolium* genome are productive, with high quality and digestible forage, but their areal of cropping is limited according to *Lolium* drought susceptibility. They are more tolerant to abiotic stresses, especially to droughty conditions, in comparison with *Lolium* species (Humphreys et al., 2018). They are also more resistant to winter than *Lolium*, but long-term survival, especially during harsh winters with little snow cover, is questionable (Kemesyte et al., 2017). Also, high temperatures in autumn, delay the beginning of the hardening period, which has a negative impact on the resistance of *Festulolium* to stressful conditions during winter (Ergon et al., 2018). Generally, *Festulolium* cultivars are characterized by high yield potential, biomass quality and multiple purposes for animal feed production on grasslands. They are usually used for intensive hay production, and they are very suitable for silage production (Curran et al., 2020; Wyss and Frick, 2019). *Festulolium* are characterized by rapid establishment and growth, which leads to weed control, as well as a very good capacity for winter survival and regrowth, which allows a high yield (Østrem et al., 2013). There are two different approaches to the breeding strategy of *Festulolium* cultivars: amphiploidisation and introgression. The first way of obtaining hybrids implies that one pair of chromosomes comes from each parent, but often *Lolium* genome dominates in *Festulolium* genetic material (Kopecky et al., 2017). Introgression implies a backcrossing strategy with ryegrass and *Lolium* genome makes a higher proportion of the *Festulolium* genomic material over several backcrossings (Humphreys et al., 2003). Both methods enable obtaining cultivars with adequate yield and dry matter quality, as well as increasing persistency. Improving dry matter yield is a primary aim of perennial grass species breeding programs. Also, in creation of new cultivars of forage grasses, significant attention is directed to the quality and digestibility. This study is aimed to investigate the forage quality of 15 different Lolioid *Festulolium* cultivars and compare them with *Lolium* and *Festuca* control cultivars under the agro-ecological conditions of Serbia.

Material and methods

This research was coordinated by the Eucarpia Fodder Crops and Amenity Section *Festulolium* Working Group and it was a part of the common *Festulolium* trial that was performed under different climatic conditions in 8 sites across the European continent. Dry matter quality of 15 different Loloid *Festulolium* cultivars and 6 standard cultivars of *Festuca* and *Lolium* species were investigated in a moderate continental climate in Kruševac, Serbia, at the experimental field of the Institute for Forage Crops Kruševac in three years. Names of cultivar are given in Table 1. Plots in the trial (7,5m^2) were completely randomized, each in 3 replications. In the second and third years samples (500 g) were taken in all plots in two cuts, and were oven-dried at 60^{o}C for 48h to determine dry matter quality. Crude protein, crude fat and ash content were determined by the Weende system (AOAC 984.13, 1990), content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to AOAC 973.18, 1997. Results were presented as two-year average values. The statistical analyses were performed using the statistical package Statistica 12.

Results and discussion

The forage quality depends on the dry matter chemical composition, which is a very important breeding criterion that is included in all forage grasses breeding programs. Forage quality has the most impact on the palatability and digestibility of the feed and their conversion in animal products. Improvement of crude protein content, ADF, NDF and ADL content, dry matter digestibility, palatability and water-soluble carbohydrates by breeding is possible and promising.
The most important impact to the forage quality has crude protein content, which mainly depends on the phase of cutting and genotype (Østrem et al., 2013). The forage quality of *Festulolium* cultivars and control *Festuca* and *Lolium* pure cultivars are presented in Table 1. and Table 2. In the first cut forage quality of the *Festulolium* cultivars was similar to *Lolium* control cultivars and better than *Festuca* pure cultivars (Figure 1). The obtained results are in accordance with a general attitude that *Lolium* has the best forage quality, then *Festulolim* cultivars and finally *Festuca* species that are characterized by the lowest quality.

Table 1. Forage quality (gkg^{-1}) of dry matter of the first cut

Cultivars	Origin	CP	CC	CF	Ash	ADF	NDF	ADL
Perun	LmFp	116.2^{g-i*}	286.2^{bc}	27.8^{h}	119.2^{a}	352.1^{ab}	577.7^{a-c}	45.3^{d-f}
Achilles	LmFp	149.0^{c}	284.5^{bc}	40.7^{bc}	107.1^{c-f}	366.3^{a}	550.6^{b-d}	61.7^{a}
Perseus	LmFp	104.4^{j}	273.9^{c-f}	34.5^{ef}	107.1^{c-f}	353.3^{ab}	552.4^{b-d}	59.6^{a}
Hostyn	LmFp	121.9^{g}	320.9^{a}	33.5^{e-g}	98.6^{f}	341.0^{a-c}	567.4^{a-d}	51.6^{b}
Becva	LmFa	113.2^{i}	278.1^{b-d}	33.5^{e-g}	104.6^{d-f}	333.0^{a-e}	561.7^{a-d}	45.9^{de}
Lofa	LmFa	128.0^{f}	278.8^{b-d}	33.7^{e-g}	112.2^{a-d}	304.0^{d-g}	547.9^{b-d}	51.0^{bc}
Fabel	LpFp	93.3^{k}	250.1^{fg}	33.7^{e-g}	107.0^{c-f}	315.0^{c-g}	526.9^{d}	42.5^{f-h}
FuRs0142	LpFp	120.1^{gh}	302.6^{ab}	37.2^{c-e}	116.9^{ab}	337.3^{a-d}	556.6^{a-d}	43.2^{e-g}
FuRs0352	LmFa	120.4^{gh}	275.7^{c-e}	32.8^{e-g}	111.3^{a-d}	352.3^{ab}	546.7^{b-d}	45.9^{de}
Agula	LmFp	138.7^{e}	244.8^{g}	40.2^{b-d}	118.9^{a}	304.2^{d-g}	543.9^{cd}	40.0^{hi}
Felopa	LmFp	169.6^{b}	245.3^{g}	46.2^{a}	117.4^{ab}	303.5^{e-g}	562.5^{a-d}	40.3^{g-i}
Sulino	LmFp	150.4^{c}	276.3^{cd}	46.6^{a}	106.0^{c-f}	304.4^{d-g}	548.0^{b-d}	39.3^{i}
Prior	LpFp	114.4^{hi}	273.3^{c-f}	33.2^{e-g}	111.8^{a-d}	327.0^{b-g}	534.2^{cd}	47.2^{d}
AberNiche	LmFp	187.1^{a}	250.6^{e-g}	40.6^{bc}	112.7^{a-d}	296.7^{fg}	544.7^{cd}	35.4^{j}
Lueur	LmFg	116.4^{g-i}	234.7^{g}	35.8^{d-f}	101.1^{ef}	306.0^{d-g}	539.8^{cd}	38.7^{i}
AberMagic	Lp2x	145.7^{cd}	235.4^{g}	42.2^{ab}	109.0^{b-e}	293.9^{g}	537.0^{cd}	39.8^{hi}
AberBite	Lp4x	114.7^{hi}	255.9^{d-g}	32.5^{fg}	114.5^{a-c}	329.4^{b-f}	594.6^{ab}	47.4^{d}
Podium	Lm2x	140.4^{de}	272.0^{c-f}	29.6^{gh}	117.4^{ab}	316.1^{c-g}	601.8^{a}	40.0^{hi}
Caballo	Lm4x	136.4^{e}	245.9^{g}	32.4^{fg}	107.4^{c-f}	332.5^{b-e}	570.9^{a-d}	48.3^{cd}
K-21	Fp2x	113.4^{i}	257.3^{d-g}	35.7^{d-f}	106.6^{c-f}	301.7^{e-g}	552.0^{b-d}	45.4^{d-f}
Kora	Fa6x	106.4^{j}	232.2^{g}	33.5^{e-g}	107.0^{c-f}	298.6^{fg}	539.9^{cd}	43.4^{e-g}

CP – crude protein; CC – crude cellulose; CF – crude fat
*Mean values with the same letters are not significantly different according to Fisher’s LSD test ($p < 0.05$)

The highest crude protein content was found for *Festulolium* cultivars AberNiche (187.1 gkg^{-1}), Felopa (169.6 gkg^{-1}) and Sulino (150.4 gkg^{-1}). *Festulolium* cultivars with the highest crude protein content are amphidiploids, created by crossing between *Lolium multiflorum* and *Festuca pratensis*. The lowest value for ADF and for ADL of all *Festulolium* cultivars were recorded for

AberNiche. Diploid *Lolium perenne* control cultivar (AberMagic) had the lowest values for ADF and NDF, which is expected according to the general fact that pure *Lolium* has the best quality performance in comparison with *Festuca* and *Festulolium* cultivars.

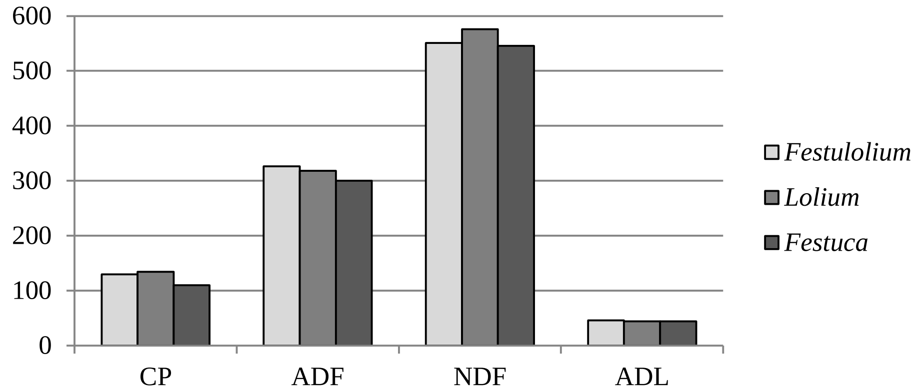

Figure 1. Dry matter quality of *Festulolium* hybrids and *Lolium* and *Festuca* pure cultivars in the first cut

Table 2. Chemical composition (gkg^{-1}) of dry matter of the second cut

Cultivars	Origin	CP	CC	CF	Ash	ADF	NDF	ADL
Perun	LmFp	131.9^{gh*}	322.6^{a}	38.9^{e-h}	161.3^{ab}	351.2^{ab}	533.5^{j}	78.3^{b-d}
Achilles	LmFp	136.5^{e-g}	326.0^{a}	42.7^{c-f}	148.6^{b-f}	373.6^{ab}	575.4^{e-i}	74.6c-g
Perseus	LmFp	140.1^{de}	312.7^{a-d}	40.7^{d-h}	150.8^{a-e}	379.3^{ab}	585.7^{c-g}	73.7^{d-h}
Hostyn	LmFp	138.4^{ef}	265.6^{gh}	40.7^{d-h}	152.5^{a-e}	344.6^{a-c}	562.2^{g-j}	60.9^{i}
Becva	LmFa	135.5^{e-g}	284.5^{e-g}	36.7^{gh}	152.2^{a-e}	353.8^{ab}	565.4^{f-j}	64.3^{ij}
Lofa	LmFa	145.5^{d}	281.7^{e-h}	41.9^{c-g}	157.9^{a-c}	340.4^{a-c}	562.7^{d-j}	70.3^{e-i}
Fabel	LpFp	136.8^{e-g}	324.2^{a}	43.1^{c-f}	136.8^{f-h}	391.4^{a}	611.9^{b-d}	67.5^{g-j}
FuRs0142	LpFp	155.7^{c}	324.1^{a}	45.5^{b-d}	159.7^{a-c}	383.1^{ab}	583.2^{d-h}	93.3^{a}
FuRs0352	LmFa	162.6^{b}	282.5^{e-g}	37.6^{f-h}	148.0^{b-g}	351.0^{ab}	621.3^{a-c}	75.7^{c-f}
Agula	LmFp	153.9^{c}	267.1^{gh}	50.7^{b}	153.6^{a-d}	365.9^{ab}	600.4^{b-f}	69.3^{f-i}
Felopa	LmFp	142.1^{de}	288.3^{d-g}	46.7^{bc}	154.4^{a-d}	384.0^{ab}	594.8^{b-g}	81.9^{bc}
Sulino	LmFp	131.0^{g-i}	274.9^{f-h}	45.8^{b-d}	163.5^{a}	350.2^{ab}	548.6^{h-j}	70.6^{e-i}
Prior	LpFp	184.3^{a}	281.7^{e-h}	50.5^{b}	159.9^{a-c}	353.8^{ab}	542.9^{ij}	73.5^{d-h}
AberNiche	LmFp	125.4^{hi}	316.0^{a-c}	35.6^{h}	151.2^{a-e}	376.5^{ab}	604.6^{b-e}	76.9^{c-e}
Lueur	LmFg	141.8^{de}	299.7^{a-f}	43.6^{c-e}	156.3^{a-c}	270.4^{c}	603.7^{b-e}	84.8^{b}
AberMagic	Lp2x	152.2^{c}	295.0^{b-f}	43.6^{c-e}	131.0^{h}	337.0^{a-c}	570.8^{e-i}	66.6^{h-j}
AberBite	Lp4x	162.5^{b}	254.6^{h}	60.8^{a}	146.6^{c-g}	341.5^{a-c}	650.9^{a}	68.2^{g-j}
Podium	Lm2x	132.6^{fg}	320.8^{ab}	41.1^{c-h}	134.9^{gh}	364.7^{ab}	630.2^{ab}	74.4^{d-g}
Caballo	Lm4x	125.0^{i}	285.8^{d-g}	40.3^{d-h}	141.9^{d-h}	375.4^{ab}	592.1^{c-g}	77.6^{b-e}
K-21	Fp2x	187.1^{a}	302.5^{a-e}	49.4^{c-g}	135.6^{f-h}	381.3^{ab}	654.9^{a}	66.9^{h-j}
Kora	Fa6x	162.5^{b}	290.7^{c-g}	50.5^{b}	139.8^{e-h}	315.9^{b-c}	582.5^{d-h}	45.2^{k}

CP – crude protein; CC – crude cellulose; CF – crude fat
*Mean values with the same letters are not significantly different according to Fisher's LSD test ($p < 0.05$)

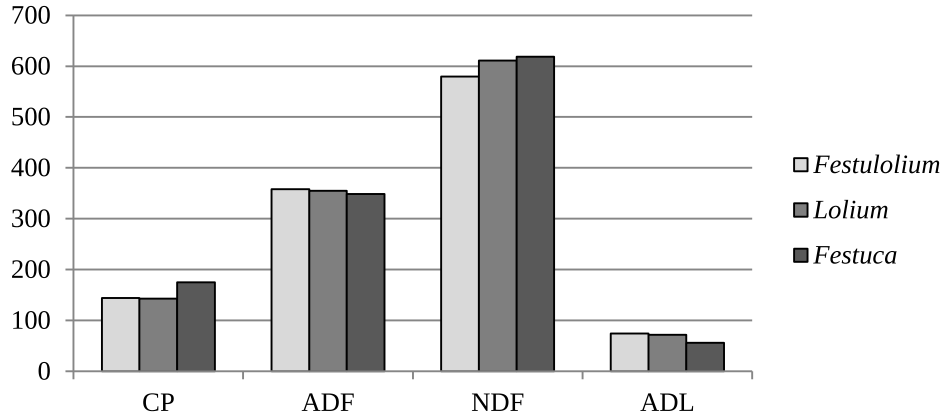

Figure 2. Dry matter quality of Festulolium hybrids and Lolium and Festuca pure cultivars in the second cut

In contrast with the general fact that pure *Festuca* cultivars have a lower quality compared to the *Lolium* and *Festulolium* cultivars, *Festuca arundinacea* pure cultivar Kora has similar values to ADF and NDF with AberMagic in the first cut. In average *Festuca* pure cultivars have lower values for ADF, NDF and ADL than *Lolium* pure and *Festulolium* cultivars. This can be explained by the fact that the species from the *Festuca* genus have a later maturity, which will affect that they later pass from the vegetative to the generative phase. The maturity time at the moment of cutting has the greatest impact on the ADF, NDF and ADL content, and therefore on forage quality. ADF and NDF content in forage grasses, as well as crude protein content, also depends on the environment where forage grasses are grown. Earlier cutting influenced the lower level of ADF and NDF and therefore higher relative feed value of forage.
Forage quality in the second cut is presented in Table 2. and the relationship between *Festulolium* cultivars and *Festuca* and *Lolium* control cultivars is in Figure 2. The *Festuca* pure cultivars had the highest crude protein content in the second cut, while for the *Festulolium* and *Lolium* cultivars, the same average crude protein content was determined. Similar trends for crude protein content were noted by Curran et al. (2020). Also, in a Solati et al. 2018 study *Festuca arundinacea* cultivar Kora has higher crude protein content in the first cut in comparison to the *Festulolium* hybrid. Also, lower ADF and consequently ADL content were determined in *Festuca* control cultivars than in *Festulolium* and *Lolium* cultivars.

Conclusion

Festulolium hybrids were created by crossing between *Lolium* and *Festuca* species in order to combine their positive characteristics, persistency and drought tolerance of *Festuca* species and biomass quality and digestibility of *Lolium* species. The best dry matter quality of *Festulolium* cultivars was determined for cultivars AberNiche and Felopa according to the fact that the highest crude protein content, as a basic indicator of dry matter quality, was noted for them. Also, the lowest value for ADF and consequently for ADL of all *Festulolium* cultivars were recorded for AberNiche. In general, analyzed *Festulolium* cultivars have a higher crude protein content in the first cut than the cultivars of *Festuca* pure species, but lower than *Lolium* species. In the second cut noted opposite results, where the *Festuca* pure cultivars had the highest crude protein content, while the *Festulolium* and *Lolium* cultivars, had the same average crude protein content. Having in mind the fact that in the total dry matter yield in perennial grasses, the first cut makes up more than 70%, *Festulolium* can be a reliable source of sufficient quantities of quality fodder.

Acknowledgment

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GENETIC DIVERSITY OF MAIZE INBREDS WITH DIFFERENT KERNEL TYPE USING SNP MARKERS

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Abstract

Analysis of genetic diversity and classification of different maize material has always been of great importance for planning maize breeding programs. In the last four decades, different molecular marker techniques have been developed and applied for these purposes. Rapid development of DNA sequencing techniques enabled discovery of molecular markers to high-throughput levels. As a result, specific SNP arrays are created which are suitable for genotyping. The aim of this study was genetic diversity analysis of 40 maize inbreeds with different kernel type (sweet and popcorn maize) and different kernel color (yellow and white) using 25k SNP Illumina Infinum Array. Genetic distances were in a range from 0.07 to 0.46 with an average value of 0.40. These results reveal high genetic variability in a chosen set of maize inbreeds. The average value of 0.31 for PIC indicate good informativeness of SNP markers. Observed heterozygosity ranged from to 0 to 0.53. Cluster analysis performed in TASSEL software clearly separated specialty maize types (sweet and popcorn) from the genotypes with standard kernel type. Similar results showed PCA and analysis in STRUCTURE software. Results of this study provide the basis for further more profound analyses about genetic diversity and structure of the chosen material.

Keywords: *maize, SNPs, genetic diversity, inbreds, kerenel type, kernel color.*

Introduction

In the era of climate change studying genetic diversity of different crops is of unprecedented importance. Maize, as one of the most important cereals in the world, has received special attention when it comes to the study of genetic diversity. Information about genetic variation helps breeders in developing new, more tolerant and high – yielding maize genotypes. Genetic diversity is very important for dissect the genetic relationship among lines, the identification of diverse parental combinations to create segregating progenies with maximum genetic variability for further selection, introgression of desirable genes from diverse germplasm into the available genetic base, and to use the local germplasm more efficiently.
Over the last four decades, a remarkable development and use of molecular markers in plants has been witnessed. Nowadays, high-throughput SNP-based molecular marker systems are routinely used in genetic diversity assessing. SNP markers are abundant, highly reproducible, and reliable. To develop these genotyping systems that cover whole genomes array and sequencing based techniques have been developed. Array-based high-throughput SNP markers have been developed for variety of crop species – soybean (Lee *et al*., 2015), barley (Comadran *et al.*, 2012), sunflower (Livaja *et al*., 2016), maize (Lu *et al*., 2009; Romay *et al*., 2013, Xu *et al*.,

2017, Boakyewaa Adu *et al.,* 2019), etc. Several types of array-based platforms have been developed for genotyping maize using Illumina and Affymetrix systems – 1536 SNPs (Yan *et al*., 2010), 50K (Ganal *et al*., 2011), 600K (Unterseer *et al*., 2014).
In this study, genetic diversity of 40 maize genotypes with different kernel types and kernel color was studied using 25k SNP Illumina Infinium array, Trait genetics, SGS with the aim to reveal their genetic structure and relationships. This information would be of great importance for improving future breeding programs.

Material and methods

Forty maize inbred lines were chosen for studying genetic diversity using 25K SNP chip. The set included inbreeds with standard kernel type from Maize Research Institute Gene bank (L1-L8), white (L9-L16) and yellow (L17-L24) colored kernel inbreeds, sweet corn (L25-L32) and popcorn (L33-L40) lines. The chosen material was sent to Trait genetics, Gatersleben, Germany in 2021., a part of SGS (Société Générale de Surveillance) company for service genotyping. To this aim, DNA was extracted from leaf tissue according to modified modified Dorokhov and Klocke (1997) procedure.
The data were loaded into TASSEL 5.2.82 software and SNP markers with minor allele frequency (MAF) less than 5%, missing rate greater than 10% and heterozygosity greater than 5% were filtered out. The final set of 21,512 SNP markers was kept for further analyses.
Power Marker software V3.2.5 (Liu and Muse, 2005) was used for calculating polymorphic information content (PIC), heterozygosity and the gene diversity. Genetic distances were estimated by the complement of identity by state (1 – IBS) with TASSEL 5.0. (Bradbury *et al.*, 2007). Neighbor joining clustering was done in the same software. Principal component analysis (PCA) was done in software R.
To assess the population structure underlying chose maize genotypes, the STRUCTURE software version 2.3.4 was used (Falush *et al*., 2003; Pritchard *et al*., 2000) applying the admixture model with correlated allele frequencies. Explored K values ranged from 1 to 5 with 10,000 burn-in steps followed by 10,000 Markov Chain Monte Carlo simulations. The estimations of parameters were repeated 5 times for each K. The most likely value for K was determined by CLumpak Best K (Kopelman *et al*., 2015).

Results and discussion

Basic diversity statistics for each SNP marker was calculated using Power marker V3.2.5. Major allele frequency was in a range from 0.31 to 0.99 with an average of 0.70. The Polymorphism Information Content (PIC) measures the ability of a marker to detect polymorphisms and in this study averaged 0.31. This value indicates good informativeness of SNP markers used in this study. Osuman *et al.* (2020) reported average PIC of 0.25 which is comparable to the results in this study but higher than 0.17 published by Silva *et al.,* (2019). The lowest PIC value detected was 0.02 and the highest 0.38. Gene diversity (GD) values varied from 0.03 to 0.5 with a mean of 0.39. Observed heterozygosity ranged from to 0 to 0.53 and the average value was 0.03. The low average value of observed heterozygosity close to zero is expected for inbred lines, as is the case in this study. Comparable, but greater average heterozygoisty (0.08) is reported in Semagn *et al*., (2012).

Genetic distances ranged from 0.07 to 0.46 with an average value of 0.40 indicating high genetic variability in a chosen set of maize inbreeds. About 82% of genetic distances among inbred lines fell between 0.40 and 0.46. The average genetic distance calculated in this study is higher than in previously published studies (Van Inghelandt *et al*., 2010; Wu *et al*., 2015). Based on pairwise genetic distance matrix among maize lines dendrogram was constructed (Figure 1). Maize inbreeds were assigned to two main clusters (A and B) and several subclusters. Cluster A was subdivided to two subclusters. The one of them comprised sweet maize and popcorn inbred lines clearly separated in two different subgroups. Cluster B comprised inbreed lines with standard kernel type and yellow kernel color.

Principal component analysis classified genotypes in three main groups. The first group included inbreeds with standard kernel type (Gene bank, yellow and white kernel color maize lines), except L8 which did not belong to any of the defined groups. The group II contained sweet maize genotypes, while popcorn inbreeds were classified in the group III.

The population structure analysis in STRUCTURE software (Figure 3.) revealed an optimal K of 3 (three subpopulations). First and the largest subpopulation consisted of inbreeds with standard kernel type, from Gene bank and maize genotypes with yellow and white kernel color. The second subpopulation consisted solely of sweet maize lines, as well as the third one comprised only popcorn inbreeds. This analysis grouped maize inbred according to their kernel type.

The above presented results suggest high consistency in grouping individuals when PCA and STRUCTURE analysis are applied. Neighbor joining cluster analysis showed somewhat different grouping of individuals. Similar results are presented in Dao *et al.,* (2014).

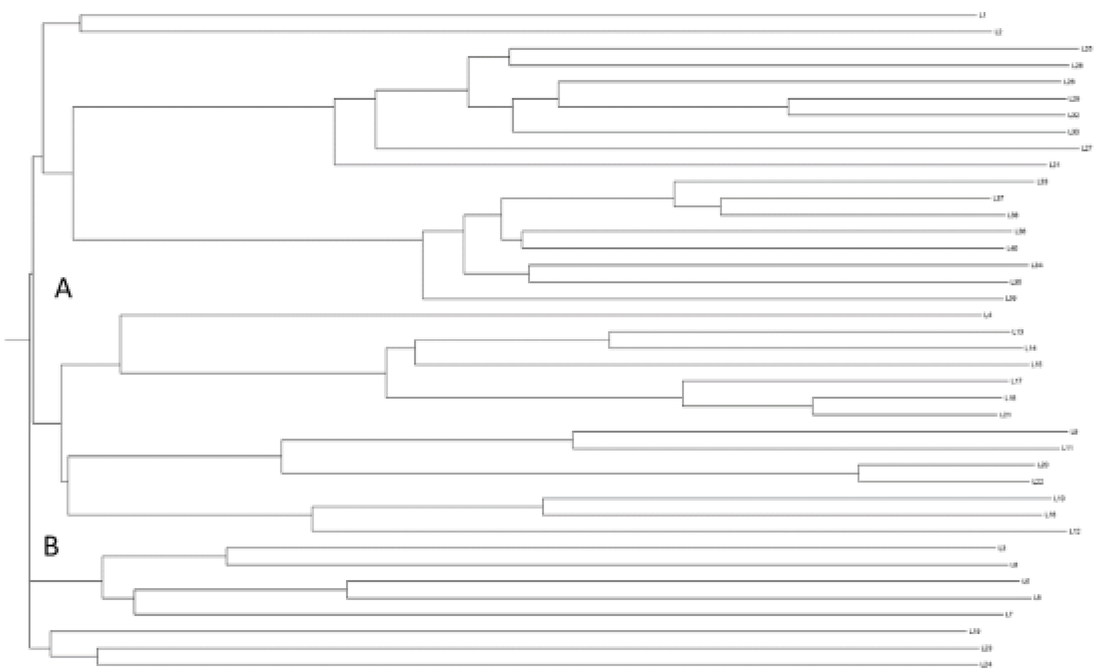

Figure 1. Dendrogram of 40 maize inbred lines based on 25k SNP maize array

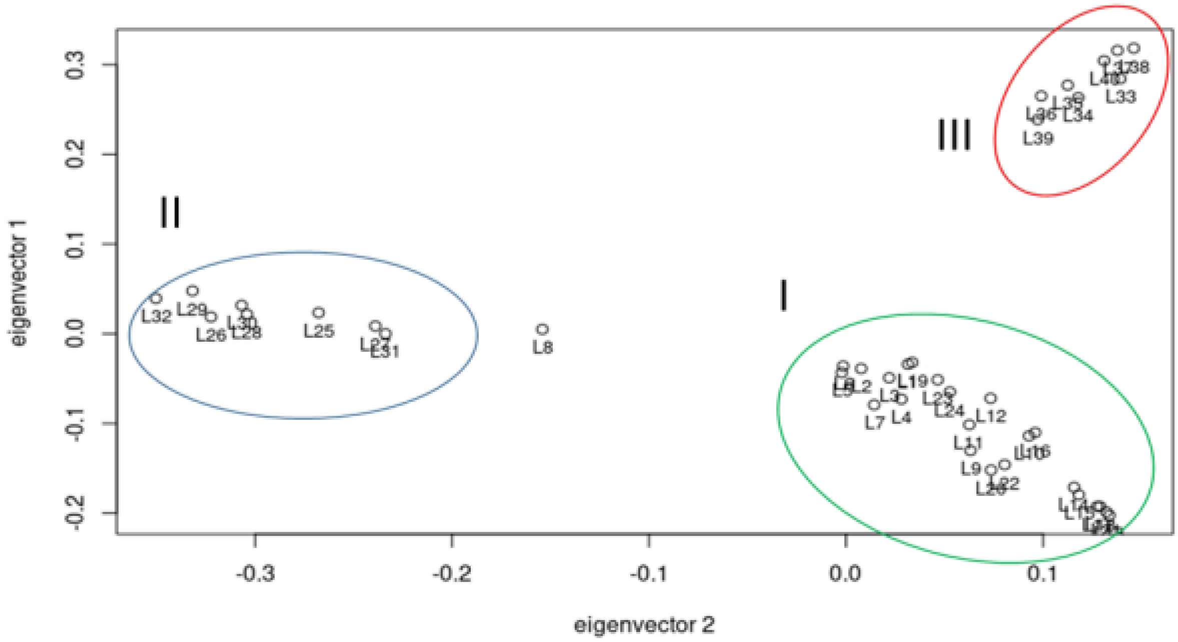

Figure 2. Principal component analysis of 40 maize inbreeds using 25k SNP maize array

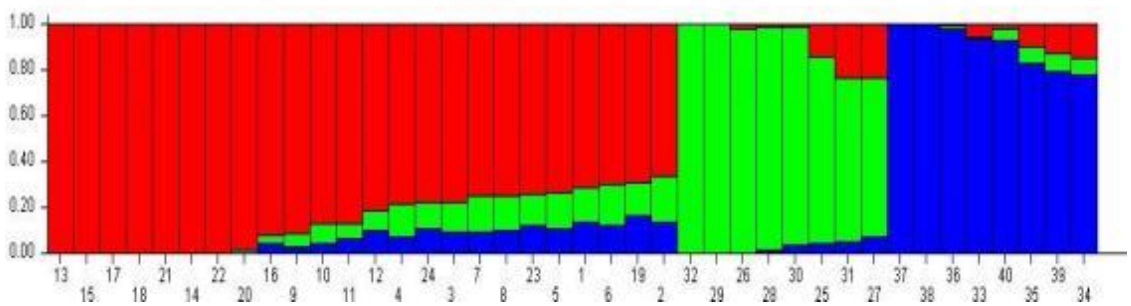

Figure 3. Population structure bar plot for K=3. Three sub-populations: 1 – red (standard kernel type maize lines), 2 – green (sweet maize lines), 3 – blue (popcorn lines) of the 40 maize inbred lines using 25k SNP maize array

The ample genetic diversity in the set of studied maize genotypes is detected according to the analysis of genetic diversity parameters. Specialty maize types (sweet and popcorn) were clearly separated from the genotypes with standard kernel type. More thorough research should be done for finer separating of genotypes with normal kernel type. A 25k SNP maize array proved to be useful in genetic diversity and structure analysis of a chosen maize inbreds set.

Conclusion

In the present study genetic diversity, population structure and relationships between 40 maize inbreeds with different kernel type (standard, sweet and popcorn maize) and different kernel color (yellow and white) were studied. Comparison of the results of three clustering methods

(Neighbor joining clustering, PCA and STRUCTURE) revealed moderate consistency in assigning maize genotypes to corresponding groups. These are just first steps in revealing genetic diversity and structure of greater panel of maize inbreeds. These results provide basis for future more profound research.

Acknowledgement

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USE OF CONTROLLED-RELEASE MINERAL FERTILIZER IN PRODUCTION OF POT GROWN *LEVISTICUM OFFICINALE* L.

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Abstract

Controlled-release mineral fertilizers (CRMF) have been developed and designed in order to improve the efficiency of fertilizers. Better efficiency in use of CRMF compared to conventional mineral fertilizers is mostly reflected in: decrease of chemical immobilization in soils which blocks uptake of nutrients by plants; lowering a fertilizer application frequency and rinsing out of nutrients in soil which consequently reduces a damaging effect on the environment. The aim of this study was to test if an application of CRMF will result in the improvement in development of aboveground biomass of pot grown lovage. The seedlings of lovage were produced in laboratory conditions, in containers placed inside a polyethylene tent (Grow Box), under the artificial lighting. With the development of the first true leaves, seedlings were transplanted into pots and submitted to treatments: CRMF- Osmocot Exact in formulation (NPK 15:9:12 +2MgO+TE), in which is gave 0,45 g N, 0,27 g P_2O_5 and 0,36 g K_2O per L of substrate (pot) and without adding fertilizer (control). After 60 days of growth in the non-heated greenhouse, for 30 plants of each treatment, the absolute dry mass of aboveground biomass (g/plant), number of branches and plant height (cm), were recorded. The highest yield of the aboveground biomass and the highest number of branches were obtained in CRMF (5.17± 0.72g and 6 to 13 branches/plant) compared to control (1.93±0.29g and 5 to 9 branches/plant), respectively. Regarding the plant height, better effect was achieved by CRMF (41.31± 3.43 cm) compared to control (29.37± 2.40 cm). Obtained results have shown positive effects of CRMF on development of aboveground biomass of pot grown lovage.

Keywords: *lovage, medicinal plant, controlled-release fertilizer.*

Introduction

Nutrient losses from mineral fertilizers have persisted at a high level for several years. This adverse phenomenon not only has economic implications, but also environmental consequences. As a result of ongoing attempts to eliminate environmental pollution, the issue of irreversible nutrient loss has lately attracted attention (Noh and Park, 2015). Some of the components are released into the atmosphere, which contributes to climate change. Carbon dioxide, nitrous oxide, and, indirectly, ammonia are gases generated by fertilizer application to soil (Wesołowska et al., 2021). According to sources, greater emission limits might result in decreased application rates or perhaps a complete prohibition on the use of urea-based fertilizers. Slow-release urea, on the other hand, may be an exception to this rule (Beig et al., 2020). Controlled-release mineral fertilizers (CRMF) have been created and designed to increase fertilizer efficiency by enabling delayed nutrient release that is timed to match plant nutritional requirements (Wesołowska et al., 2021). These fertilizers release biogenetic components over some period of time, depending on materials used for coating affected by temperature and soil moisture in decreasing coating thickness (Christianson, 1988; Morgan, Cushman and Sato, 2009). This prevents fertilizer activation at the time of application and/or planting, which is common when using basic mineral fertilizers. In this manner, a high concentration of salts in the substrate is avoided, which in the case of producing seedlings in containers and pots, frequently results in plants degradation (Jelačić, Beatović and Lakić, 2007a). Better efficiency in use of CRMF compared to conventional mineral fertilizers is mostly reflected in: decrease of chemical immobilization in soils which blocks uptake of nutrients by plants; lowering a fertilizer application frequency and rinsing out of nutrients in soil which consequently reduces a damaging effect on the environment (Lubkowski, 2016). *Levisticum officinale L.* is a perennial plant native to south-western Asia and southern Europe. It belongs to the *Apiaceae* family. It has a powerful flavor and has long been exploited in the culinary and food industries. Aside from its culinary interest, *L. officinale* has also been utilized as a medical plant due to its carminative, spasmolytic, diuretic effects, in treating urinary tract infections, and as an antiseptic for treating wounds (Spréa et al., 2020). The aim of this study was to test if an application of CRMF will result in the improvement in development of aboveground biomass of pot grown lovage.

Material and Methods

Production of seedlings. The seeds of lovage (*L. officinale* L.) used in this experiment originated from the MAP collection of the Institute for Medicinal Plants Research " Dr Josif Pančić", in Pančevo, Serbia (44° 52’20.0” N, 20°42’04.7” E). Production of seedlings started in February 2021 in the laboratory of the Institute’s Department for Research and Development in Agriculture, in Belgrade, Serbia (44°49′ N, 20°28′ E). The seedlings have been produced in styrofoam containers with 160 cells, filled with a substrate “Cultivo I SF” (Gramoflor, Romania) of following characteristics provided by the manufacturer: structure 0 - 5 mm, fertilizer NPK 18:10:20+Mg+me in dose of 1 kg/m3, RADIGEN®- Jost GmbH (slow-release micronutrient) in dose of 50 g/m3, hydrogel (wetting agent) in dose of 1 kg/m^3. After the sowing, the containers were kept inside a polyethylene tent (Grow Box), under the following growing conditions: the artificial lighting produced by cool fluorescent tubes with a 12-hour photoperiod; the relative humidity of 40 to 60 %; the air temperature was from 20 °C to 24 °C, while the substrate was kept moist and its temperature was 21±2 °C. Monitoring of the air temperature and relative humidity in the Grow Box was provided by the use of HAXO-8 Data logger and for substrate temperature by Testo 110 thermometer.

Hardening-off process. With the emergence and development of the first true leaves, in the beginning of the second week of March 2021, the 3 weeks long hardening-off process (adaptation) started, as recommended by Davies et al. (2017). Containers were taken outside the Grow box and left inside the laboratory, and were occasionally taken outside in order to ensure adaptation of seedlings to lower air temperatures, reduced relative air humidity and natural light irradiance. The plants were watered with tap water.

Applied treatments and plant growth in greenhouse. By the end of March, the containers were transferred to a greenhouse and seedlings were transplanted into plastic pots (ø 13 cm), filled with 1 L peat substrate (Cultivo I SF), and submitted to treatments: in dose of 3g per L of substrate of the controlled-release mineral fertilizer (CRMF)- Osmocot Exact in formulation

(NPK 15:9:12 +2MgO+TE), in which is gave 0,45 g N, 0,27 g P_2O_5 and 0,36 g K_2O per L of substrate(pot) and without adding fertilizer (control). The pots with plants were placed on a black “agrotextil” film covering the greenhouse ground. The plants grew in the non-heated greenhouse, at 30% shade, under the average daily T 24±2 ℃ and under drip irrigation with a flow rate of 1 liter per hour for each pot. Irrigation was applied every two days for 20 min.

Harvest, morphological parameters and statistical analysis. After 60 days plants were harvested and, in the laboratory conditions, the brunches number counting and plant’s height measuring (cm) were conducted. The absolute dry aboveground biomass (g/plant) was recorded after the harvested plant material have been dried at 105 °C to a constant mass. To compare the achieved yields, the mean values of plant’s height and aboveground biomass were compared by the use of Student’s t-test ($p<0.05$). The obtained results were statistically analyzed by the use of Data Analysis Tool package in Excel 2016 software.

Results and Discussion

The use of CRMF had a significant effect on the development of aboveground biomass in pot grown lovage, compared to the control (Figure 1). Plants in CRMF treatment had 63 % more mass than plants in control treatment, while plants in control treatment had 29 % less height than plants in CRMF treatment (Table 1). In CRMF, plants produced more branches, with the greatest number of plants having 8 branches in treatment and the greatest number of plants having 5 branches in control.

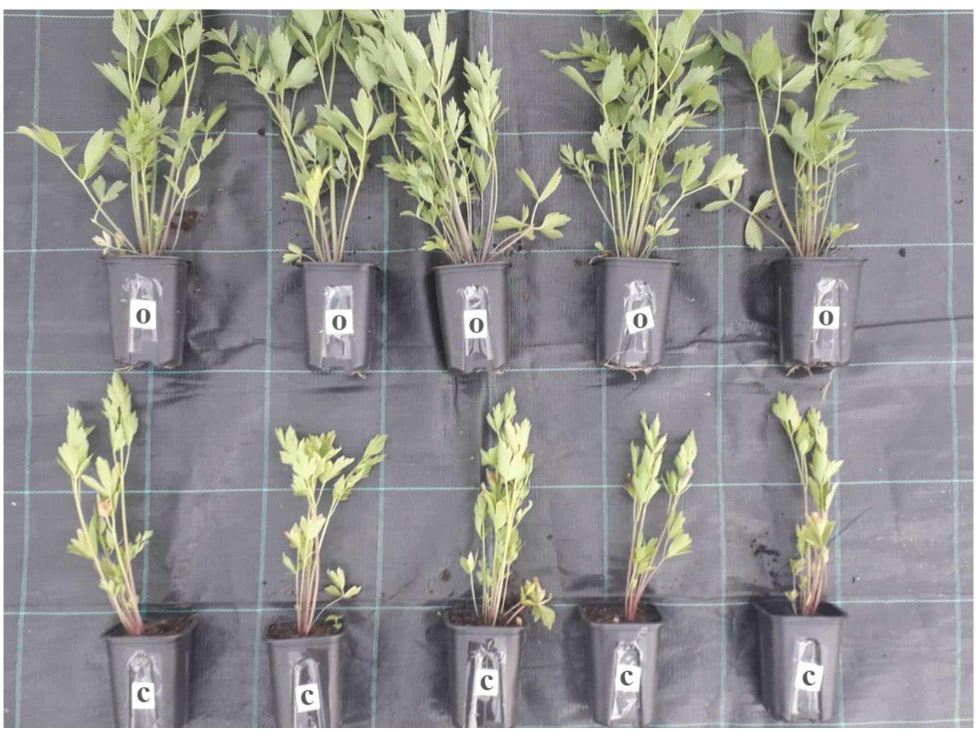

Figure 1. Effect of CRMF on development of aboveground biomass of pot grown lovage, after 60 days of growth in the non-heated greenhouse. Pots marked with o-CRMF; c-control treatment.

Table 1. The average values of plant height, aboveground biomass, and the interval of variation in branch number in potted *L. officinale* plants grown for 60 days.

Plant specie	**Treatment**	**Plant height** [cm]	**Number of branches** I_v (Min-Max)	**Plant mass** [g]
L.officinale	**CRMF**	41.31± 3.43*	6-13	5.17± 0.72*
	Control	29.37± 2.40	5-9	1.93±0.29

Mean values marked with * within the same column are significantly different according to the two-tail t-test ($p < 0.05$).

CRMF have been widely used for over three decades in the container production of seedlings (speedling method) and pots (pot system) in the development of vegetable and horticultural plants (Sharma, 1979). In example, Vujošević et al. (2007a) studied the impact of different dosages (0, 1, 2, 3, and 4 g per L of substrate) of CRMF (Osmocot Exact) in formulation (NPK 15: 9: 9: MgO + Me) on *Tagetes patula* L. and *Salvia splendens* L. seedlings. The obtained results revealed that a CRMF in rate of 4g/L of supstrate had the most impact on the seedling’s height (cm), number of lateral branches and aboveground mass (g), followed by similar impact of used rate of 3g per L of supstrate. Reseach in use of CRMF (NPK 15: 9: 9: MgO + Me) in production of *Gazania rigens* L. (Vujošević, et al., 2007b) also sugested usage of dose of 4g per L of supstrate for producing seedlings with the highest plant aboveground mass (g) and number of buds. It is noticed that in both previosly metioned researchers, higher dosage (3g and 4g per L of substate) of used fertilizer resulted in redused number of flowers since applied fertilizer is reacher in N than in P. P is important element that improves flower formation and seed production (Kumar, Kumar and Patel, 2018). In our study, used CRMF (NPK 15:9:12 +2MgO+TE), in dose of 3g per L of substrate produced satisfactory results in the aboveground biomass development of *L. officinale* L., and this dose was indicated by manifactores as an optimal usege dose. Researchers have also investigated the influence of a CRMF on the quality of medicinal and aromatic pot seedlings. A study has been done by Jelačić et al. (2006) on the utilization of CRMF (Osmocote Exact), in the formulation NPK 16+11+11+3MgO+TE, in production of *Ocimum basilicum* L. and *Melissa officinalis* L. seedlings. Based on the results, it can be concluded that doses of 3 and 4g per L of a substrate are the most effective in terms of vegetative development of basil and lemon balm seedlings, which is in accordance with our results and also is recommended by manufacturers in the use of CRMF (Osmocote Exact), in formulations of NPK 16+11+11+3MgO+TE, NPK 15:9:12+2MgO+TE and NPK 15:9:9+MgO+TE. The applied 3 g per L dose of CRMF fertilizer in formulation of NPK 15:9:9 +MgO+TE as the optimal dose was also verified in studies conducted with other medicinal and aromatic species like *Salvia officinalis* L. (Jelačić et al., 2007a), *Rosmarinus officinalis* L. (Jelačić et al., 2007b) and *Hyssopus officinalis* L. (Beatović et al., 2007).

Conclusion

The results of the study demonstrate that CRMF has a considerable favourable influence on the development of lovage seedlings. The use of these fertilisers results in high-quality seedlings. The use of 3g per L of substrate of CRMF in the production of lovage provides the greatest results in terms of above-ground mass gain.

Acknowledgments

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OPTIMIZATION OF THE PROTOCOL FOR *IN VITRO* PROPAGATION OF AUTOCHTHONOUS PLUM GENOTYPE ‘METLAŠ’

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Abstract

Fast and cost-effective clonal propagation of planting material is possible to achieve by application of tissue culture *in vitro*. In this way, problems associated with traditional propagation, such as rapid spreading of diseases, lack of initial material caused by dependence on seasonal growth or low propagation coefficient can be overcome. This paper deals with optimization of micropropagation of autochthonous plum ‘Metlaš’ (*Prunus domestica* L.) originated from Guberevci (Municipality Knić, Serbia). ‘Metlaš’ is very often considered to be the same genotype as ‘Okruglica’ (syn. ‘Dragačica’), but they can be clearly distinguished according to plant habit, stone and fruit characteristics. To optimize multiplication stage, the influence of benzyladenine (BA at 0.5, 1.0, 1.5 and 2.0 mg l^{-1}) and thidiazuron (TDZ at 0.25, 0.5, 1.0 and 1.5 mg l^{-1}) on the multiplication capacity (multiplication index, length of axial and lateral shoots) was examined. Rooting ability of shoots (rooting rate, number and length of roots, and height of rooted plants) was monitored on half strength Murashige and Skoog (MS) medium containing 1.0 mg l^{-1} indole-3-butyric acid (IBA) or 1-naphthaleneacetic acid (NAA), each combined with 0.1 mg l^{-1} gibberellic acid (GA_3). TDZ applied at 0.25 mg l^{-1} gave the highest multiplication index (9.1), while the longest axial (14.1 mm) and axillary shoots (8.6 mm) were obtained on medium with 1.5 mg l^{-1} BA. Although both auxins proved to be efficient in rhizogenesis (rooting rates being 85.7% and 95.2%), higher values of all rooting parameters were observed in the presence of IBA. Rooted shoots were successfully acclimatized.

Keywords: *Prunus domestica* L., *In vitro, Multiplication, Rooting, Acclimatization*.

Introduction

Plum is a very important fruit species native throughout the Northern Hemisphere but mostly in the temperate zone. According to the production, plum is ranked as the fourth most important cultivated fruit tree crop in temperate climate (after apple, pear and peach), accounted for 7% of total word production of temperate fruits (FAOSTAT, 2019). Today, the most globally cultivated plum species are the hexaploid ($2n = 6x = 48$) European plums (*Prunus domestica* L.) and the diploid ($2n = 2x = 16$) Japanese plums (*Prunus salicina* Lindl.) (Sottile, 2022). Contrary to Japanese plums which are widely grown for fresh consumption, European plums are commercially grown worldwide for a number of uses including fresh consumption, cooking, baking, drying, canning, distilling (brandy production), as well as other types of processing. However, cultivated European plums show very limited intra-specific genetic variability (Zhebentyayeva et al., 2019), due to modernization of agriculture, loss of local ecotypes, inbreeding and/or use of a limited number of founders in breeding processes (Sottile et al, 2022).
While other breeding programs face narrowing of genetic diversity of plums, Serbia as well as other regions of former Yugoslavia is rich in old plum cultivars, primitive forms and

autochthonous biotypes (landraces) (Milošević et al., 2010; Vukojević et al., 2012) suitable for selection and breeding of both cultivars and *Prunus* rootstocks (Paunović and Paunović, 1994). Still, this rich plum germplasm is directly treated due to continuous introduction of improved newly-bread cultivars as well as to the climate change and increased rate of infection with Sharka virus (Plum pox virus – PPV) which affect many local cultivars and biotypes of plums (Botu et al., 2012). Considering the importance of these valuable plum genetic resources there is an urgent need to develop new concepts of its sustainable conservation, management and utilization. Beside that, there has been an increased interest in establishing new commercial orchards of these autochthonous cultivars as they display higher adaptability to local agroecological conditions and are suitable for low-input farming. Beside being used for production of high quality brandies (Popović et al., 2015), fruits of these genotypes have high nutritional value and antioxidant activity (Tomić et al., 2019) and could be suitable for fresh use as well (Milošević and Milošević, 2012).
In vitro approaches are useful tools for rapid clonal propagation of true-to-type, disease-free and uniform plants (Vujović et al., 2020) as well as for medium- to long-term conservation of vegetatively propagated plants such as fruit tree species (Engelmann, 2004). Micropropagation has found the widest practical application of all *in vitro* techniques in fruit growing, so it has become a standard method of propagation for many fruit species, especially for vegetative rootstocks, berry fruit species and other fruit tree species that are grown on their own roots such as autochthonous plum species. Successful *in vitro* clonal propagation is determined by many factors such as genotype, mineral composition of nutrient medium (Ružić et al., 2003), type and concentration of carbon sources (Ružić et al., 2008; Yaseen et al., 2012), light conditions and plant growth regulators (PGRs) (Ružić and Vujović, 2008).
The aim of this paper was to develop efficient protocol for micropropagation of virus-free autochthonous plum ‘Metlaš’ (*Prunus domestica* L.) through: i) optimization of multiplication stage by using two cytokinins – N^6-benzyladenine (BA) and thidiazuron (TDZ) applied at different concentrations, ii) evaluation of rooting ability of *in vitro* shoots on medium containing different auxins – indole-3-butyric acid (IBA) or 1-aphthaleneacetic acid (NAA), and iii) monitoring of acclimatization ability of both *in vitro* rooted and unrooted shoots under the ‘mist’ system in greenhouse.

Material and Methods

Field grown virus-free clone of autochthonous plum cultivar ‘Metlaš’ (*Prunus domestica* L.) originated from Guberevci (Municipality Knić, Serbia) was used as a source of initial material for establishment of aseptic culture. Aseptic culture of this genotype was established according to the procedure previously described by Vujović et al. (2021). Upon establishment of aseptic culture, uniform single shoots were multiplied on Murashige and Skoog (1962) medium (MS) of constant PGR composition: 1 mg l^{-1} BA, 0.1 mg l^{-1} NAA and 0.1 mg l^{-1} gibberellic acids (GA_3).
To optimize multiplication, the influence of type and concentration of cytikinins (BA and TDZ) on the multiplication capacity and shoot quality was examined in the fifth subculture. Cytokinins were applied at following concentrations: i) BA at 0.5, 1.0, 1.5 and 2.0 mg l^{-1}; ii) TDZ at 0.25, 0.5, 1.0 and 1.5 mg l^{-1}. All media contained 30 g l^{-1} sucrose and 7 g l^{-1} agar. The pH value was adjusted to 5.7 before autoclaving at 121°C, 150 kPa for 20 min. Shoots were subcultured twice at a 28 day-interval on the medium of the same PGR composition, and therefore multiplication parameters were determined in the second subculture. The following parameters were monitored:

multiplication index (the number of newly formed axillary shoots >5 mm per initial shoot tip), length of axial shoots and length of lateral shoots. Some specific issues, such as leaf color, leaf and callus size, leaf roll, incidence of chlorosis, or necrosis along with occurrence of fasciation and hyperhydricity were also monitored.

Rooting was performed on the MS medium with mineral salts reduced to ½-strength and organic complex unchanged. Rooting treatments included two PGR combinations: i) 1 mg l^{-1} IBA and 0.1 mg l^{-1} GA_3, and ii) 1 mg l^{-1} NAA and 0.1 mg l^{-1} GA_3. The percentage of rooted plants was determined after 28 days along with the number and length of roots, and height of rooted plants.

Each treatment in multiplication and rooting stages included 42 uniform shoots (three replicates of two culture vessels with seven shoots each). Shoot cultures were grown in 100 ml culture vessels containing 50 ml of multiplication or rooting medium, at 23 ± 1°C and 16-h photoperiod (light intensity, 41 µmol m^{-2} s^{-1}).

Both rooted and unrooted shoots were removed from culture vessels, washed carefully with water to remove adhering medium, transferred to plastic pots containing sterile soil substrate (Klassmann Steckmed – mixture of white sod peat, white peat and perlite) and acclimatized on a ‘mist’ bench in a greenhouse for two weeks (Mist system type ‘Electronic leaf’, MC Company, Belgrade).

All data were analyzed by ANOVA, followed by Duncan’s Multiple Range Test ($P < 0.05$) for means separation. Data presented in the form of percentage were subjected to arcsine transformation.

Results and Discussion

Determination of the most optimal types and concentrations of PGRs is one of the most important aspects in plant tissue culture especially in proliferation stage. It is well known that cytokinins play multiple roles in the plant development such as promotion of cell division and cell expansion, plant protein synthesis stimulation and the activities of some enzymes (Arab et al., 2014). A wide range of cytokinin types and concentrations are effective for *in vitro* culture even though the requirements among species are different. Some investigators have reported that benzyladenine (BA) and thidiazuron (TDZ) are the two cytokinins most commonly used in stone fruit micropropagation (Ružić and Vujović, 2008). BA is frequently applied in *Prunus* rootstock micropropagation (Vujović et al., 2018), while TDZ has also been reported to be appropriate for *in vitro* proliferation of some *Prunus* spp. (Arab et al., 2014).

Monitoring of multiplication capacity of autochthonous plum ‘Metlaš’ revealed that TDZ, although applied at lower concentrations, is more efficient for micropropagation of this genotype comparing to BA (Table 1). Namely, multiplication index of shoots grown on media with TDZ ranged between 5.9 and 9.0 which was significantly higher than multiplication indexes obtained on media with BA (2.5–5.8). The highest multiplication index was achieved with lowest TDZ concentration (0.25 mg l^{-1}) (Fig. 1a). The increase in TDZ concentration has led to a gradual and significant decline in multiplication capacity of shoots. In contrast, increase in BA concentration to 1.5 mg l^{-1} and above caused significant increase in the multiplication index (Fig. 1b). Although all cytokinins stimulate cell division, axillary bud formation and shoot multiplication, it is well known that the effect of different cytokinins is highly genotype dependent (Dobránszki and Teixeira da Silva, 2010). Similarly to our results, some investigators reported that TDZ at low concentration is more effective than purine adenine derivatives in *Prunus* micropropagation (Espinosa et al., 2006; Canli and Tian, 2008). However, Tang et al. (2002) and Ružić and Vujović (2008) reported that BAP is more efficient than TDZ in *P. avium* and *P. cerasus,* although high concentrations of cytokinins of adenine type are often necessary for growth and differentiation in *Prunus* spp. (Arab et al., 2014) which has been confirmed in our study.

According to Huetteman and Preece (1993), TDZ may inhibit shoot elongation and cause shortening of internodes. In our research axial and lateral shoots grown on media containing TDZ were significantly shorter compared to those cultivated on media supplemented with BA at concentration of 1.5 mg l^{-1} and above. Fasciation or hyperhydricity, phenomena that are often associated with TDZ application in tissue culture (Kadota and Niimi, 2003) were not noticed even on shoots grown on medium with the highest TDZ concentration.

The rooting stage in micropropagation is very important as it directly affects greenhouse survival and acclimatization success of *in vitro* plants. Rooting difficulties occur in micropropagation of both fruit-bearing and ornamental species belonging to *Prunus* genus (Wiszniewska et al., 2016). Despite the attempts directed towards higher effectiveness of the rhizogenesis, European plums usually exhibit poor rooting ability of *in vitro* induced shoots, which could be the major drawback in commercial micropropagation. According to Tian et al. (2007), use of 1-naphthaleneacetic acid (NAA) at higher concentrations instead of indole-3-butyric acid (IBA) can increase rooting efficiency of *P. domestica L*. Vujović et al. (2020) also reported that shoots of autochthonous plum ‘Crvena Ranka’ cultured on the medium supplemented with NAA displayed higher rooting ability (60%) in comparison with those grown on the medium containing IBA at the same concentration (20%). Contrary to those results, ‘Metlaš’ exhibited a much better rooting performance (Tab. 2; Fig. 1c-d), rooting rate being between 85.7% (medium with NAA) and 95.2% (medium with IBA). Also, plantlets rooted on medium with IBA had significantly longer roots compared to those rooted on medium with NAA.

Following *in vitro* rooting shoots were successfully acclimatized (90,0%), while percentage of acclimatization of unrooted shoots was markedly lower (15,0%).

Table 1. Multiplication parameters of autochthonous plum genotype ‘Metlaš’ on MS medium (Murashige and Skoog, 1962) of different plant growth regulator (PGR) composition.

PGR composition of medium (mg l^{-1})	Multiplication index	Length of axial shoot (mm)	Length of lateral shoots (mm)
[a]BA 0.5	2,7 f	12,3 b	6,2 bc
BA 1.0	2,5 f	11,7 b	5,7 c
BA 1.5	3,2 e	14,1 a	8,6 a
BA 2.0	5,8 d	14,0 a	6,4 b
[b]TDZ 0.25	9,0 a	12,1 b	6,7 b
TDZ 0.5	7,9 b	12,7 b	6,5 b
TDZ 1.0	6,2 c	11,9 b	6,8 b
TDZ 1.5	5,9 d	11,9 b	6,5 b

Mean values for each parameter followed by the same letter are not significantly different according to Duncan’s Multiple Range Test ($P < 0.05$); [a]BA – N^6-benzyladenine; [b]TDZ – thidiazuron.

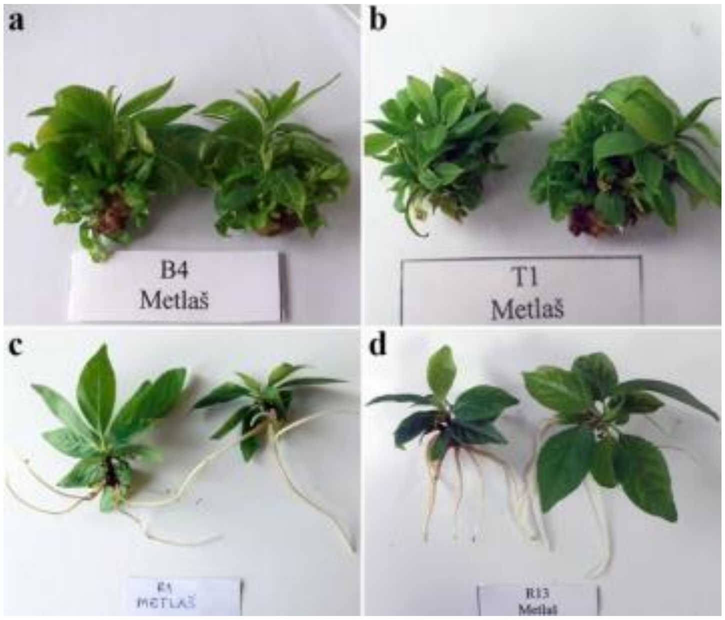

Figure 1. Shoots of autochthonous plum 'Metlaš' in the multiplication stage on MS medium with 2.0 mg l^{-1} BA (a) and 0.25 mg l^{-1} TDZ (b), and in the rooting stage on half-strength MS medium containing 0.1 mg l^{-1} GA_3 in combination with 1.0 mg l^{-1} IBA (c) or 1.0 mg l^{-1} NAA (d).

Table 2. Rooting parameters of autochthonous plum genotype 'Metlaš' on half-strength MS medium (Murashige and Skoog, 1962) of different plant growth regulator (PGR) composition.

PGR composition of medium (mg l^{-1})	Rooting rate (%)	Number of roots	Root length (mm)	Rooted shoots length (mm)
[a]IBA 1.0 + [b]GA_3 0.1	95.2 a	3.5	5.2 a	12.9
[c]NAA 1.0 + GA_3 0.1	85.7 b	3.1	3.0 b	12.5

Mean values for each parameter followed by the same letter are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$); [a]IBA – indole-3-butyric acid; [b]GA_3 – gibberellic acid; [c]NAA – 1-aphthaleneacetic acid.

Conclusions

In this paper, we presented an optimized protocol for successful *in vitro* propagation of indigenous plum genotype 'Metlaš'. Analysis of the effect of cytokinin type and concentration on multiplication phase indicates that TDZ, although applied at lower concentrations, is more efficient in micropropagation of this genotype than BA. As regards of rhizogenesis, 'Metlaš' displayed high rooting ability with both IBA and NAA. Nevertheless, considering rooting percentage and root length, IBA could be recommended as more effective. The results obtained can find practical application in commercial laboratories for clonal propagation of planting material of genotype 'Metlaš' for establishment of new commercial orchards.

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MULTIVARIATE ANALYSIS OF AGRONOMIC TRAITS IN MID-SEASON SOYBEAN VARIETIES

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Abstract

Principal Component Analysis (PCA) is a useful tool for processing multiple data, which are often encountered in breeding practice. This method is suitable for the evaluation of genotypes on the basis of multiple traits and graphical presentation of relationships between traits. This study included 16 soybean mid-season genotypes (maturity group I), originated from different regions of the world, maintained in soybean collection of Maize Research Institute Zemun Polje. Field trials were carried out at two locations, during two years, according to a RCB design with three replications. The genotypes were evaluated in respect to eight major agronomic traits: *PH* – plant height, *NN* – node number, *PN* – pod number, *SN* – seed number, *TSW* – 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content. First two PCA axes encompassed a large portion of the variance of standardized data (75,9%). Biplot distinguished genotypes of potential importance for various breeding targets. Two genotypes stood out with the largest 1000 seed weight. One variety formed a larger number of pods and the seed number per plant as compared to the group average, achieving the highest grain yield per plant. Two genotypes were among the most productive ones, with a larger number of pods as well as a higher 1000 seed weight, compared to the average. The most promising variety was Laura, which had a high yield and higher protein content than the average, and could be used as a potential germplasm source for the simultaneous improvement of both traits. Correlations among traits determined by PC biplot were in accordance with Pearson's correlation coefficients.

Keywords: *soybean, quantitative traits, multivariate analysis, correlations*

Introduction

The areas under soybean in Serbia are continuously increasing in the last decade. The total soybean harvested area in Serbia in 2020 was 236758 ha, with an average yield 3,175 t/ha (FAOSTAT, 2022). Although the seed yield is one of the most important traits in soybean breeding programs, released cultivars have to adjust to the requirements of processing industry regarding chemical composition and technological quality of the grain (Peric *et al.*, 2018).The success of breeding programs depends on the availability of a genetic pool with adequate diversity (Gwinner *et al.*, 2017), and possibility to identify potential sources for the enhancement of agronomic traits through different breeding methods. Promising genotypes should simultaneously unite a set of positive traits, which could elevate yield to fulfil the market demands (Cruz, 2013). Multivariate exploratory techniques, such as PCA (Principal Component Analysis) can be used for selecting superior genotypes, simultaneously analyzing important agronomic traits and the relationships among them, as well as identifying the most influencing traits in a selection process (Leite *et al.,* 2018). PCA is a widely used method for evaluation of

genotypes regarding multiple traits in soybean and other crops (Yan and Rajcan, 2002; Mohamadi and Amri, 2011; Mirosavljevic *et al*., 2015; Peric *et al*., 2018).
The identification of genotypes that are at the same time superior for negatively correlated traits presents a special challenge. The aim of this study was: to evaluate genotypes on the basis of multiple traits and identify potential breeding sources among them; examine the correlations between agronomically important traits and suggest a reliable selection criterion by applying the method of PCA.

Material and methods

Experimental material for this study included sixteen soybean accessions belonging to maturity group I (mid-season genotypes), maintained in soybean collection of Maize Research Institute "Zemun Polje". Part of the examined genotypes was developed in Serbia (domestic ones), while the other part originated from different geographical regions of the world (introductions). The field trials were set up during two growing seasons (2011 and 2012), at two locations in Serbia (Zemun Polje and Pančevo), according to a randomized complete block design with 3 replications. The experimental plot size was 5 m^2 with two rows per plot The standard agricultural practice was applied. The soil type at the Zemun Polje location was slightly calcareous chernozem, i.e. carbonate chernozem on the loess terrace at the Pančevo locality. During the both research years, total rainfalls were significantly lower and average temperatures much higher than multiyear average, so 2011 was characterized as moderately dry, while 2012 was marked as extremely dry year. The samples consisted of 30 plants per genotype were collected at the R8 stage, by random selection of 30 plants per genotype, and scored for eight agronomically important traits: *PH* –plant height, *NN* – node number, *PN* – pod number, *SN* – seed number, *TSW* – 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content. Seed samples were analyzed for protein and oil content (% on a dry matter basis) with grain analyzer Infraneo, Chopin Technologies®, based on the near-infrared transmission spectroscopy (*NIRT*). Principal Component Analysis (PCA) was performed as the data reduction technique, in order to identify minimum number of traits which contributes to maximum variation, and to rank genotypes on the basis of PC scores. Pearson's correlation coefficients between agronomically important traits were calculated and compared to correlations displayed by PC biplot.

Results and Discussion

Two axes of PCA biplot (PCA1 and PCA2) of soybean genotypes maturity group I explained 75.9% of the variance of the standardized data (Figure 1). Dispersion of genotypes along the first axis (PC1) was mainly based on *PROT*, *OIL*, *PH*, *NN*, *PN* and *SN*, while second axis (PC2) separated genotypes by *TSW* and to the lesser extent by *SYP*.
Genotypes intermediate for most of the analysed traits (Hodgson 78 and Danubian) had short vectors and were positioned near the coordinate origin. Biplot highlighted two cultivars with the largest *TSW*- Balkan and Ardin. The Balkan genotype formed a larger *PN* and *SN* per plant, as compared to the group mean, achieving the highest *SYP*. The high yield of this genotype was expected, considering that it was a domestic variety used as a standard in trials for new varieties registration in maturity group I, until 2013. Genotypes Brock and Parker ranked among the most productive genotypes, forming higher *PN* as well as *TSW* compared to the average, while for the

SN per plant these two varieties were mostly intermediate. Cultivar Laura also stood out in terms of high yield and *TSW* as compared to the group average, while it was intermediate for the *PN* and *SN* per plant. High-yielding genotypes were mostly intermediate for protein content and synthesized high oil content, except for the Laura variety, which had a higher yield and higher than average protein content, representing a possible source of germplasm for simultaneous improvement of both traits. Genotypes Ika, Shine, A 1937, Ravnica and NK 15 50 formed a very high *SN* and *PN* per plant, but by *TSW* these genotypes were below the group average, while for *SYP* mostly intermediate or slightly above the group average, as well as for the *OIL*.
Varieties Chornaya, Daniela and Krizia had *PROT* significantly above the group average, but the lowest grain yield and the lowest *OIL* made these genotypes less important for breeding for simultaneous improvement of several traits. Nevertheless, these 3 genotypes can be used in breeding for improved seed protein content.

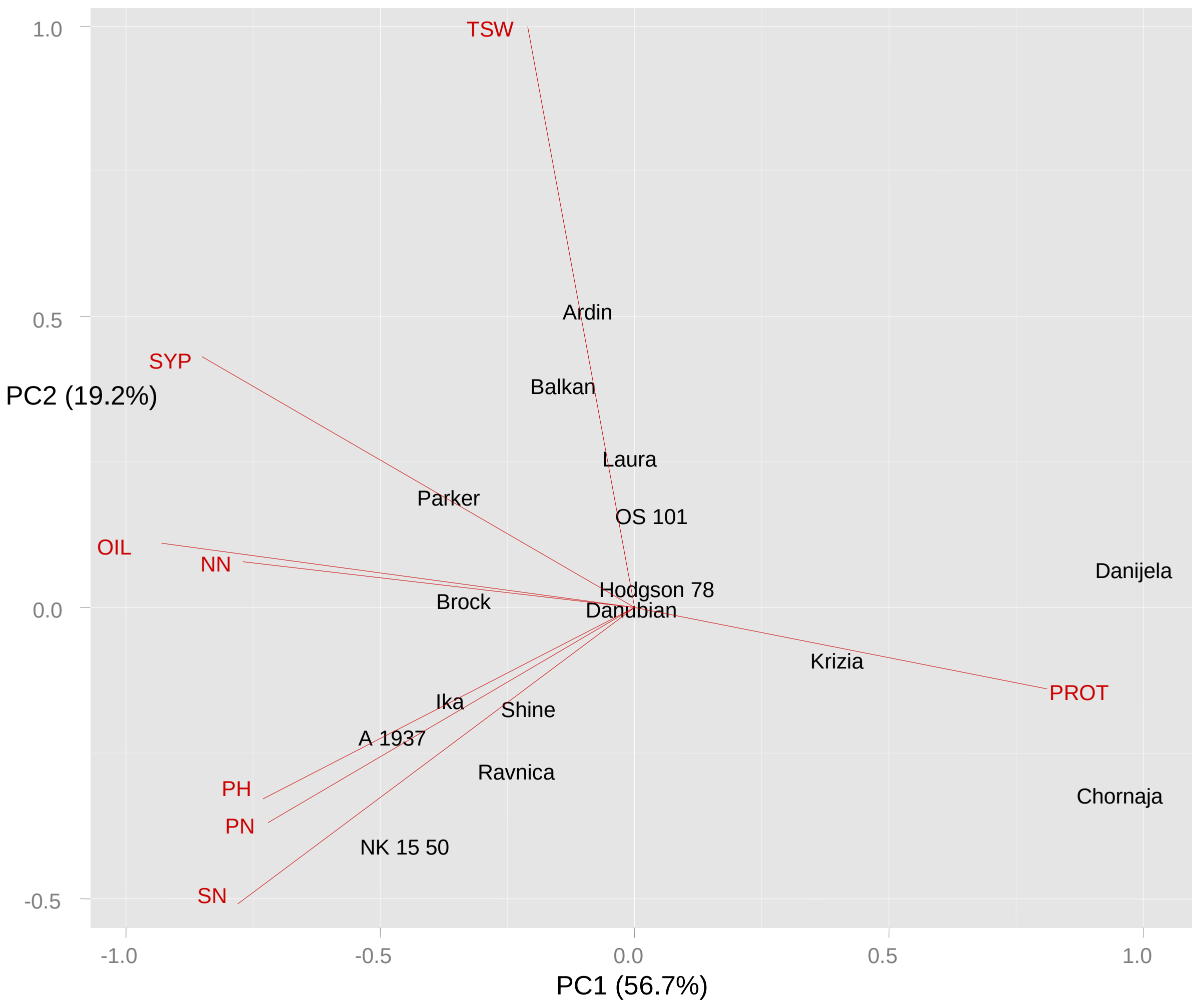

Figure 1. PCA biplot of 16 soybean genotypes maturity group I evaluated for important agronomic traits

The angles between trait vectors on PCA biplot figured out that the *SYP* showed positive correlation with *OIL*, *NN*, *PH*, and *PN* and *SN* per plant while negatively associated with *TSW* and *PROT*. The correlations between the traits shown by the biplot were in accordance with *Pearson's* correlation coefficients (Table 1), regarding vector directions (correlation sign) and the angles between vectors (correlation strength), as well.
The highest correlation coefficient was determined between *OIL* and *PROT*, indicating negative and highly significant association (-0.91***). *GYP* showed negative high significant correlation with *PROT* (-0.69**) and positive highly significant correlation with *OIL* (0.75**). Negative correlation of yield and protein, on one hand, and positive between yield and oil, on the other, as well as negative association of protein and oil has been confirmed in many studies (Taški-Ajdukovic *et al.*, 2010; Li and Burton, 2002, Popovic *et al.*, 2013), indicating a significant problem in development of high yielding soybean varieties with a satisfying level of protein and oil in grain. Similar findings were reported by Peric *et al.* (2018), *GYP* was in a highly positive and highly significant correlation with *TSW* (0.63**), and a medium positive significant correlation with the *SN* (0.56*) and *PN* per plant (0.55*). By comparing the correlation coefficients between yield and three main yield components (*SN*, *PN* and *TSW*), it could be concluded that *TSW* was the component with the greatest influence on yield. At the same time, the very weak negative and insignificant association of *TSW* with *PN* (-0.34) and *SN* (-0.28), suggests that selection of larger seed genotypes within maturity group I could possibly improve yield without significantly reducing the number of seeds and number of pods per plant.

Table 1. *Pearson's* correlation coefficients between the traits

PH	0.72**	-0.23	-0.06	0.20	0.04	-0.06	-0.05
NN		-0.06	0.13	0.29	0.25	0.16	-0.11
PN			0.81**	-0.32	0.51**	-0.21	0.17
SN				-0.15	0.78**	-0.43*	0.07
TSW					0.49**	0.08	-0.29
SYP						-0.37*	-0.09
PROT							-0.57**
	NN	PN	SN	TSW	SYP	PROT	OIL

PH –plant height, *NN* – node number *PN* – pod number, *SN* – seed number, *TSW* – 1000 seed weight, *SYP* – seed yield per plant, *PROT* – protein content, *OIL* – oil content
*P < 0.05; ** P <0.01

Conclusion

Application of PCA for simultaneous analysis of important agronomic traits in mid-season soybean genotypes revealed potential valuable sources for breeding for different goals. Five higly-productive genotypes were identified, superior by both yield and yield components. The most promising variety was Laura, which had a high yield and higher protein content than the average, and has been used in other breeding programs, presenting a potential germplasm source for the simultaneous improvement of both traits. Correlations among agronomic traits based on PC biplot were in agreement with *Pearson's* correlation coefficients, suggesting that *TSW* could be efficient selection criterion in breeding for soybean seed yield.

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EFFECTS OF *BACTERIA AND ENZYME* MIXTURE INOCULANTS ON QUALITY OF HIGH-MOISTURE MAIZE GRAIN SILAGE

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Abstract

The objective of this study was to evaluate the effect of applying lactic acid bacteria (LAB) and enzymes mixture inoculants (Sil-All and Silaprilis) on the chemical composition and fermentation of high-moisture grain silage of two maize hybrids Zenit and ZP 735. Maize hybrids were harvested at 68-72% of dry matter. Commercial inoculants were prepared and sprayed following the manufacturer's specifications. Silages were stored in glass jars with a special valve filled with water in the middle of the lid. Significant differences between hybrids were found for ash, crude protein, pH, and acetic acid. The hybrid Zenit had significantly higher ash (14.9 g kg^{-1} dry matter (DM)), pH (4.03), and acetic acid (6.3 g kg^{-1} DM), and significantly lower crude protein (89.0 g kg^{-1} DM) than hybrid ZP 735 (12.5 g kg^{-1} DM, 3.98, 5.1 g kg^{-1} DM and 101.2 g kg^{-1} DM, respectively). Compared to control, LAB+enzymes mixture inoculants stimulated ensiling of high-moisture maize grain. Inoculants decreased the contents of ammonia nitrogen and acetic acid, and pH value, and increased the contents of dry matter, ash, crude protein, crude fat, and lactic acid during silage fermentation than control. Accordingly, the application of LAB+enzymes mixture inoculants is justified and they can be recommended for high-quality silage production in feeding livestock.

Keywords: *grain maize silage, lactic acid bacteria+enzymes mixture inoculants, chemical composition, fermentation parameters.*

Introduction

Maize grain is an important component of livestock feed ingredients due to its availability and nutritional quality. In Serbia, the production of maize is high, with a yield of 7.9 million tons in 2020 *(Statistical Yearbook of the Republic of Serbia, 2021)*. The practice is to store dry grains of maize. However, high-moisture maize grain also had a high nutritional value and energy content, is highly digestible for livestock, and doesn't require drying. The maize grain contains high starch content (60-72%) with a ratio of amylose to amylopectin from 20:80 to 30:70. The higher the content of amylopectin makes it favorable for livestock feeding because it is easy to digest (Copeland *et al*., 2009). It is suitable for silage making and can be used as a supplement for feeding dairy cows and fattening cattle and as a basic raw material (with a proportion of 50 to 70%) for feeding pigs and poultry. Ensiling high-moisture maize grain increases ruminal and total-tract starch digestibility compared with dry maize (Ferraretto *et al*., 2013) and may increase lactation performance by dairy cows due to the increased surface area for bacterial enzymatic degradation and the breakdown of the hydrophobic starch-protein matrix (Castro *et al*., 2019).

However, the ensiling of wet grain carries a greater risk of fungal contamination and spoilage compared to other forages because moisture content at harvest may vary leading to a loss of nutrients (Hoffman and Ocker, 1997). For this reason, good ensiling practices are mandatory which includes the use of silage additives (bacterial inoculants, enzymes, and their combinations). The bacterial inoculants reduce pH thereby favoring the growth of aerobic microorganisms and preventing the growth of undesirable aerobic organisms (Zielińska and Fabiszevska, 2018). Also, they reduce dry matter and nutrient loss and improve the aerobic stability in fermented silage (Queiroz *et al*., 2013). In the world market for improving the quality of maize silage fermentation, silage bacterial inoculants containing homo-fermentative *Lactobacillus plantarum*, *L. acidophilus*, *Pediococcus pentosaceus*, *P. acidilactici*, and *Enterococcus Enterococcus faecium* and/or hetero-fermentative bacteria like *Lactobacillus buchneri* have been developed. It has been proven that the homofermentative inoculants enhance fermentation and improve starch digestibility in feed high-moisture maize (Saylor *et al*., 2020). Lactobacillus plantarum dominates in silage inoculants (Oliveira *et al*., 2017). These bacteria in silages is being highly competitive with epiphytic lactic acid bacteria decrease pH and increase lactic acid production preventing dry matter and nutritional losses (Guan *et al*., 2020), but usually reduces the aerobic stability of silage (Silva *et al*., 2018). Consequently, dual-purpose inoculants were developed containing homofermentative and heterofermentative lactic acid bacteria. However, LAB from inoculants does not have a consistent effect on fiber degradation into fermentable sugars due to which enzymes are added to help bacteria produce lactic acid and render the silage more digestible during feed out (McDonald *et al*., 1991; Sheperd and Kung, 1996). Enzyme addition to silage inoculants aims to break down forage fiber during fermentation into soluble sugars, and so help bacteria produce lactic acid, which helps to lower silage pH (Ordaz, 2017).
The study aimed to analyse the effects of lactic acid bacteria+enzymes mixture inoculants on the chemical composition and fermentation parameters of high-moisture maize grain silage.

Materials and Methods

The study was carried out at the Institute for Animal Husbandry, Zemun, Serbia in 2019. Maize hybrids Zenit and ZP 735 were harvested at 68-72% of dry matter which is the appropriate point for grain silage. Approximately 100 kg of grains were processed by a hammer mill to a 3-4 mm particle size. Three replicated piles containing approximately 10 kg of samples were prepared for each treatment (total of 24 piles). Samples were sprayed uniformly with a solution of two lactic acid bacteria+enzyme mixture inoculants according to the manufacturer’s specifications. Inoculant Sil-All (Alltech, UK) contains *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Pediococcus pentosaceus* and *Propionibacteria acidipropionici* bacteria together with amylase, cellulase, β-glucanase and xylanase enzymes. Inoculant Silaprilis (Timac Agro, USA) contains *Lactobacillus plantarum*, *Pediococcus acidilactici* and *Propionibacteria acidipropionici* bacteria together with ß-glucanase and xylanase. Also, control silage (non-inoculated) was included. Silages were stored in glass jars (1.5 l). The jars had a special valve filled with water in the middle of the lid. The valve was served to create an anaerobic fermentation environment. The silos were stored at 22–25°C in dark conditions for a period of 60 days. *After 60 days* of ensiling, *silage samples were taken for analysis.* The contents of dry matter (DM), ash, crude fat, crude protein, acid (ADF) and neutral detergent fibres (NDF) were determined according to AOAC (2000). The content of cellulose was determined by the Weende method. The content of soluble

nitrogen/total nitrogen was measured by the method of Licitra *et al*. (1996). The content of NH_3-N/total nitrogen was determined by the distillation method using a Kjeltec 1026 analyser. The GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) was used for the determination of the lactic, acetic, and butyric acids content. The pH data were obtained with a digital HANNA HI 83141 Portable PH Meter.
Analysis of variance (ANOVA) was used to compare datasets, using Statistical software Statistica version 10 (StatSoft, Tulsa, Oklahoma, USA). The randomized complete block analysed the trial with three replicates. Tukey's test ($P \leq 0.05$) was used to compare means.

Results and Discussion

The chemical composition of the maize grain sample before ensiling is shown in Table 1.

Table 1. Chemical composition of wet grain maize sample before ensiling

Parameter	Zenit	ZP 735
Dry matter (DM) (g kg^{-1})	680.5	680.9
Ash (g kg^{-1} DM)	15.11	13.5
Crude fat (g kg^{-1} DM)	54.8	57.3
Crude protein (g kg^{-1} DM)	100.0	105.1
Cellulose (g kg^{-1} DM)	41.9	41.5
Acid detergent fiber (ADF) (g kg^{-1} DM)	39.0	39.5
Neutral detergent fiber (NDF) (g kg^{-1} DM)	240.0	246.2

As shown in Tables 2 and 3, the maize hybrids differ considerably in the contents of ash, crude protein and acetic acid, and pH value. Hybrid Zenit was found to have significantly higher contents of ash (14.9 g kg^{-1} DM) and acetic acid (6.3 g kg^{-1} DM), pH value (4.03), and significantly lower content of crude protein (89.0 g kg^{-1} DM) compared to hybrid ZP 735 (12.5 g kg^{-1} DM, 5.1 g kg^{-1} DM, 3.98, and 101.2 g kg^{-1} DM, respectively). So, the chemical composition and fermentation characteristics of wet grain silage high may be affected by the hybrid difference.
The contents of dry matter (582.3 g kg^{-1} DM), ash (14.9 g kg^{-1} DM), crude protein (102.6 g kg^{-1} DM), crude fat (41.0 g kg^{-1} DM), and lactic acid (72.7 g kg^{-1} DM) were significantly higher in silage treated with Silaprilis compared to control (561.6 g kg^{-1} DM, 13.0 g kg^{-1} DM, 81.4 g kg^{-1} DM, 38.2 g kg^{-1} DM, and 67.5 g kg^{-1} DM). Contrary, the contents of ammonia nitrogen (49.0 g kg^{-1} DM), acetic acid (5 g kg^{-1} DM), and pH value (3.97) were significantly lower in silage treated with inoculant Silaprilis compared to control (80.4 g kg^{-1} DM, 6.1 g kg^{-1} DM, and 4.04, respectively). Similar conclusions have also been reported by Rafiuddin *et al*. (2021), their study found that the application of Sil-All ® 4X4 (*L. plantarum*, *Enterococcus faecium*, *Pediococcus acidilacti*, and *Lactobacillus salivarius* + α-amylase, cellulase, hemicellulose and pentosanase) could reduce dry matter loss, decrease pH and increase protein content and lactic acid in silage maize.
The silages treated with inoculants, especially with Silaprilis decreased dry matter losses. This indicated good fermentation and preservation of nutrients. Higher ash content indicates that bacteria from inoculants use soluble components and so increase ash content, similarly to Đorđević *et al*. (2020). When ensiling, it is very important to maintain a high level of protein in silages. As evident from the results, the silages treated with inoculants had a higher protein

content compared to the control. This can be related to the fact that the addition of inoculants in silages increased lactic acid fermentation and sugar utilization and reduced pH and proteolytic activity, as evidenced by the research by Abdul Rahman *et al*. (2017). Also, Xing *et al*. (2009) reported that the sharp decline in pH of inoculated silage with inoculant and enzymes was the major reason for reducing the protein degradation which contributes to higher dry matter content.

Table 2. Chemical composition of maize by wet grain silage (untreated silage and silage treated with inoculants).

Factor		DM g kg^{-1}	Ash g kg^{-1} DM	Crude protein g kg^{-1} DM	Crude fat g kg^{-1} DM	Cellulose g kg^{-1} DM	NDF g kg^{-1} DM	ADF g kg^{-1} DM
Hybrid (A)	Zenit	575.5	14.9^{a}	89.0^{b}	39.2	34.1	190.8	33.9
	ZP 735	564.2	12.5^{b}	101.2^{a}	40.7	34.2	198.6	33.3
Inoculation (B)	Control	561.6^{b}	13.0^{b}	81.4^{b}	38.2^{b}	34.6	177.1	31.6
	Sil-All	565.7^{ab}	13.2^{ab}	101.3^{a}	40.6^{ab}	33.9	203.1	34.3
	Silaprilis	582.3^{a}	14.9^{a}	102.6^{a}	41.0^{a}	34.0	203.9	34.9
F test	A	ns	**	*	ns	ns	ns	ns
	B	*	**	*	*	ns	ns	ns
	A×B	ns	**	ns	*	*	ns	ns

DM – dry matter; NDF – Neutral detergent fiber; ADF – Acid detergent fiber; Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$); ns – non significant;
* – significant at 5 % level of probability; ** – significant at 1% level of probability.

Table 3. Fermentation characteristics of maize by wet grain silage (untreated silage and silage treated with inoculants).

Factor		NH_3-N g kg^{-1} TN	pH	Lactic acid g kg^{-1}	Acetic acid g kg^{-1}	Butyric acid g kg^{-1}
Hybrid (A)	Zenit	69.7	4.03^{a}	69.6	6.3^{a}	0.10
	ZP 735	54.8	3.98^{b}	71.2	5.1^{b}	0.17
Inoculation (B)	Control	80.4^{a}	4.04^{a}	67.5^{b}	6.1^{a}	0.17
	Sil-All	57.4^{ab}	4.00^{b}	70.9^{ab}	5.0^{b}	0.11
	Silaprilis	49.0^{b}	3.97^{c}	72.7^{a}	5.0^{b}	0.12
F test	A	ns	**	ns	**	ns
	B	*	**	*	*	ns
	A×B	ns	*	ns	*	ns

DM – dry matter; TN – total nitrogen; Distinct letters in the row indicate significant differences according to Tukey's test ($P \leq 0.05$); ns – non significant; * – significant at 5 % level of probability; ** – significant at 1% level of probability.

Crude fat is an important source of energy for animals. Our data showed greater crude fat content in silages treated with inoculants than in control. However, a significant difference was detected between the Silaprilis treatment and control. There were no significant differences among the inoculant treatments in terms of cellulose, NDF, ADF, and butyric acid. Similarly, Ranjit and Kung (2000) found that the silage inoculants have no effect on the ADF and NDF content of maize silage. The content of NH_3-N/total nitrogen ranged from 49.0 g kg^{-1} (Silaprilis treatment)

to 80.4 g kg^{-1} DM (control) and was less than 100 g kg^{-1} which suggests that harmful bacteria were effectively inhibited. The pH of the silage varied from 3.97 to 4.04, indicating that the silages were adequately fermented and achieved good fermentation. Acceptable silages generally contain > 65% lactic acid, <4% acetic acid, and <0.5% butyric acid of dry matter (Shaver, 2003). Based on the results obtained for acids, all silages met good fermentation quality. The greater lactic acid production in the high moisture silage of maize treated with Silaprilis is most likely related to the greater concentration of enzymes in him. A lower concentration of butyric acid showed that the silages has not undergone clostridial fermentation. It is understandable because the spores of *Clostridium* spp do not grow if the pH of silage is 4 or less (Cai *et al*., 1998).
We observed an interaction between hybrid, and inoculant for ash, crude fat, cellulose, acetic acid, and pH value. The interaction between hybrid and inoculant resulted in a significant increase in ash, crude fat, and cellulose and in a significant decrease in acetic acid, and pH value.

Conclusions

The choice of maize hybrids can affect the quality of high-moisture grain silage. Our results showed that the hybrid ZP 735 had a higher content of crude protein and lower contents of ash and acetic acid, and pH compared to hybrid Zenit. Compared to control, bacteria+enzyme mixture inoculants stimulated ensiling. Inoculants decreased the ammonia nitrogen and acetic acid contents, and pH value, and increased dry matter, ash, crude protein, crude fat, and lactic acid contents, especially Silaprilis. Accordingly, the application is justified and they can be recommended for high-quality silage production.

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ACCURACY DETECTION OF INTEL® REALSENSE D455 DEPTH CAMERA FOR AGRICULTURAL APPLICATIONS

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Abstract

Depth cameras are very important for automation applications in agriculture, especially in robotics and drone applications. But they are still developing, new sensor systems and additions are being tried by the manufacturers and end-users. Because of this reason, this article focuses on the Intel® RealSense D455 Depth Camera. To summarize, the calculation of depth from the stereo is explained in the article. Stereo depth cameras are made up of two fixed cameras that point in the same direction but are separated by a predetermined distance known as the baseline. Additionally, the measurement and management software is important for getting good results with firmware updates on the depth cameras. Intel® uses its own depth camera management software kit which contains a group of software called RealSenseTM 2.0 Software Development Kit. It also supports different programming languages with some examples in it. The Intel® RealSenseTM D400 series depth cameras are mainly used on drones, robots, homes, and surveillance, as well as virtual reality, and finally on PC peripherals. Because of this reason, an appropriate experiment was carried out to assess the feasibility of using one of these devices, for robots and drones, for agricultural applications. The primary conclusion of this paper is that Intel® RealSense Depth camera D455 produces a significantly higher-quality depth image than the cameras in D400 series depth cameras, with more discernible objects and obstacles.

Keywords: *Intel® realsense, Depth camera, Depth measurement, Device performance, Agricultural applications.*

Introduction

Depth measurement in cameras started with Microsoft Kinect. When Microsoft introduced the Kinect, portable, consumer-grade RGB-D devices increased in popularity. It was the first to provide hardware-accelerated depth calculation via a USB connection, paving the way for widespread usage of RGB-D sensors in computer vision, human-computer interaction, and robotics. RGB-D sensors with subpixel disparity precision, aided lighting, and outdoor capabilities were unveiled by Intel® in the year 2015 (Keselman et al., 2017). Then they go on to develop by Intel®. Now, to determine depth, as the last depth camera series of Intel®, Intel® RealSenseTM D400 cameras use stereo vision. The stereo vision solution includes a left imager, a right imager, and an optional infrared projector. In low-textured environments, the infrared projector generates a static infrared pattern that is not visible to the naked eye. For each pixel, the depth imaging (vision) processor correlates points on the left and right images and calculates a shift between the left and right images to arrive at the depth value for each pixel in the image. Processing the depth pixel data yields a depth frame. A depth video stream is formed by the addition of subsequent depth frames (Anonymous, 2022).

Drones, robots, home and surveillance, virtual reality, and PC peripherals are just a few of the applications for the Intel® RealSenseTM D400 series depth cameras. Tadic et al. 2019 studied the application of Intel® real-sense cameras to create depth pictures in robots, for instance. They wanted to see whether depth cameras might help with object recognition in photos by providing more information about the image's depth. Using depth cameras in the context of painting robots will be briefly discussed in the article. When it comes to robotics, the RealSense depth camera is an essential tool since it allows robots to identify and remove obstacles from walls. Their experiments revealed that the camera D415 offers substantially more accurate depth information than the camera D435, which was previously believed. In another study in the literature, unmanned aerial vehicle (UAV) photos were used by Li et al. (2021) to quickly recognize and locate longan fruits. Longan fruits on tall longan trees that produce in the mountain orchard landscape can't be picked easly by ground fruit-picking robots. A deep learning-based technique for quickly and correctly identifying and finding fruit harvesting sites on branches was suggested in their study by the researchers. Once the Intel® Realsense D455 camera has been installed on an UAV by them, then longan images collected from the front side of the UAVfor training and testing purposes. For Intel®'s real sense depth cameras, Park et al. (2020) researched the rectification of depth photographs. They told about the infrared projector and an infrared camera are used to create depth pictures by the Intel® RealSense depth camera. The depth may be precisely measured using infrared radiation. A postprocessing method is included in the Intel® RealSense SDK for improved performance. For the Intel® RealSense D400 series depth cameras, Grunnet-Jepsen et al. (2019) also worked on enhancing the subpixel linearity. They stress how critical it is to find an answer to the correspondence conundrum. They also stress that depending on how far away from its correct counterpart each pixel in the right image is from the appropriate one, it is necessary to adjust that pixel's position in the left image accordingly.

This article focused on the Intel® RealSense Depth Camera D455. In a nutshell, the stereo depth computation is described. In a stereo depth camera, the baseline denotes the distance between two fixed cameras facing in the same direction. Frame by frame, the pictures collect by the left and right cameras that are compare to identify whether elements of the photos are identical. The axis formed by the two imagers, they shift more for close objects than for distance ones.

Materials and methods

Intel® Realsense D455 Depth Camera

RealSense D400 series is a depth camera series with stereo vision from Intel® RealSenseTM (Figure 1). A stereo depth module and a vision processor are included in the subsystem assembly and are linked to the host processor by USB 2.0/USB 3.1 Gen 1 or MIPI[1]. System integrators may use the camera subsystem in a broad range of products because of its compact size and simplicity of integration. A stereo depth module, an RGB sensor with color image signal processing, and an Inertial Measurement Unit are all included in the Intel® RealSenseTM D400 series of integrated depth cameras (IMU). The depth cameras are perfect for makers, educators, hardware prototypes, and software development because of their mobility and simplicity of setup. A cross-platform and open-source SDK, the Intel® RealSenseTM SDK 2.0, is supported by the Intel® RealSenseTM D400 series (Anonymous, 2022).

Figure 1. Intel® RealSense™ Depth Camera D455 (Anonymous, 2022)

Depth camera SKU properties, Intel® RealSense™ Depth Camera D455 mechanical dimensions, depth image formats (USB 3.1 Gen1) – D455, and depth field of view of D455 can be seen in Table 1 – 4, respectively.

Table 1. Specifications for the SKU of the D455 depth camera (Anonymous, 2022)

D400 series Depth Cameras	Intel® RealSense™ Depth Camera D455
Depth module	Intel® RealSense™ Depth module D450
Baseline	95mm
Left/Right Imagers Type	Wide
Depth FOV HD (degrees)	H:87±3 / V:58±1 / D:95±3
Depth FOV VGA (degrees)	H:75±3 / V:62±1 / D:89±3
IR Projector	Wide
IR Projector FOV	H:90 / V:63 / D:99
Color Sensor	OV9782
Color Camera FOV	H:87±3 /V:58±1 /D:95±3
IMU	6DoF

Table 2. The dimensions of the Intel® RealSense D455Depth Camera (Anonymous, 2022)

Dimension	Min	Nominal	Max	Unit
Width	123.5	124	124.5	mm
Height	28.5	29	29.5	mm
Depth	25.5	26	26.5	mm
Weight				gr
Flatness Tolerance	-	0.2	-	mm

Table 3. The D455 depth image format is part of USB 3.1 Gen1 (Anonymous, 2022)

Format	Resolution	Frame Rate (FPS)
Z [16 bits]	1280x720	5,15,30
	848X480	5,15,30,60,90
	640x480	5,15,30,60,90
	640x360	5,15,30,60,90
	480x270	5,15,30,60,90
	424x240	5,15,30,60,90

Table 4. D455’s Depth of Field (Anonymous, 2022)

Format	D455
Horizontal FOV (VGA 4:3)	74
Vertical FOV (VGA 4:3)	62
Diagonal FOV (4:3)	88
Horizontal FOV (HD 16:9)	86
Vertical FOV (HD 16:9)	57
Diagonal FOV (HD 16:9)	94

Camera System Block Diagram

The Vision processor D4 and the Depth module are the two most important parts of the camera system. Both USB2.0/USB 3.1 Gen1 and MIPI[1] can be used to link the Vision processor D4 to the host processor, and the host processor may be incorporated into the motherboard. The Depth module contains left and right imagers for stereo vision with the optional IR projector and RGB color sensor. Color Image Signal Processor (ISP) on the Host Processor or D4 Board transmits data from the RGB color sensors to the vision processor D4 (Anonymous, 2022).

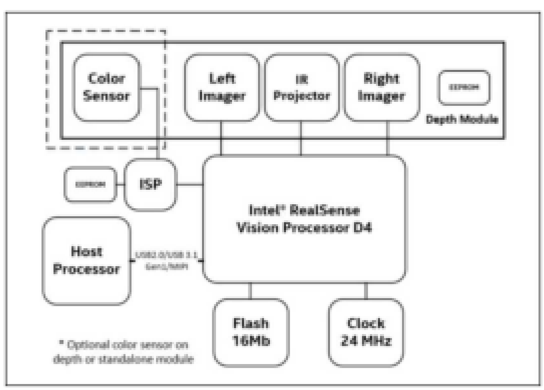

Figure 2. Block diagram of the D4 Vision Processor Camera System (Anonymous, 2022)

Infrared Projector

To improve depth perception in low-contrast settings, the infrared projector projects a steady infrared pattern onto the scene. When used properly, the infrared projector conforms with laser safety standards for class 1 devices. The power supply and laser safety circuitry are included in the stereo depth module. Standard or wide refers to the infrared projector's projected field of view (Anonymous, 2022).

Color Sensor

The stereo depth module's color sensor offers texture information in addition to the color picture. For example, texture information may be utilized to produce a color point cloud or to reconstruct 3D models by layering them on top of a depth picture (Anonymous, 2022).

Method

The smallest possible Z-depth

The Vision Processor D4's depth data is based on the Minimum-Z Depth value, which measures the distance between the depth camera and the scene (Table 5).

Table 5. The smallest possible Z-depth (Anonymous, 2022)

Resolution	D450
	Min-Z (mm)
1280x720	520
848X480	350
640x480	320
640x360	260
480x270	200
424x240	180

Definition of Depth of Quality

A consistent set of criteria based on accuracy, data validity, and temporal stability are used to measure depth quality (Figure 3). However, even though the module has a specified depth field of view (FOV), it only measures within 80% of this FOV, which is called the area of interest (Table 6). With this ROI, the intended use area and the field for qualifying optical parameters are most closely aligned (Anonymous, 2022).

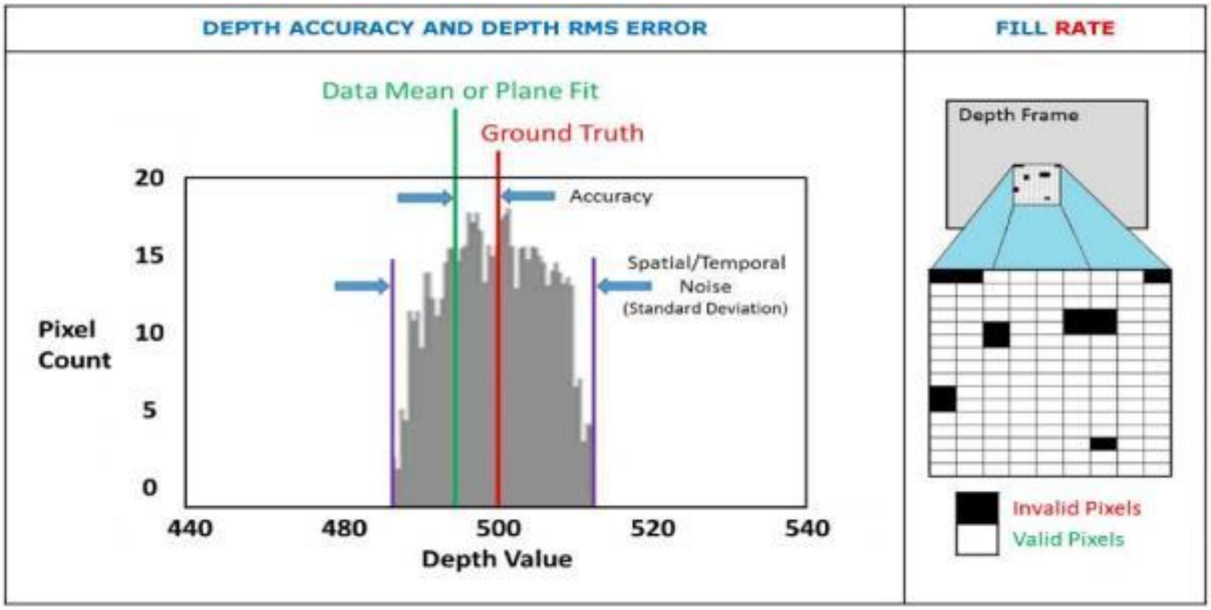

Figure 3. The depth quality metrics chart (Anonymous, 2022)

Table 6. Definition of the depth of quality (Anonymous, 2022)

Metric	**D455 (up to 4 Meters and 80% ROI, HD Resolution)**
Z-accuracy (or absolute error)	< 2%
Fill rate	> 99%
RMS Error (or Spatial Noise)	< 2%
Temporal Noise	< 1%

Intel® RealSenseTM 2.0 Software Development Kit

A cross-platform library for the Intel® RealSenseTM D400 Series is provided via the Intel® RealSenseTM SDK 2.0. (Figure 4). It can be found at https://github.com/Intel®RealSense/librealsense, an open-source project. Intel® RealSenseTM 2.0 Software Development Kit consists of Intel® RealSenseTM Viewer, view, record, and replay depth streams as well as create camera configurations and other controls may be done with this program. Also, the Depth Quality Tool, the fill rate, the standard deviation of the Z accuracy, and the distance to the plane can all be checked using this program for their correctness. Debug Tools, camera debugging is made easier with the help of these command-line tools, which gather data and produce log files. Many prominent programming languages and environments are supported through wrappers, such as ROS, and Python. Matlab, node.js. LabVIEW (Anonymous, 2022).

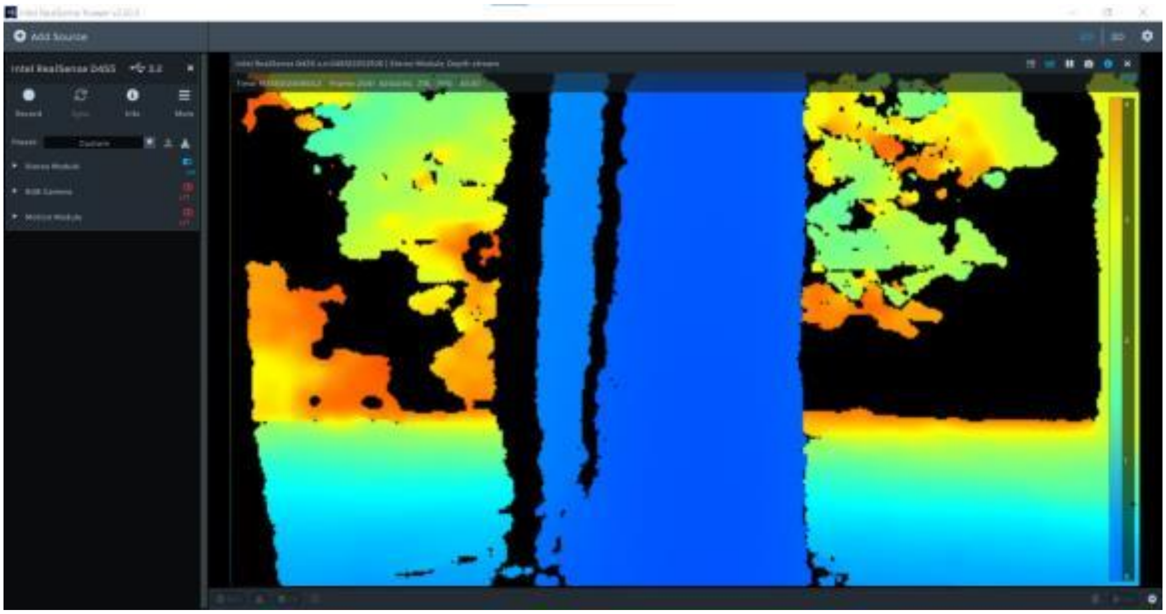

Figure 4. The interface of the Intel® RealSense SDK 2.0

Depth Band

Since the fields of view of the left and right imager do overlap, stereo vision will provide depth information at the right boundary of the frame (Figure 5). The depth band is narrower in images taken from a greater distance than in those shot from a closer distance (Anonymous, 2022).

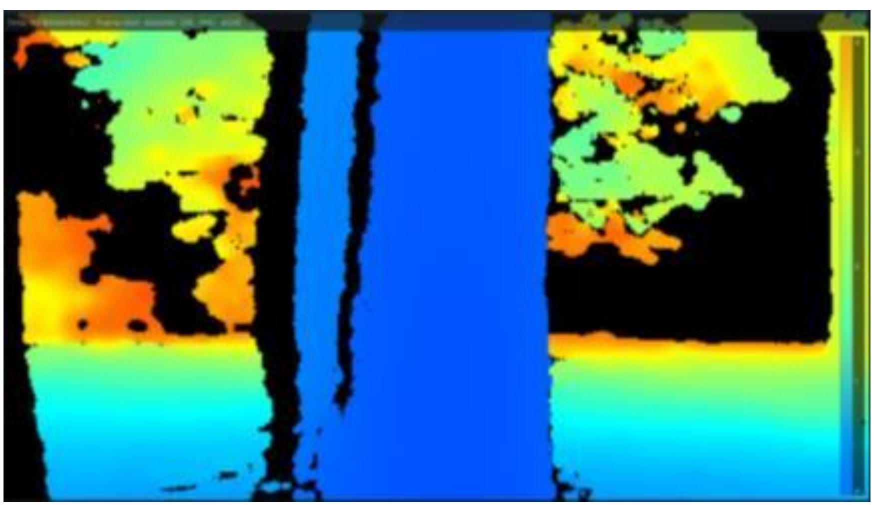

Figure 5. The depth band on the right (Anonymous, 2022)

Depth Field of View at Distance (Z)

To compute depth FOV at any distance, the equation below may be utilized (Z).

$$Depth\ FOV = \frac{HFOV}{2} + tan^{-1}\left(tan\left(\frac{HFOV}{2}\right) - {}^{B}/_{Z}\right) \qquad (1)$$

Depth FOV = Depth Field of View
HFOV = Horizontal Field of View of Left Imager on Depth Module

B = Baseline
Z = Distance of Scene from Depth Module (Anonymous, 2022).

As the distance between the scene and the depth module grows, so does the incorrect depth band in the overall depth picture. depth image. Both an incorrect and a valid depth map were used to create the final result of the combined depth image (Figure 6).

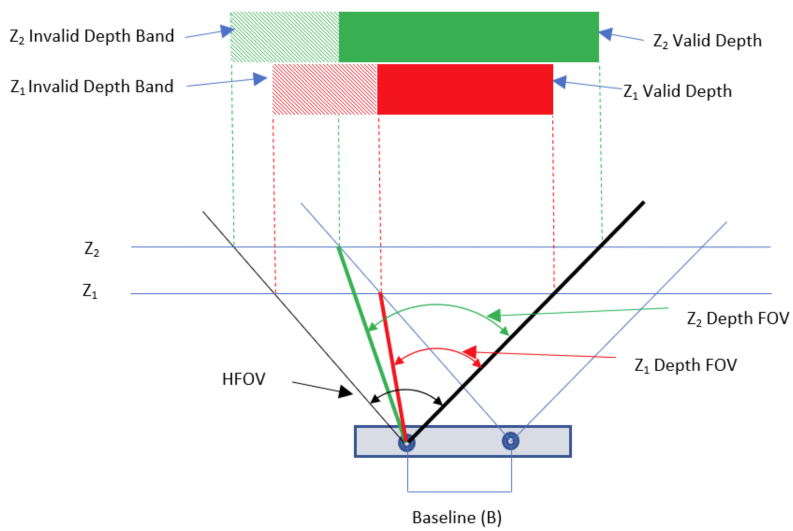

Figure 6. The relationship between depth field of view and depth map (Anonymous, 2022)

In this research, 15 depth measurements were done at 424x240 resolution, 30 FPS for the set of 6 m color correction by using an Intel® Realsense D455 depth camera between 0,5 to 4 m from a trunk surface. The depth range was selected between 0,5 to 4 m because this depth range is enough to get instant responses from electronic equipment of the agricultural robots, drones, etc. Actual depth measurements were made using a tape measure.

Results and Discussion

Model summary and analysis of variance can be seen in Tables 7 and 8, respectively. Also, the regression equation and coefficient between real and measured depth measurements can be seen in figure 7. According to figure 7, the regression coefficient between real and measured depth measurements is 99,8%. The regression coefficient shows us that we can securely use this depth camera for the automation of agricultural system applications. Using an Intel® Realsense D455 depth camera and UAV images, Li et al. (2021) studied the fast detection and location of longan fruits and found that the center of the string fruit boundary box's depth data is taken as the true distance value in long-range scenes; when the depth data are empty, the average value of all effective depth data along the vertical centerline of the string fruit boundary box's centerline is considered. The depth data of the center point of the fruit branch boundary box is used as the real distance value in the close-range scene, and when the data are empty, the depth data of the center point of the biggest single fruit bounding box is used as the true distance value, it should be noted. As a result, in agricultural applications, distance measurements are critical for target identification. Intel® realsense cameras may be used to generate depth images in robotics, and Tadic et al. (2019) found that the camera D415 provides a far better depth picture than the camera D435, allowing for a clearer distinction between objects and barriers based on the depth of an image. In light of these considerations, it is essential to know the features of the cameras used in agriculture.

Table 7. Model Summary

S	R-sq	R-sq(adj)
0,0458245	99,84%	99,83%

Table 8. Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	17,4727	17,4727	8320,78	0,000
Error	13	0,0273	0,0021		
Total	14	17,5000			

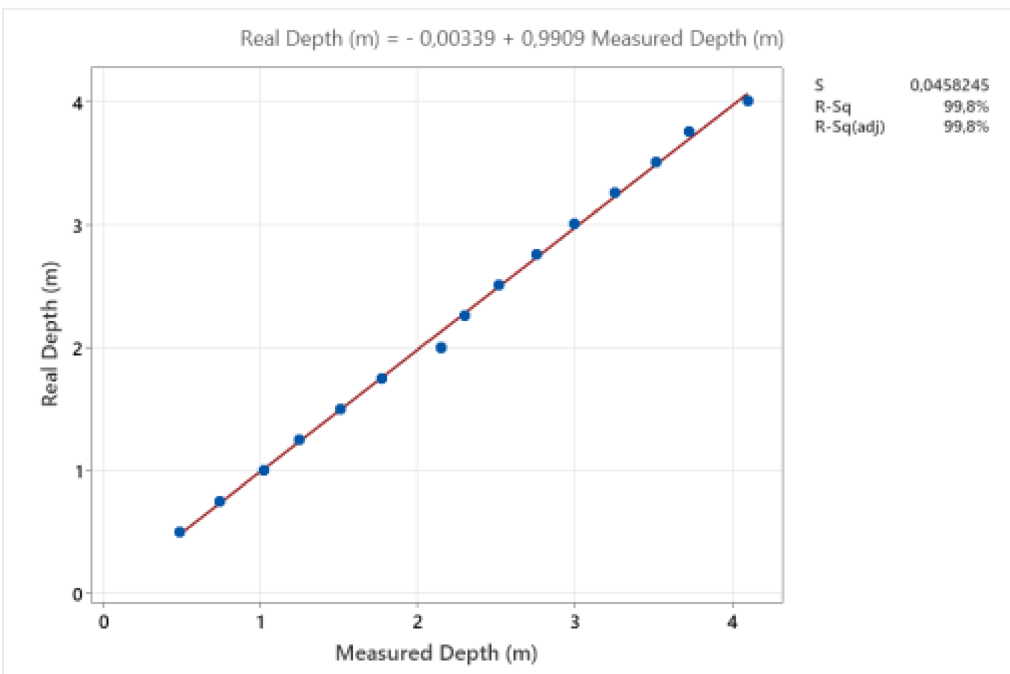

Figure 7. The regression equation and coefficient between real and measured depth measurements.

Conclusion

The goal of this article is to provide a quick overview of Intel®’s RealSense D455 depth camera’s functioning and features. The D455 camera was used to perform appropriate tests, and the findings were analyzed. The experiment’s goal was to show that robotic vision systems can be developed utilizing depth cameras. The first and most important stage in designing a system with digital image processing algorithms that allows the robot, drones, etc. in agriculture to automatically identify impediments in their area of movement is the employment of a depth camera and the development of a depth image.

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DETERMINATION OF AGRONOMIC CHARACTERISTICS AND CARVACROL RATES OF B CLONES OF IMPROVED ISTANBUL OREGANO (*Origanum vulgare* subsp. *hirtum*)

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Abstract

The popularity of the *Origanum* genus increases due to proven beneficial effects, including antioxidants, antimicrobials and antifungals effects. There are many agronomical and breeding studies about improved new varieties and cultivation techniques of oregano have been increasing last few decades. *Origanum vulgare* subsp. *hirtum,* which is commonly found in Turkey flora, has been standing out with high essential oil content compared with other *Origanum* species. In this study, the results of the year 2020 field trial of B clones of the breeding study initiated to improve varieties with clone selection method in Istanbul oregano are presented. In the study, plant height (cm), fresh herb yield (g/plot), drug herb yield (g/plot), drug leaves yield (g/plot), leaf/stem ratio (%), essential oil ratio (%) and essential oil yield (g/plot) of 100 genotypes of B clones belonging to the five populations of Istanbul oregano grown in Aydın ecological conditions were investigated. In the study 20 genotypes were examined in each population, drug leaves yields for the populations of A, B, C, D and E were 22.2-139.9, 34.7-658.6, 44.4-221.2, 49.2-287.5 and 62-175.2 g/plot, respectively. The essential oil contents of the A, B, C, D and E populations also ranged between 2.8-6.35, 2.95-6.25, 3.1-5.3, 3.5-5.8 and 4.0-5.80%, respectively. Among the examined genotypes, it was determined that the most important component of the essential oil was carvacrol and the ratio varied between 53.60-82.34%. As a result, all clones were compared in terms of agronomic traits and carvacrol ratios and promising genotypes were selected to generate C clones.

Keywords: *Origanum vulgare subsp. hirtum, clone selection, yield, essential oil, carvacrol.*

Introduction

The popularity of the *Origanum* genus is increasing every passing day because proven many scientifically beneficial effects, especially antioxidants, antimicrobial and antifungal effects. Turkey flora is very rich in *Origanum* genus that contains 21 species, 24 taxon and 13 hybrids. The endemism rate of this genus in Turkey is 67% (Başer, 2001; Celep and Dirmenci 2017; Arabacı et al., 2021). Turkey has the leading position in the world oregano trade, with approximately 80% supplied from Turkey. Oregano production is concentrated mainly in the western provinces (Denizli, Manisa, Uşak, Kütahya, Aydın) and southern provinces (Antalya and Hatay) of Turkey. During the last 20 years, the cultivation area of oregano in Turkey has increased from 5250 hectares to 19957 hectares, and the annual cultivated oregano production has reached 21174 tons from 7000 tons (Turkstat, 2022).

Cultivation and breeding studies on merchantable *Origanum* species have been increased in recent years. *Origanum vulgare* subsp. *hirtum* which is commonly found in Turkey's flora, has been standing out with high essential oil content compared with other *Origanum* species.
Origanum vulgare subsp. *hirtum* is a perennial, herbaceous or semi-shrub plant with natural distribution in Albania, Greece and Turkey. Stems usually softly hairy, leaves densely glandular-dotted, flowers dense and firm, branches and spiculae not soft. White and pink flowers, rural calyx technically and five-stepped, corolla 3-10 mm and stamens short. Oregano seeds are very small and thousand grain weight is 0.2 to 0.3 g. The seed color is brown and shape is round-oval. This species which blooms in July and August, is widely found in Thrace, Western and Southern Anatolia (Davis, 1982; Baytop, 1999; Karık et al., 2007; Bozdemir, 2019). *Origanum vulgare* subsp. *hirtum* is cross-pollinated (Allogamy) plant. With respect to this, there are inter-popular high variation among the plants. Excising variability among individual plants provides a great advantage for plant breeders, especially by creating source populations. *Origanum vulgare* subsp. *hirtum* is a plant that can be propagated via both generative and vegetative organs. In vegetative propagation the clone characteristics preserved because used same plant material that obtained from cuttings of rootstock plants (Bayram and Arabacı, 2021). The aim of this study was determine some agronomical characteristics and carvacrol ratios of B clones selected from the populations of *Origanum vulgare* subsp. *hirtum* plant created in Aydın ecological conditions. Field study results of B clones of the breeding study initiated that improved varieties by clone selection method in Istanbul oregano (*Origanum vulgare* subsp. *hirtum*) are presented in the study.

Materials and Methods

The field were conducted on the area of the Research and Practice Farm of the Field Crops Department, Faculty of Agriculture in Adnan Menderes University in 2020. The province of Aydın, where the research was conducted, has a typical Mediterranean climate, with a total precipitation of 643.3 mm and an average temperature of 17.7 ºC for the long years. The analysis of trial field soil indicated that texture soil was sandy-loamy, pH 8.43 and organic matter ratio was 1.30%. In this study the research materials originated from the Ida (Kaz) Mountains flora. Within the scope of the breeding work initiated with seeds obtained from this flora, single plants belonging to five populations selected in order to improve varieties according to the clone selection method with the project numbered 113O285 by TÜBİTAK titled “Selection of High-Efficiency and High-Quality Clones in Selected Istanbul Oregano (*Origanum vulgare* subsp. *hirtum*) Populations” investigated throughout the year. As continues of previous study, the materials of this study obtained from superior genotypes in terms of yield and essential oil quality were selected among these single plants (A clones) as B clones. In order to create B clones, cutting and planting processes were carried out between 21-23 October 2019 in a total of 100 clones for 5 populations, 20 of which were selected from each population. 40 pieces of cuttings were taken from each clone and 4000 pieces of cuttings were taken for all clones. The cuttings to be rooted were cut in 12-15 cm lengths and the leaves at the bottom were cut off and planted in viols filled with a mixture of peat and perlite. After planting, viols were irrigated and placed in the greenhouse. In order to ensure the rooting of the cuttings, plants irrigated regularly and the necessary maintenance were carried out on time. In Aydın ecological conditions, study field established for planting of seedlings. Before planting of seedlings, 5 kg/da P_2O_5 (Triple Super Phosphate) was given as the basic fertilizer in the trial area. Half of the nitrogen fertilizer

(5 kg/da Ammonium Sulphate) was applied before planting. In 15-17 April 2020, the seedlings were planted in the field at 50×40 cm planting density and in plots with 40 plants from each clone. In the experiment, each plot was arranged in a way that it consisted of 4 rows of 4 m in length and 10 plants in each row. Accordingly, plots of 8 m^2 were created for each clone.
After planting, the seedlings were irrigated. During the experiment, the water needs of the plants were provided with a drip irrigation system. In the experiment, weed control was done with hoe, and no diseases or pests were encountered during the development of the plants. Necessary maintenance procedures were carried out on the plants during the vegetation period. It was harvested between 21-22 July 2020 when the plants reached the full flowering period. In the second and third rows of each plot, one plant was left from the beginning and the end of the rows and the remaining plants were harvested. Accordingly, the harvest area of each parcel is 3.2 m^2.
In the research; plant height (cm), fresh herb yield (g/plot), drug herb yield (g/plot), drug leaves yield (g/plot), leaf/stem ratio (%), essential oil rate (%), essential oil yield (g/plot) and carvacrol ratio (%) were investigated. The analyses of the essential oil were performed in the Medicinal Plants Laboratories of Ege University, Faculty of Agriculture, Department of Field Crops. The essential oil rates were determined by volumetrically method on the air dried leaves samples using the Neo Clevenger apparatus. The essential oil rates were stated as milliliter /100 g (%) on air dried leaves (Wichtl, 1971). The essential oil components were determined with GC-MS (Shimadzu 2010 Plus QP-5050 Quadrapole Detector) at the SDU Experimental and Observational Research and Application Center.
The findings were evaluated statistically using the TARIST package program and minimum, maximum, mean, variance, standard deviation, S$\bar{x}$ and CV values were determined.

Results and Discussion

Statistical analysis results of some agronomical and quality characteristics of B clones belonging to five populations (A, B, C, D and E populations) of Istanbul oregano grown in Aydın ecological conditions are presented in Table 1-5. Statistical values of some agronomical and technological characteristics examined in 20 B clones of population A are given in Table 1. In this study, plants belonging to A population plant height 24.5-39 cm, fresh herb yield 88-578.9 g/plot, drug herb yield 32-226.9 g/plot, drug leaves yield 22.2-139.9 g/plot, leaf/stem ratio 52.4-81.3%, essential oil ratio was 2.8-6.35% and essential oil yield was between 0.98-6.51 g/plot.

Table 1. Statistical Values of Some Properties Examined in B Clones belonging to the of Population A in *Origanum vulgare* subsp *hirtum*

Parameters	Num. of Plant	Min.	Max.	Mean.	Variance	Standard Deviation	S$\bar{x}$	CV
Plant Height (cm)	20	24.500	39.000	32.837	15.4695	3.9331	0.8795	11.9777
Fresh Herb Yield (g/plot)	20	88.000	578.910	326.895	18249.3859	135.0903	30.2071	41.3253
Drug Herb Yield (g/plot)	20	32.000	226.930	128.288	2237.3799	47.3010	10.5768	36.8711
Drug Leaves Yield (g/plot)	20	22.160	139.930	85.646	909.3647	30.1557	6.7430	35.2099
Leaf/Stem Ratio (%)	20	52.410	81.330	67.421	38.4071	6.1973	1.3858	9.1920
Essential Oil Ratio (%)	20	2.800	6.350	4.780	0.6567	0.8104	0.1812	16.9531
Essential Oil Yield (g/plot)	20	0.980	6.510	4.077	2.2647	1.5049	0.3365	36.9166

Average plant height of 20 clones of the Istanbul oregano B population was 32.5 cm, fresh herb yield was 578.5 g/plot, drug herb yield was 228.6 g/plot, drug leaves yield was 156.0 g/plot, was leaf/stalk ratio 71.5% essential oil ratio was 4.48% and essential oil yield was recorded as 7.06

g/plot, drug herb yield was 46-1172.5 g/plot, drug leaf yield was 34.7-658.6 g/plot, and essential oil ratio was 2.95-6.25% (Table 2).

Table 2. Statistical Values of Some Properties Examined in B Clones belonging to the of Population B in *Origanum vulgare* subsp *hirtum*

Parameters	Num. of Plant	Min.	Max.	Mean.	Variance	Standard Deviation	$S\bar{x}$	CV
Plant Height (cm)	20	16.670	45.000	32.545	32.1926	5.6739	1.2687	17.4341
Fresh Herb Yield (g/plot)	20	132.000	3152.000	578.472	397361.4048	630.3661	140.9541	108.9709
Drug Herb Yield (g/plot)	20	46.000	1172.540	228.587	54837.5247	234.1741	52.3629	102.4442
Drug Leaves Yield (g/plot)	20	34.720	658.640	156.040	17038.0841	130.5300	29.1874	83.6519
Leaf/Stem Ratio (%)	20	56.170	79.510	71.487	24.2416	4.9236	1.1009	6.8874
Essential Oil Ratio (%)	20	2.950	6.250	4.480	0.9064	0.9521	0.2129	21.2514
Essential Oil Yield (g/plot)	20	1.110	27.990	7.062	33.6415	5.8001	1.2969	82.1257

According to some statistical evaluations made on the properties examined in the clones in Population C of *Origanum vulgare* subsp. *hirtum;* fresh herb yield 158.6-1046.2 g/plot; drug herb yield 62.6-355.7 g/plot; drug leaves yield 44.4-221.2 g/plot, essential oil content 3.1%-5.3% and essential oil yield 2.1-8.7 g/plot between were varied (Table 3).

Table 3. Statistical Values of Some Properties Examined in B Clones belonging to the of Population C in *Origanum vulgare* subsp. *hirtum.*

Parameters	Num. of Plant	Min.	Max.	Mean.	Variance	Standard Deviation	$S\bar{x}$	CV
Plant Height (cm)	20	20.000	51.250	34.736	56.4882	7.5159	1.6806	21.6371
Fresh Herb Yield (g/plot)	20	158.550	1046.150	498.728	54287.8371	232.9975	52.0998	46.7184
Drug Herb Yield (g/plot)	20	62.550	355.690	182.046	6311.8316	79.4470	17.7649	43.6413
Drug Leaves Yield (g/plot)	20	44.440	221.160	131.061	2883.7271	53.7003	12.0078	40.9736
Leaf/Stem Ratio (%)	20	61.760	80.350	72.608	17.1560	4.1420	0.9262	5.7046
Essential Oil Ratio (%)	20	3.100	5.300	4.270	0.4404	0.6636	0.1484	15.5410
Essential Oil Yield (g/plot)	20	2.090	8.670	5.495	4.6033	2.1455	0.4798	39.0453

Statistical values of clones belonging to Population D are given in Table 4. As to average values in the investigated features; The drug herb yield was determined as 242.1 g/plot, drug leaves yield 164.5 g/plot, essential oil rate 4.54% and essential oil yield 7.43 g/plot. The range of variations for the properties examined were established between 72 and 494.8 g/plot, 49.2-287.5 g/plot, 3.5-5.8% and 2.5-14 g/plot, respectively. (Table 4).

Table 4. Statistical Values of Some Properties Examined in B Clones belonging to the of Population D in *Origanum vulgare* subsp *hirtum.*

Parameters	Num. of Plant	Min.	Max.	Mean.	Variance	Standard Deviation	$S\bar{x}$	CV
Plant Height (cm)	20	20.000	45.000	34.917	40.3322	6.3508	1.4201	18.1882
Fresh Herb Yield (g/plot)	20	170.670	1113.000	646.269	80686.8529	284.0543	63.5165	43.9530
Drug Herb Yield (g/plot)	20	72.000	494.760	242.134	11714.9468	108.2356	24.2022	44.7006
Drug Leaves Yield (g/plot)	20	49.200	287.520	164.541	4597.6735	67.8061	15.1619	41.2093
Leaf/Stem Ratio (%)	20	58.110	74.610	68.818	23.3403	4.8312	1.0803	7.0203
Essential Oil Ratio (%)	20	3.500	5.800	4.538	0.4747	0.6890	0.1541	15.1843
Essential Oil Yield (g/plot)	20	2.530	14.040	7.426	10.4177	3.2276	0.7217	43.4642

It was determined that the average of the fresh herb yield, drug herb yield, drug leaves yield and leaf/stem ratio in the E population of Istanbul oregano were 433.2 g/plot, 176.1 g/plot, 118.8

g/plot, 68%, respectively. It was determined that the minimum value of essential oil ratio was 4.00%, maximum value was 5.80% and average value was 4.81%, essential oil yield varied between 2.64-9.03 g/plot and the average value was 5.71 g/plot (Table 5).

Table 5. Statistical Values of Some Properties Examined in B Clones belonging to the of Population E in *Origanum vulgare* subsp. *hirtum.*

Parameters	Num. of Plant	Min.	Max.	Mean.	Variance	Standard Deviation	$S\bar{x}$	CV
Plant Height (cm)	20	17.500	52.500	31.188	59.1047	7.6880	1.7191	24.6500
Fresh Herb Yield (g/plot)	20	201.600	772.920	433.238	18900.7210	137.4799	30.7414	31.7331
Drug Herb Yield (g/plot)	20	96.000	299.890	176.069	3125.8964	55.9097	12.5018	31.7544
Drug Leaves Yield (g/plot)	20	61.950	175.180	118.834	1403.3055	37.4607	8.3765	31.5237
Leaf/Stem Ratio (%)	20	40.640	78.300	68.001	64.4056	8.0253	1.7945	11.8018
Essential Oil Ratio (%)	20	4.000	5.800	4.813	0.2797	0.5289	0.1183	10.9895
Essential Oil Yield (g/plot)	20	2.640	9.030	5.705	3.4628	1.8609	0.4161	32.6179

During the selection among B clones to identify C clones, belonging to each clone; The results of fresh herb yield, drug herb yield, essential oil ratio and essential oil yield were taken into consideration and the essential oil component analysis was performed on 31 selected clones. It was determined that the major component of the essential oil in Istanbul oregano was carvacrol in all 31 clones selected from B clones belonging to A, B, C, D and E populations. In addition to the major component, it was found out that γ-terpinene, cymol, α-terpinene, β-myrcene, α-pinene and thymol constitute the main components (Figure 1).

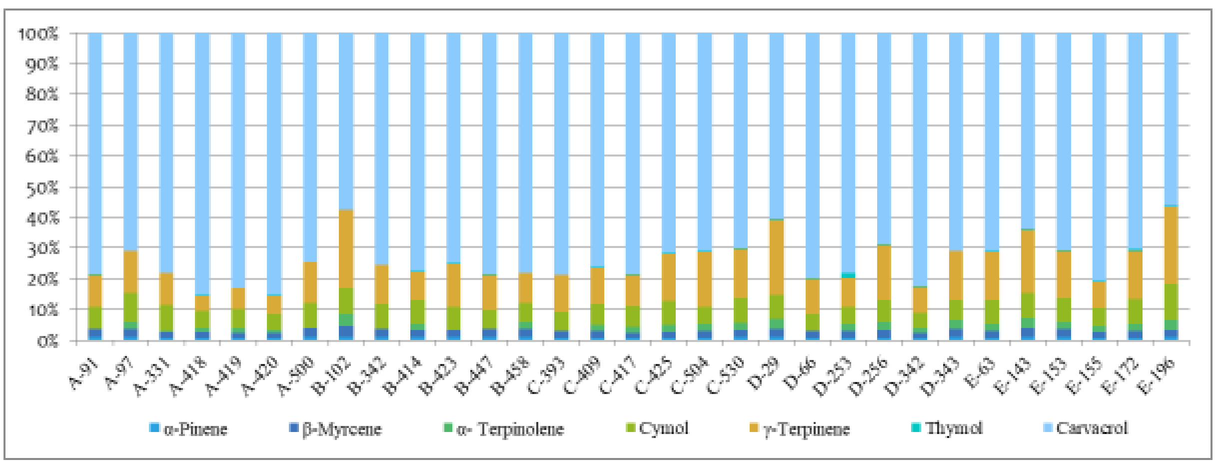

Figure 1. The essential oil composition of genotypes of selected B clones.

The ratio of carvacrol varied between 67.04% and 82.34% compared to clones in population A, the lowest ratio was obtained from clone A-97 and the highest ratio from clone A-420. The lowest value for carvacrol ratio was found in clone B-102 with 54.58% and the highest value was found in clone B-458 with 73.82% when 6 clones of B population were investigated. The carvacrol ratio of the clones selected among the B clones of the examined C population was found between 67.16% (clone C-504) and 75.62% (clone C-417). It has been observed that the carvacrol ratio in the clones of the D population of Istanbul oregano varies between 57.54% and 79.23%, the clone no D-29 has the lowest rate and the clone no D-342 has the highest rate. It was noted that the carvacrol value of the clones belonging to the E population varied between 53.60-

77.47%, and the highest value of carvacrol was reached with clone E-155 and the lowest value with clone E-196 (Table 6 and Figure 1).

Table 6. Carvacrol Ratios of Clones of Selected Populations from B Clones (%).

Clone No	Carvacrol (%)	Clone No	Carvacrol (%)	Clone No	Carvacrol (%)	Clone No	Carvacrol (%)	Clone No	Carvacrol (%)
A-91	72.28	**B-102**	54.58	**C-393**	74.42	**D-29**	57.54	**E-63**	68.49
A-97	67.04	**B-342**	70.10	**C-409**	73.59	**D-66**	75.34	**E-143**	60.72
A-331	74.12	**B-414**	72.82	**C-417**	75.62	**D-253**	74.38	**E-153**	68.21
A-418	82.02	**B-423**	69.09	**C-425**	69.08	**D-256**	64.97	**E-155**	77.47
A-419	79.72	**B-447**	72.10	**C-504**	67.16	**D-342**	79.23	**E-172**	66.90
A-420	82.34	**B-458**	73.82	**C-530**	67.43	**D-343**	67.77	**E-196**	53.60
A-500	69.66								

Within the 31 selected clones; According to result of the selection the fresh herb yield, drug herb yield, drug leaves yield, essential oil ratio and essential oil yield results as well as the essential oil component results, a total of 20 clones, 4 from each population have been determined in order to create C clones.

Origanum vulgare subsp. *hirtum*, a perennial plant, has a height of 15-73 cm (Davis, 1982; Tanker and Tanker, 1976; Marzi, 1996; Baytop, 1999; Janke and De Armond, 2004; Sarıhan et al., 2006; Sancaktaroğlu and Bayram, 2011; Bozdemir, 2019). The plant height values obtained from this study were compatible with the literature studies. In this study, fresh herb yield varied between 88-3152 g/plot, drug herb yield varied between 32-1172. 5 g/plot, drug leaves yield varied between 22.2-658.6 g/plot in inter-populations as well as it varied intra-populations. It has been noteworthy that fresh herb yield and drug herb yield differ in compared with the previous studies in the literature (Janke and De Armond, 2004; Sarıhan et al., 2006; Karık et al., 2007; Sancaktaroğlu and Bayram, 2011). When analyzed studies conducted especially in different population, Tınmaz et al. (2002) was found between 84-310 kg/da, for the first year Karık et al. (2007) was determined between 106-246 kg/da and Sancaktaroğlu and Bayram (2011) were establish between 273-539 kg/da. The result of the drug leaf yield indicated that the result of this study was in harmony with the values of previous studies by calculating as a unit of kilogram (205.8 kg/da).

According to previous researches, essential oil ratio of *Origanum vulgare* subsp. *hirtum* were Kokkini et al. (1997) 1.0-3.1%, Russo et al. (1998) 2.25-5.69%, Baytop (1999) 4-5%, Başer (2001) 1-7%, Tınmaz et al. (2002) 1-6.1.1%, Zeybek and Zeybek (2002) 5%, Oflaz et al. (2004) 3.6-4.4% reported. The essential oil ratios of A, B, C, D and E populations examined in the study were detected as 2.8-6.35%, 2.95-6.25%, 3.1-5.3%, 3.5-5.8% and 4.0-5.80%, respectively. In this study, the rates of essential oil show that similarity with the results of other researchers.

It has been determined that carvacrol is the major component in the chemical composition of essential oil of the clones belonging to all populations examined in this study and it value varied between 53.60-82.34%. In studies carried out in different populations of *Origanum vulgare* subsp. *hirtum*, the rate of carvacrol was determined 7.5-82.9% by Tınmaz et al. (2002) 42.9%-73.5%, Oflaz et al. (2004), %5.3-88.6 Karık et al. (2007), %2.89-88.3 Sancaktaroğlu and Bayram (2011) reported that they found it between found it between. Carvacrol values in our study were found to be higher than the lower limit of previous studies and parallel to the upper limit.

Conclusion

It was determined that the coefficient of variation of the fresh herb yield, drug herb yield, drug leaves yield and essential oil yield values of the five populations examined in *Origanum vulgare* subsp. *hirtum* (Istanbul Oregano) was quite high compared to other characteristics.
Also, the existence of inter- and intra-population alterations in terms of these characteristics has also attracted attention. This result explains the wide variability. Research results showed that the possibility of obtaining promising clones in terms of yield and quality characteristics for breeding work.
In the study, 20 genotypes were ascertained by forming the C clones of the clone selection, as a result of the selection made by considering some agronomical and technological features of the B clones examined. In the future, it seems possible to improve high quality varieties in consequence of multi-local yield trials of the genotypes that create the C clones.

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EFFECT OF DEFICIT IRRIGATION ON ANTIOXIDANT, ANTIRADICAL AND FLAVONOID CONTENTS OF *Origanum vulgare* subsp. *hirtum* GENOTYPES

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Abstract

Istanbul oregano (*Origanum vulgare* subsp. *hirtum*) is one of the important medicinal plants naturally grown in Turkey. It is a valuable antioxidant plant from the Lamiaceae (Labiatea) family and native to the Mediterranean climate. Nowadays, plants belonging to the *Origanum* genus have attracted many attentions of consumers as a spice plant due to their high antifungal, antimicrobial and antioxidant properties. The extracts of Istanbul oregano have one of the most effective antioxidant activities among aromatic herbs. Plants exposed to insufficient water conditions have their photosynthesis decreased, growth and development negatively affected. This is an important factor that causes yield losses up to 50% in plants exposed to deficit water conditions. According to the World Resources Institute (WRI) 2020 data, there will be serious water shortages in Turkey along with other countries. Tolerant plants have more potential for higher levels of both osmolytes and antioxidants and reprogram their metabolism to enhance their antioxidant capacity in insufficient water conditions. In this study, the effects of three different deficit irrigation conditions (100%, 67% and 33%) on antioxidant (FRAP), antiradical (DPPH) and flavonoid properties of 12 different Istanbul oregano genotypes were investigated in 2020 and 2021. According to results, FRAP (Ferric Reducing Antioxidant Power), DPPH (2,2-diphenyl-1-picrylhydrazyl) and flavonoid values in 2020 were determined between 47.8-84.6%, 24.6-57.2%, 77,1-165.5 mg Rutin/g and values in 2021 were determined between 51.0-69.9%, 45.1-75.9%, and 105.8-182.4 mg Rutin/g respectively. The highest values were obtained at 33% irrigation condition for all properties.

Keywords: *Deficit irrigation, Genotype, Antioxidant capacity, Medicinal plant, Origanum vulgare subsp. hirtum.*

Introduction

Istanbul oregano (*Origanum vulgare* subsp. *hirtum*) is one of the important medicinal plants naturally grown in Mediterranean climate (Goliaris et al., 2002; Ietswaart, 1980). Plants of the *Origanum* are popularly used as a spice plant (Azizi et al., 2009). 60% of the known Origanum species in the world have distributed in Turkey (Arabacı et al., 2016; Avcı 2006; Başer 2001).
The extracts of Istanbul oregano have most effective antioxidant activities among aromatic herbs (Azizi et al., 2009; Bakkali, 2008; Kulisic, 2004; Kokkini, 1997). It is stated that the high antioxidant property of *Origanum* may be due to high levels of carvacrol and thymol components in plant (Azizi et al, 2009; Yanishlieva et al.,1999; Aeschbach et al., 1994; Lagouri et al., 1993).
It has been reported that the water requirement of plants for cultivation is going to increase by 40-250% towards the end of the 21st century. It was stated that the reason is increasing evapotranspiration during the growing season and the changes in the phenology of the plants. For this reason, the need for irrigation water will increase even more due to global warming and drought stress will increase intensely (Shchedrin et al., 2018).

Plants exposed to insufficient water conditions have their photosynthesis decreased, growth and development negatively affected. This is an important factor that causes yield losses up to 50% in plants exposed to deficit water conditions (Bray et al., 1997).
The damage caused by stress factors varies depending on the plant species, tolerance, and adaptability (Madhova et al., 2005). Considering that plants encounter many stress factors throughout their lives, it is very important to elucidate stress-related mechanisms and to develop tolerant species and varieties. (Kadıoğlu, 2004).
Aim of this study is to determine the effects of deficit irrigation conditions on antioxidant (FRAP), antiradical (DPPH) and flavonoid properties of 12 different Istanbul oregano genotypes.

Material and Methods

The study material was *Origanum vulgare* subsp. *hirtum* plants naturally distributed in Mount Ida, Turkey. The origins of the populations used in the study were shown in Table 1.

Table 1. Origins of *Origanum vulgare* subsp. *hirtum* populations used in the study (Arabacı et al., 2016).

No	Population	Coordinates		Altitude (m)	Aspect
		x	y		
1	A	542066	4422267	586	South
2	B	545202	4423405	599	Southeast
3	C	546586	4426023	627	South
4	D	524032	4394952	359	(Stream bed)
5	E	525416	4394939	381	North


Fig 1. Istanbul Oregano (*Origanum vulgare* subsp. *hirtum*) plants.

In order to determine soil moisture, soil samples were taken from the soil layers by soil probe according to the principles determined in Petersen and Calvin (1965).

Soil moisture (%) (Pw) was calculated using PW=(Wet soil)-(Dry soil)/(Dry soil) x 100. The formula dn= (TK-Pw)/100x(γt)xD was used to calculate the amount of irrigation for plants (TK: Field capacity, Pw: Current moisture, γt: Soil bulk weight, D: soil depth.).

Antioxidant activity (FRAP)

Antioxidant activity was performed according to Benzie and Strain (1999). Extraction process was carried out by using drug leaf samples with 80% ethanol. The FRAP solution was prepared using 0.054 g $FeCl_3$, 38% HCL and 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine. 1.5 ml of FRAP solution, 0.05 ml of sample and 0.15 ml of distilled water were added into the cuvettes and their measurements were carried out spectrometrically at 593 nm.

Antiradical analysis (DPPH)

Antiradical activity was determined according to Brand-Williams et al. (1995). Extraction process was carried out by using drug leaf samples with 80% ethanol. The DPPH solution was prepared using 2,2-diphenyl-1-picrylhydrazil and the inhibition (%) values of the samples were calculated by comparing with the control. Measurements were performed spectrometrically at 515nm.

Flavonoid Analysis (mg Rutin/g)

Total flavonoid content was performed according to Zhishen et al. (1999). Extraction process was carried out by using drug leaf samples with 80% ethanol. 0.3 ml of 5% $NaNO_2$ was added to the samples and waited for 5 minutes, then 0.3 ml of 10% AlCl3 was added and waited for another 6 minutes. At the end of 6 minutes, 2 ml of 1 molar NaOH was added, and red color was obtained. Measurements were performed spectrometrically at 510 nm.

Results and Discussion

It was determined that FRAP (%) values increased in 2020 and 2021 with the decrease of irrigation doses (Fig. 2). Antioxidants play major role in preventing reactive oxygen species that occur in the cell and harm plant cells. Plants reduce the negative conditions caused by drought stress in plant tissues and cells with antioxidants in and it provide tolerance. It can be said that this situation can be used as as selection criteria to select tolerant genotypes.

Considering the irrigation doses from the applications, it was determined that the DPPH values increased with the decrease at irrigation level (Fig. 2). We determined same result in FRAP results. Since DPPH and FRAP methods are both used as antioxidant determination methods, same results were expected. In our study, it was found that both methods have similar results.

In terms of irrigation doses flavonoid values also have similar results with FRAP and DPPH and was determined that the flavonoid values have increased with the decrease of the irrigation dose.

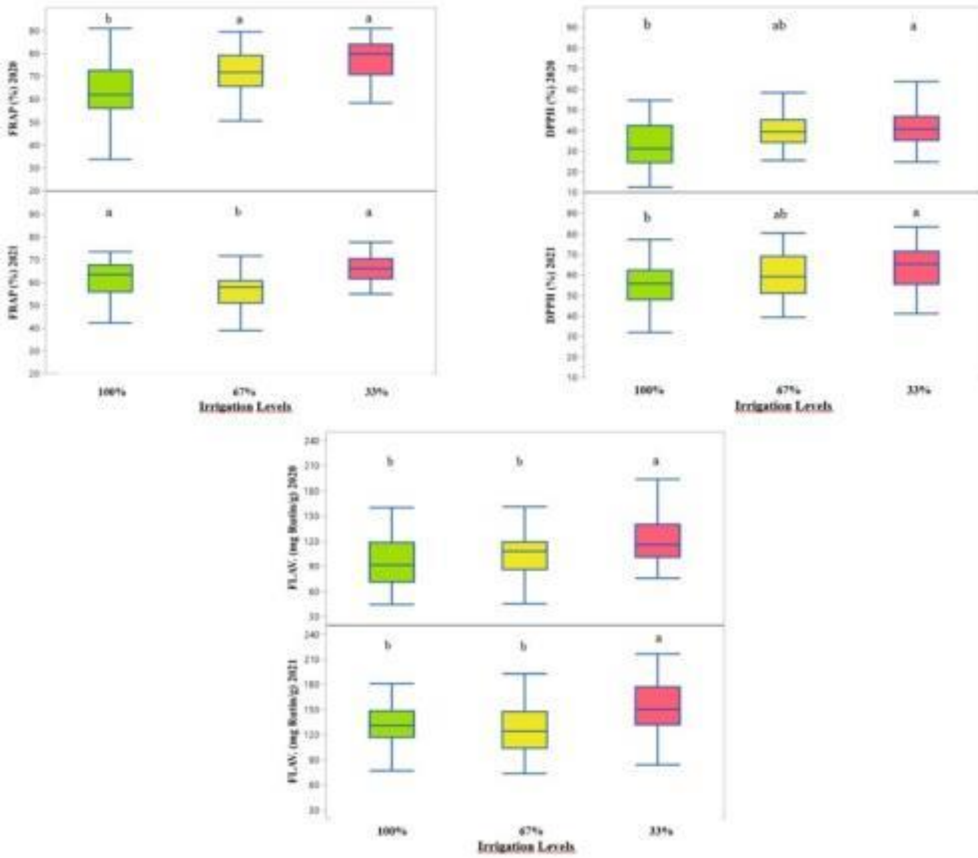

Fig 2. Box plot graph of irrigation level effects on FRAP, DPPH and flavonoid values.

According to 2020 results, the highest FRAP average value 75.7% was obtained from B-201 and C-290 genotypes, the lowest FRAP was obtained from the D-99 with 59.9%. Considering 2021 values, the highest average value was obtained from the C-425 and E-302 genotypes with 64.9%, while the D-92 genotype has the lowest average value with 58.0% (Fig. 3.).

While the highest DPPH average value in 2020 was obtained from the genotype A-210 with 46.8%, the lowest DPPH value was determined from the genotype C-548 with 32.4%. According to 2021 average values, the genotype E-400 has the highest value with 63.1%, while the genotype E-11 has the lowest value with 56.4% (Fig. 3.).

Among the genotypes in 2020, the highest flavonoid average value was obtained from the C-425 genotype with 133.4 mg Rutin/g, while the lowest flavonoid was determined from the genotype D-92 with 85.4 mg Rutin/g. Considering the 2021 average values, C-425 and E-302 genotypes have highest value with 150.4 mg Rutin/g, while the genotype B-575 has lowest value with 123.1 mg Rutin/g (Fig. 3.).

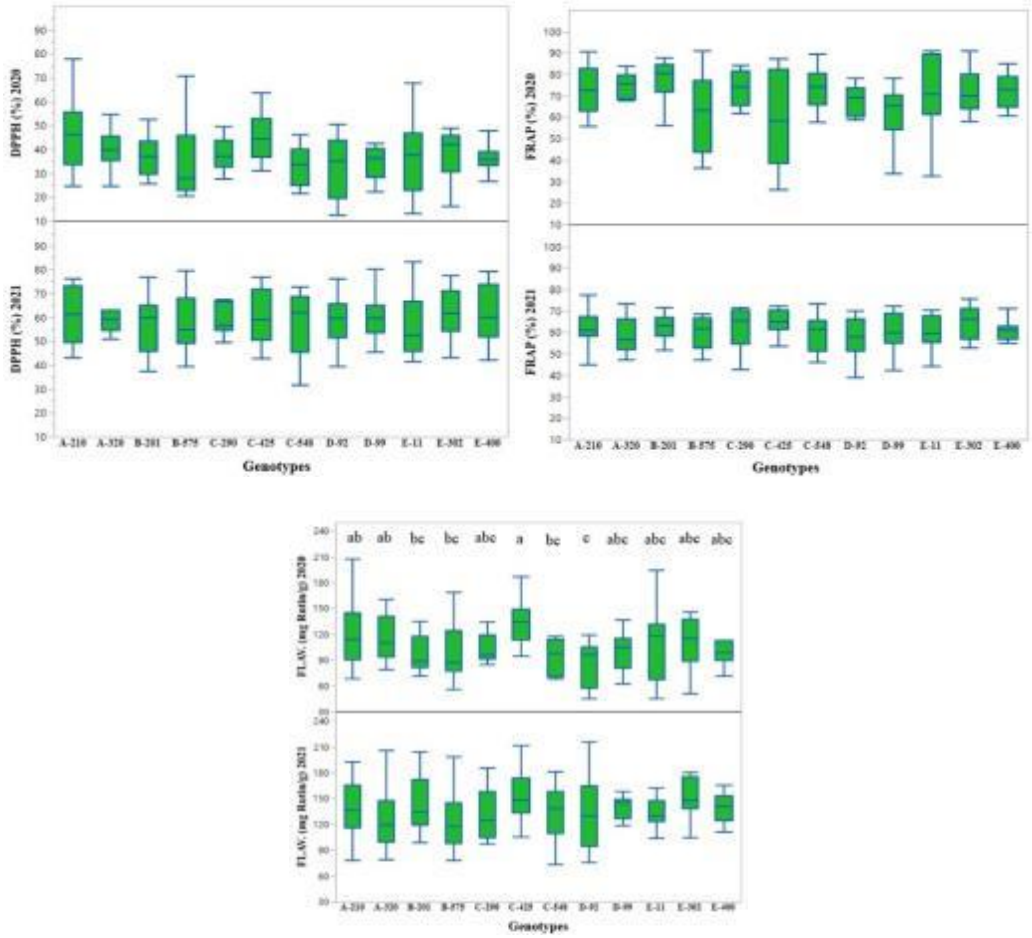

Fig 3. Box plot graph of FRAP, DPPH and flavonoid values variation by genotypes

Conclusions

Many studies underline there will be drought stress in future due to lack of irrigation water. It is important to understand plants response to lack of water and determine drought tolerant plants. In this study, it was determined that there was an increase in antioxidant properties with the decrease of limited irrigation doses. Plants have developed a defense mechanism by increasing the synthesis of antioxidants with drought stress of Istanbul oregano. It can be said that genotypes which have higher antioxidants properties have higher drought-tolerant potential. In addition, antioxidant (FRAP), antiradical (DPPH) and flavonoid measurements can be used as selection criteria to select drought-resistant plants.

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SIGNIFICANCE OF FERTILIZATION AND SOIL TYPE FOR THE DEVELOPMENT OF SUNFLOWER (*HELIANTHUS ANNUUS*)

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Abstract

Sunflower (*Helianthus annuus*) represents an important oil crop and may have a certain production potential under optimal input conditions. The study was conducted in a pot experiment with two soil types – Eutric Fluvisol and Pellic Vertisol with the aim to evaluate the effect of different norms and combinations of nitrogen, phosphorus, potassium, and silicon fertilizers and to estimate their impact on the vegetative development of the medium-early hybrid Sunflower (*Helianthus annuus* L.) – Sumiko HTS. The experiment included 15 fertilization treatments and one control variant, with three replications. Data was obtained on the height of the plants on the 34th, 47th, 57th and 67th day, from the beginning of the vegetation, as well as weights of fresh biomass from the above ground part and roots. According to the obtained experimental data, the agronomic characteristics were affected significantly by treatments. The optimal vegetative development was reached in the variants - $N_{200}P_0K_{140}Si_{800}$ and $N_{300}P_{80}K_{210}Si_{400}$ for the Eutric Fluvisol. In the other type of soil - Pellic Vertisol, respectively variants - $N_{400}P_{160}K_{140}Si_{800}$ and $N_{200}P_0K_{140}Si_{800}$, were those in which plant development was more favorably influenced by the applied fertilization. The analysis confirmed that the norm of 200 mg N/ pot, 140 mg K/pot in combination with 800 mg Si/ pot in both soil types, was the most appropriate for the growth of sunflower.

Keywords: *Fertilization rates, Nitrogen, Phosphorus, Potassium and Silicon fertilizers, Growth rate of sunflower biomass*.

Introduction

Bulgaria is characterized by a great variety of soil types, which is due to the different, climatic and geological conditions. Conducting agrochemical research with different soil types is essential to achieve high efficiency in optimizing fertilization, which is important for maintaining soil fertility. In our country, many farmers focus mainly on nitrogen fertilization. According to some scientists (Hellal et al., 2012; White et al., 2017) nitrogen is a limiting factor for crop development and to reduce its excessive use it is necessary to study it's relationship with silicon. Silicon (Si) is one of the most common elements in the earth's crust and the main structural element of the soil. The agricultural export of Si from crop cultivation and harvest may deplete soils' labile Si pools (Clymans et al., 2011, Vandevenne et al., 2012, Barão et al., 2014). Si management in agricultural ecosystems thus offers a possibility to enhance crop production. . Silicon (Si) is not considered a basic nutrient for most plants and is not usually included in commercially available fertilizers (Epstein, 1994). Today in Japan, Si is considered an important agronomic element, as its beneficial effects improved growth and quality, increased

photosynthesis, reduced transpiration and increased plant resistance to abiotic and biotic stresses, are well established in several crops. (Ma and Takahashi, 2002).
Sunflower is an important oilseed crop. Balanced nutrition is key to achieving stable, high-quality yields for any crop. There are few studies related to the optimization of sunflower nutrition, and there is almost no information on silicon fertilization. This crop is characterized by exceptional requirements for micronutrients. It is very important that the stem grows, which must be resistant to lodging and ensure long-term transfer of metabolites to the growing seeds, and this is mainly its role. Silicon is not classified as a key element for plant growth, but many studies have described its beneficial properties in a variety of soil and climatic conditions, including low levels of nutrient-accessible forms of nutrients in the soil (Pavlovic et al., 2021). Recent studies confirm that Si is an important element that increases the plant's tolerance to stress caused by unexpected changes in the environment. Si (in the form of amorphous silica) strengthens the walls of epidermal and vascular tissues. Si strengthens plant tissues and reduces water loss (Savant et al., 1999). Most of the silicon in plants is found in insoluble form. Soluble silicon at high concentrations is not toxic to plants. Plant species differ significantly in their ability to accumulate Si (Epstein and Bloom, 2008). Therefore, some plant species are minimally affected by Si uptake compared to others (Coskun et al., 2019). Silicon fertilizer has improved plant-silica nutrition, which enhances plant protection properties against diseases and adverse climatic conditions (Snyder et all., 2016). It is important to clarify the influence and contribution of silicon on the effectiveness of essential macronutrients in the cultivation of sunflower oilseeds.
The main goal of the study was to determine the importance of the norms of the main nutrients of plants (N, P, K and Si) and to combine them to optimize the nutrition of sunflower.

Material and Methods

Under controlled conditions in a greenhouse on the territory of ISSAPP "N. Pushkarov” we performed a pot experiment with sunflower (*Helianthus annuus* L.) as the main crop. The starting soils were Eutric Fluvisol and Pellic Vertisol (FAO, 2015). Eutric Fluvisol are productive soils that form along large rivers in different climates. The main activities to increase their fertility, in addition to fertilization, are aimed at preventing them from flooding, swamping and salinization. Pellic Vertisol are the heaviest soils in Bulgaria and have unfavorable water-physical properties: when wet they swell strongly, and when dry they shrink and crack.
The experiment included 15 treatments of fertilization and one control. Before seeding, fertilizers with different amounts of active substances in mg / pot were added to the experimental pot (Table 1). The imported quantities of mineral fertilizers were ammonium nitrate (34% N), triple superphosphate (46% P), potassium sulfate (45% K) and diatomaceous earth (92% SiO_2) (Table 1).

Table 1. Scheme of the pot experiment – amount of fertilizer, active substances in mg/pot

Treatment	Active elements in mg/pot			
№	N	P	K	Si
1	0	0	0	0
2	0	160	140	800
3	400	160	140	800
4	200	0	140	800

5	200	320	140	800
6	200	160	0	800
7	200	160	280	800
8	200	160	140	0
9	200	160	140	2000
10	200	160	140	800
11	300	240	70	400
12	300	80	210	400
13	300	80	70	1200
14	100	240	210	400
15	100	240	70	1200
16	100	80	210	1200

Data on the height of the plants were obtained at 34^{th}, 47^{th}, 57^{th} and 67^{th} day from the beginning of the experiment, and the weights of the above ground part and the roots of the plants were recorded at 57 and 67 day. The statistical processing of the obtained results was prepared by Statgraphics (ANOVA).

Results and Discussion

The main soils are of medium humus class and are characterized by a neutral soil reaction (pH 7.4 6.2). Eutric Fluvisol has a low content of total and mineral nitrogen. Although the total nitrogen content of Pellic Vertisol is 0.139% and this characterizes it as relatively well stored, the levels of mineral nitrogen are low. Both soils have a low supply of mobile phosphorus and absorbable potassium (Table 2).

Table 2. Agrochemical characteristic of Eutric Fluvisol and Pellic Vertisol

Type soil	pH		NH_4^++NO_3^-	Total N	P_2O_5	K_2O	Organic matter
	H_2O	KCl	$mg.kg^{-1}$	%	$mg.100g^{-1}$		%
Eutric Fluvisol	7.4	6.3	11.52	0.052	8.09	14.35	2.58
Pellic Vertisol	6.2	5.4	12.67	0.139	0.20	30.11	3.02

The effect of imported mineral fertilizers on the height and yield of fresh sunflower biomass varies depending on the concentration of N, P, K and Si in the soil. During the vegetation of sunflower plants, measurements of the heights were performed on the 34^{th}, 47^{th}, 57^{th} and 67^{th} day from the beginning of the experiment (Table 3). After a one-way analysis of variance, the plants grown on Eutric Fluvisol measured on days 34^{th} and 47^{th}, in variants 14 ($N_{100}P_{240}K_{210}Si_{400}$), 6 ($N_{200}P_{160}K_0Si_{800}$) and 10 ($N_{200}P_{160}K_{140}Si_{800}$) were the highest. On the first date of plant measurement, variants 8 ($N_{200}P_{160}K_{140}Si_0$) and 13 ($N_{300}P_{80}K_{70}Si_{1200}$) statistically fall into one homogeneous group (a), which showed the lowest results. In another group (b) - 6 ($N_{200}P_{160}K_0Si_{800}$) and 10 ($N_{200}P_{160}K_{140}Si_{800}$), in which the highest values are reported. Plants grown on Pellic Vertisol grow better, all higher than the control version on both dates. On this type of soil the best treatments on the 34^{th} day were 3 ($N_{400}P_{160}K_{140}Si_{800}$) and 7 ($N_{200}P_{160}K_{280}Si_{800}$), where there is potassium, these two variants fall into one homogeneous group (c). On the 47^{th} day, the highest plants were in variants 9 ($N_{200}P_{160}K_{140}Si_{2000}$) and 13

($N_{300}P_{80}K_{70}Si_{1200}$), which may be due to higher silicon levels. Si improves the water, physical and chemical properties of this type of soil, as well as maintaining nutritional value substances in plant-available forms (Snyder et all., 2016).
In the next two measurements (days 57 and 67), the highest plants grown on Eutric Fluvisol were in variant 4 (90.3 and 120 cm) and in variant 16 the lowest values (65.4 and 104 cm). Plant height growth was significantly reduced by increasing the rate of silicon, regardless of the applied levels of nitrogen, phosphorus and potassium (Pavlovic et. al., 2021). Rather, silicon strengthens stem strength (Richmond and Sussman, 2003). On the 67th day, the highest values of heights were obtained in variants 4 ($N_{200}P_0K_{140}Si_{800}$) and 8 ($N_{200}P_{160}K_{140}Si_0$), forming a group - (d), which included 4 more variants, in which the plant heights were between 116 and 118 cm. The same in these variants were the norms of nitrogen - N_{200} and Si_{800} mg/ pot.

Table 3. Influence of fertilization treatments on the height of sunflower plants (One-way analysis)

Treatment	Height of sunflower plants (cm)							
	34 day		47 day		57 day		67 day	
	Eutric Fluvisol	Pellic Vertisol	Eutric Fluvisol	Pellic Vertisol	Eutric Fluvisol	Pellic Vertisol	Eutric Fluvisol	Pellic Vertisol
1	33.1^{ab}	20.1^{a}	48.0^{ab}	41.4^{a}	64.3^{a}	64.6^{a}	93.1^{a}	91.3^{a}
2	31.3^{ab}	28.4^{abc}	50.0^{ab}	47.2^{ab}	69.5^{abc}	63.3^{a}	114.7^{cd}	107.3^{bc}
3	28.5^{ab}	43.4^{c}	51.4^{ab}	63.3^{c}	79.0^{bcd}	89.3^{fg}	116.3^{d}	117.0^{bcde}
4	30.1^{ab}	26.1^{abc}	51.3^{ab}	54.9^{bc}	90.3^{d}	90.3^{fg}	120.0^{d}	128.0^{def}
5	31.0^{ab}	36.7^{bc}	55.2^{ab}	57.7^{c}	80.2^{cd}	79.9^{cdef}	117.0^{d}	121.3^{cdef}
6	35.3^{b}	34.6^{abc}	58.6^{b}	56.2^{bc}	80.6^{cd}	72.5^{abc}	118.0^{d}	123.3^{def}
7	30.4^{ab}	44.5^{c}	51.8^{ab}	62.9^{c}	81.0^{cd}	87.4^{defg}	116.7^{d}	123.0^{def}
8	22.0^{a}	38.1^{bc}	45.8^{a}	62.9^{c}	71.2^{abc}	91.7^{g}	118.3^{d}	133.7^{f}
9	32.4^{ab}	41.4^{bc}	52.2^{ab}	63.6^{c}	75.5^{abc}	90.2^{fg}	106.0^{bc}	131.0^{ef}
10	35.7^{b}	40.3^{bc}	57.1^{ab}	61.2^{c}	82.0^{cd}	88.5^{efg}	111.0^{bcd}	128.3^{def}
11	30.8^{ab}	25.1^{ab}	52.7^{ab}	47.6^{ab}	75.2^{abc}	67.2^{ab}	111.0^{bcd}	113.7^{bcd}
12	32.6^{ab}	37.8^{bc}	56.3^{ab}	59.0^{c}	78.1^{bcd}	84.1^{cdefg}	117.3^{d}	130.3^{ef}
13	22.0^{a}	33.3^{abc}	48.8^{ab}	63.7^{c}	69.2^{abc}	87.9^{defg}	111.7^{bcd}	127.0^{def}
14	34.0^{ab}	31.1^{abc}	56.8^{ab}	54.7^{bc}	76.4^{abc}	84.7^{defg}	110.7^{bcd}	121.7^{cdef}
15	27.8^{ab}	33.0^{abc}	50.0^{ab}	55.2^{bc}	72.4^{abc}	77.0^{bcde}	111.7^{bcd}	126.0^{def}
16	25.9^{ab}	26.2^{ab}	47.4^{ab}	54.4^{bc}	65.4^{ab}	76.4^{bcd}	104.0^{b}	104.0^{ab}

Values are means of 3 replicates. The different letters, symbolize statistically significant differences among the 3 treatments, according to the Duncan’s multiple range test, for $p \leq 0.05$.

On the 57th and 67th day, in the second type of soil (Pellic Vertisol), the highest were again the plants of variant 8 ($N_{200}P_{160}K_{140}Si_0$) – 91.7 and 133.7 cm and 9 ($N_{200}P_{160}K_{140}Si_{2000}$) – 90.2 and 131.0 cm. The other variants form intermediate groups, without statistically significant differences.
In both soil types, from the beginning of the vegetation, the amounts of 200 mg N/ pot in combination with 800 mg Si/ pot, had a positive effect on the growth of sunflower plants. Therefore, the combination of N_{200} and Si_{800} had a positive effect on the amount of biomass accumulated, which is most likely due to the improved mechanisms of nitrogen uptake, assimilation and remobilization by plants (Detmann et al., 2012).

One-factor analysis of variance of fresh sunflower biomass weights on Eutric Fluvisol, reported on the 67th day of the growing season, showed that the variants were very heterogeneous and fell into 6 homogeneous groups (Table 3). Statistical processing of the weights of plants grown on Pellic Vertisol arranges them into 4 homogeneous groups. The control variants for both soil types remained in a separate group (a) with the lowest weight (Table 4).
Sunflower forms twice as many roots, which contain 1.5 times more nitrogen and 5 times more potassium, compared to other spring crops (Nikolova, 2010; Saldzhiev, 2004).
The highest result was reported for plant biomass on Pellic Vertisol in variant 11 ($N_{300}P_{240}K_{70}Si_{400}$) - 115.24 g, at levels of N_{300} and Si_{400} in the soil. These levels were optimal for this soil because in variant 12 ($N_{300}P_{80}K_{210}Si_{400}$) plant weights were also high – 110.19 g. For plants grown on Eutric Fluvisol, the highest weight was obtained in variant 8 ($N_{200}P_{160}K_{140}Si_{0}$) - 100.49 g, it was in a separate group (f), where silicon is excluded, but N_{200}, P_{160} and K_{140} were in optimal amounts. The other variants were arranged in intermediate groups.

Table 4. Influence of fertilization rate on the weight of fresh biomass from the aboveground part and roots of sunflower plants (One-way analysis)

Variants	Eutric Fluvisol		Pellic Vertisol	
	F.W. of plants (g) 67 day	F.W. of roots (g) 67 day	F.W. of plants (g) 67 day	F.W. of roots (g) 67 day
$N_0P_0K_0Si_0$	47.14^{a}	1.99^{a}	29.49^{a}	1.05^{a}
$N_0P_{160}K_{140}Si_{800}$	83.03^{bcdef}	7.78^{bcd}	50.44^{ab}	4.29^{abc}
$N_{400}P_{160}K_{140}Si_{800}$	91.96^{cdef}	5.00^{abcd}	109.42^{cd}	5.16^{abc}
$N_{200}P_0K_{140}Si_{800}$	93.51^{def}	6.53^{abcd}	89.24^{cd}	2.41^{ab}
$N_{200}P_{320}K_{140}Si_{800}$	77.21^{bcde}	2.96^{ab}	108.64^{cd}	8.14^{cdef}
$N_{200}P_{160}K_0Si_{800}$	86.14^{bcdef}	4.03^{abc}	94.67^{cd}	5.11^{abc}
$N_{200}P_{160}K_{280}Si_{800}$	75.66^{bcd}	9.08^{cd}	90.79^{cd}	5.47^{abc}
$N_{200}P_{160}K_{140}Si_0$	100.49^{f}	9.30^{d}	98.94^{cd}	6.02^{abcd}
$N_{200}P_{160}K_{140}Si_{2000}$	81.09^{bcdef}	4.02^{abc}	97.39^{cd}	7.64^{cdef}
$N_{200}P_{160}K_{140}Si_{800}$	69.45^{b}	4.16^{abcd}	97.39^{cd}	6.30^{bcd}
$N_{300}P_{240}K_{70}Si_{400}$	86.14^{bcdef}	4.92^{abcd}	115.24^{d}	11.73^{ef}
$N_{300}P_{80}K_{210}Si_{400}$	98.16^{ef}	4.68^{abcd}	110.19^{cd}	10.60^{def}
$N_{300}P_{80}K_{70}Si_{1200}$	88.85^{bcdef}	5.82^{abcd}	100.1^{cd}	6.69^{bcde}
$N_{100}P_{240}K_{210}Si_{400}$	72.94^{bc}	4.56^{abcd}	80.7^{bc}	7.53^{bcdef}
$N_{100}P_{240}K_{70}Si_{1200}$	88.08^{bcdef}	6.77^{abcd}	101.66^{cd}	12.63^{f}
$N_{100}P_{80}K_{210}Si_{1200}$	80.32^{bcdef}	4.35^{abcd}	76.44^{bc}	11.03^{def}

F.W.= Fresh Weight; Values are means of 3 replicates. The different letters, symbolize statistically significant differences among the 3 treatments, according to the Duncan’s multiple range test, for $p \leq 0.05$.

Contrary to expectations, the tallest plants in both soil types did not have the highest weights. This is again related to the different amounts of mineral fertilizers introduced into the soil. For example, variant 6 ($N_{200}P_{160}K_{0}Si_{800}$) in soil type Eutric Fluvisol, in which the 118 cm tall plants (one of the largest) had a lower weight, which may be due to deficiency of potassium. As vegetation progresses, plants in both soil types grew better in variants 3 ($N_{400}P_{160}K_{140}Si_{800}$), 4 ($N_{200}P_{0}K_{140}Si_{800}$) and 12 ($N_{300}P_{80}K_{210}Si_{400}$). Obviously, the need for more nitrogen at a later stage of sunflower development is better met than the norm of 300 mg N/ pot, this corresponds to the results of other authors (Ahmad et al., 2017).

Conclusions

Optimal vegetative development is achieved with the variants - $N_{200}P_{0}K_{140}Si_{800}$ and $N_{300}P_{80}K_{210}Si_{400}$ for Eutric Fluvisol. In the other type of soil - Pellic Vertisol variants - $N_{400}P_{160}K_{140}Si_{800}$ and $N_{200}P_{0}K_{140}Si_{800}$, were those in which plant development is more favorably affected by the applied fertilization.

The opposite was the case with the highest Si norms in Eutric Fluvisol soil, for example variant 9 ($N_{200}P_{160}K_{140}Si_{2000}$) with the highest Si level, in which plant heights were among the lowest - only 106 cm. While with Pellic Vertisol the highest plants were reported in this variant, which is due to the improvement of water, physical and chemical properties of this type of soil.

The analysis confirms that the norm of 200 mg N/ pot, 140 mg K/ pot in combination with 800 mg Si / pot in both soil types, most stimulate the growth of sunflower.

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SIGNIFICANCE OF FERTILIZATION FOR WINTER WHEAT DEVELOPMENT AND YIELD IN A FIELD EXPERIMENT ON ALLUVIAL MEADOW SOIL

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Abstract

The main objective of the study was to determine the influence of the main macro-elements: nitrogen (N), phosphorus (P), potassium (K) and silicon (Si) on the development of winter wheat, “Sadovo 1” variety and their optimal values in the conditions of field experiment on alluvial-meadow soil on the experimental field in Tsalapica, Plovdiv district. The distribution of the variants by plant height within the 175 days was in 6 homogeneous classes, while with increasing the vegetative development of wheat the differences between the variants decreased. Thus, within the 195 days there were 5 homogeneous classes, and when harvesting wheat on the 241 day, only 3 groups. All fertilizer variants felt into homogeneous class B and were significantly different from the control. The results show that the yield was influenced by the element potassium and the phosphorus-potassium interaction, while the interaction between nitrogen and silicon had practically no significant effect. The lowest yields were in the control variant, V9, homogeneous group **a,** and they were statistically different from all the other variants, while the highest yields were obtained in V7 ($N_{100}P_{160}K_{120}Si_{28}$), followed by V3 ($N_{100}P_{160}K_{60}Si_{28}$) and V4 ($N_{200}P_{160}K_{60}Si_{14}$). The increase in yields in the variants with fertilization relative to the control was high and ranged between 56 and 71%. According to the obtained data, the smaller norm of nitrogen (N100) was more efficient than N200 in the conditions of the 2021 year. The regularities established by the statistical processing of plant height during the wheat growing season were poorly correlated with grain yield data depending on the treatments of fertilization. Fertilization with SiO_2 did not prove to be very efficient in increasing plants yields, probably due to physico-chemical interactions with soil components and lack of enough time for dissolution and translocation in the test plants.

Key words: *macro-elements fertilization, field experiment, winter wheat, alluvial-meadow soil.*

Introduction

Winter wheat production in 2020 amounts to 4 711 000 tonnes decrease of 23.5% compared to 2019 due to adverse climatic conditions in the crop development process, which led to a decrease in average yield (by 23.7%). The areas sown with winter wheat in 2020 were 1 207 994 ha – 0.5% more on an annual basis, of which 1 200 175 ha were harvested. The relative share of harvested areas with wheat from total harvested areas with cereals was 61 %. The largest share of wheat areas is the North-East region – 22.2%, followed by northwestern with 22%. (Agrarian Report, 2021).

The wheat harvest in Bulgaria is increasing by over 50% in 2021 compared to 2020, crossing the border of 7.1 million tonnes of production. This is the best level in the last five years (Vateva, 2021.) Our agriculture is characterized by a long restructuring process aimed at achieving highly efficient and environmentally friendly soil management and ensuring the food security of the population. This process shall be supported by the rules and requirements of direct payment schemes and transitional national aid linked to compliance with commitments to implement good agricultural practices. According to data from a survey of the Agrostatistics Department in 2020, the wheat cultivation areas after wheat are 6.5% and for 2019 this value is 7.5%. Nearly 74.3% of the areas with wheat were sown after trenched crops and this value is 67.8% for the previous year. Another good indicator is that 1.7% of fallow is included in wheat crop rotation in 2020.

Management in agriculture is the management of human activity not only for the conservation and improvement of the natural environment, but also for the development of efficient production. The achievement of this objective is directly linked to ensuring the optimal diet of agricultural crops. Here comes the role of agrochemistry and fertilization It must take into account the need to preserve the ecological equilibrium of the soil created during the soil process and be consistent with the physiological needs of the crop during the different phases of its development. This will be achieved with well-founded optimised fertilization.

In the literature there is accumulated data on quantity, quality and components of cereal yield; growth, development and parameters of crops; reutilization of carbon, nitrogen and phosphorus elements; efficiency of nitrogen use by industrial mineral fertilizers. There are studies giving a detailed description of the many wheat varieties studied (Vasileva and Ur, 2014; Bocharnikova, 2012; Uhr et al., 2021, Davies, et al. 2020, Valeva & Stamenov, 2017). The effectiveness of the essential nutrients imported depends not only on the amount of individual elements, but also on the complex interaction between them. Silicon is widespread in soils, but its use as fertilizer is not studied in our country. It’s use can contribute to increasing the quantity and quality of yields, as well as increasing the resistance of wheat to adverse climatic conditions, diseases and enemies (Bocharnikova, 2012).

This study aims to determine the impact of the main macroelements: nitrogen (N), phosphorus (P), potassium (K) and silicon (Si) and soil type on wheat development and their optimal values in field experiment on Alluvial-meadow soil of the experimental field in Tsalapiza, Plovdiv region.

Materials and Methods

On 17.11.2020 a field experiment with winter wheat, variety "Sadovo 1", was set up at the ISSAPP "N. Pushkarov" station in Tsalapiza, Plovdiv region. The imported quantities of mineral fertilizers with the main tilling of the soil are triple superphosphate (46 % P), potassium sulphate (45 % K) and *diatomaceous earth* (92 % SiO_2) and also 1/3 of the dose ammonium nitrate (34 % N). Fertilization rates are presented in Table 1. (Table 1). On 8 March 2021, spring feeding of wheat with the remaining quantity (2/3 of the dose) of ammonium nitrate took place. The experiment includes 8 fertilization variants and one control variant in 3 repetitions.

Table 1. Active substance in kg/ha

Variants	Factors			
№	N	P	K	Si
1	100	80	60	14
2	200	80	60	28
3	100	160	60	28

4	200	160	60	14
5	100	80	120	14
6	200	80	120	28
7	100	160	120	28
8	200	160	120	14
9	0	0	0	0

The soil type is Aluvial-meadow soil, according to the soil classification in Bulgaria (Koinov, 1987) and defined as Eutric Fluvisol (FAO, 2015). It is characterized by low content of total nitrogen and organic matter, with a slightly acidic to neutral reaction in the surface soil layer, with low cation exchange capacity in the arable horizon and average values in the sub-soil layer (Koleva et al., 2001). The analyses were done by the following methods: Humus - by oxidation during heating (Konova, 1963); pH-potentiometrically in H_2O and KCl (Arinushkina, 1962); Mineral N by Bremner and Kiney method (Bremner,1965a; Bremner,1965b); mobile forms of phosphorus and potassium (P_2O_5 and K_2O), by the acetate method (Ivanov, 1986). The agrochemical characteristics of the soil are presented in Table 2.

Table 2. Agrochemical characteristics of Alluvial-meadow soil from an experimental field in Tsalapitsa

Site	pH		∑ $N\text{-}NH_4+NO_3$	Total N	P_2O_5	K_2O	humus
	H_2O	KCl	mg/kg	%	mg/100g		%
0-30 cm	7,4	6,8	11,52	0,056	8,09	14,35	1,16
30-60cm	7,3	6,4	16,70	0,061	5,91	15,35	1,20

Data on plant height in cm at 175, 215 and yield on the 241-th day since the beginning of the trial are presented in Table 3 and Figure 1. They were analysed using the analysis of variance method (Statgraphics, 2018), following the procedure of (Sadovski, 2021). In order to identify the differences between the variants, a Tukey test and the least statistical differences test (LSD) were used, respectively, at 0,05 (5%) and 0,01 (1%) significance level.

Results and Discussion

The phenological observations carried out during wheat vegetation and the experimental data obtained on plant height and grain yields at harvesting are presented in Table 3.

As of 11.05.2021, at the first deadline for measuring the height of plants, there were quite high differences between variants. A single-factor analysis of variance found a proven difference between the variants at a confidence level $p \leq 0,05$ and their distribution in 6 homogeneous classes – **a, b, c, d, e** and **f** (Table 3) within the first 175 days. For the variants, which are in separate homogeneous classes, the LSD = 10.32 at a confidence level of 95%). With the lowest values of plant height were the variants V9 (control), 38.5 cm, forming a homogeneous group **a**. Similar results for the development of wheat and yields from unfertilized experimental parcels are obtained by other authors (Tsenov et al, 2011, Tsenov, N.& D. Atanasova, 2013, Nenov et al, 2020). The highest value was achieved for the variant B8, 105 cm, falling into a homogeneous class **e**. This was the variant with imported high nitrogen rate (N_{200}), the high doses of P_{160} and K_{120} and the low dose of silicon (Si_{14}) in the experiment. Statistically different from the others were the plants in the variant **5** ($N_{100}P_{80}K_{120}Si_{14}$), forming a homogeneous group **b**, 85 cm. All the other variants (V1, V2, V3, V6 and V7) were placed in intermediate homogeneous classes **cde**, **de**, **bc**, **bcd** and **bcde**, and the differences between them are not statistically different (Figure 1).

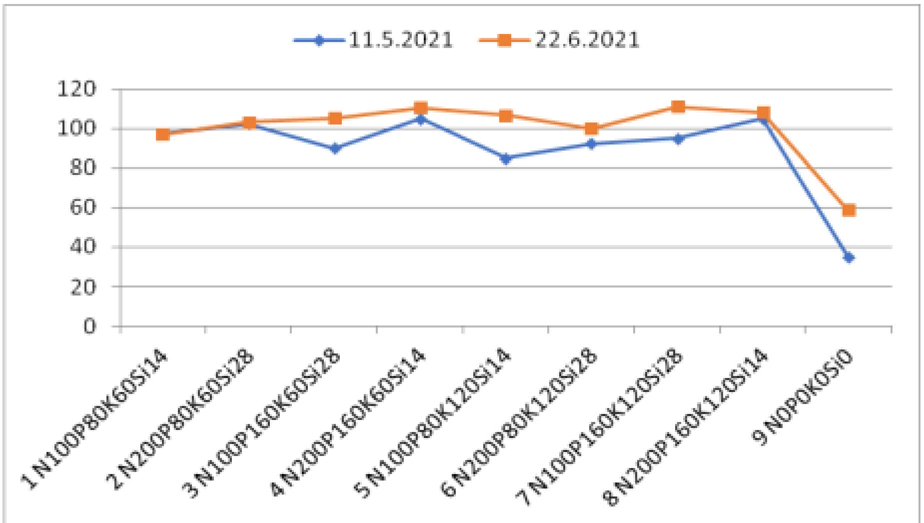

Figure 1. Plant’s height (cm) during the vegetation of wheat, “Sadovo 1” variety in the field experiment with Alluvial-meadow soil, Tsalapitsa, 2020/2021.

Within the next period, 215 days from the beginning of the trial, some similar interactions were established, a total of 5 homogeneous groups. The variants, in separate homogeneous classes, which were statistically different in this period are 4 (LSD = 8.96). These were the control variant, group a with the lowest values of 59 cm and the variant V1, group b, 97cm. V4 and V7 with an average plant height of 110.6 and 111.1 respectively form a homogeneous group d. The remaining 5 variants (V2 and V3, B5 and V8, V6) fell into 3 intermediate groups, bcd, cd and bc, respectively. It was also clearly seen from Figure 1 that the differences in plant heights between variants within this period decreased. This trend of reducing disparities was also confirmed by data on grain yields at harvest, 07.07.2021 (Table 3).

In the last period, 241 days the data for wheat yield formed only 3 homogeneous classes with LSD = 3109.6 between different variants at a confidence level of 95%. The lowest it was expected were the yields in the control variant, B9, homogeneous group a and they were statistically different from all the other variants, except B6 falling into intermediate group ab. The regularities established by the statistical processing of plant height during the wheat growing season were poorly correlated with grain yield data depending on the treatments of fertilization. The increase in yields in fertilized variants relative to the control was high and ranged from 56 to 71% and it was evident from table 3 that the smaller norm of nitrogen (N100) was more efficient than N200. Wheat yields are significantly affected by both the level of agrotechnics including the norms of nitrogen fertilization and the amount of rainfall that has fallen. Probably insufficient rainfall during the active spring vegetation of the 2021 year (from the beginning of the stem elongation stage to full maturity (Z30-Z99) contributes to better work of norm N100 than N200 in the alluvial meadow soil.

Table 3. Effect of fertilization on the height and yield of wheat, "Sadovo 1" variety during the vegetation in the experimental station Tsalapitsa, 2020/2021

Variants	Height of plants, cm		Yield, kg.ha^{-1}	Increase of yield, %
	11.05.2021	22.06.2021	07.07.2021	
1 $N_{100}P_{80}K_{60}Si_{14}$	97.5 cde	97.0 b	6688.79 b	64.55
2 $N_{200}P_{80}K_{60}Si_{28}$	102.5 de	103.3 bcd	7150.47 b	66.84
3 $N_{100}P_{160}K_{60}Si_{28}$	90.0 bc	105.3 bcd	7367.13 b	67.82
4 $N_{200}P_{160}K_{60}Si_{14}$	105.0 e	110.6 d	6865.16 b	65.47
5 $N_{100}P_{80}K_{120}Si_{14}$	85.0 b	106.8 cd	6392.98 b	62.91
6 $N_{200}P_{80}K_{120}Si_{28}$	92.5 bcd	99.9 bc	5374.3 ab	55.89
7 $N_{100}P_{160}K_{120}Si_{28}$	95.0 bcde	111.1 d	8157.09 b	70.94
8 $N_{200}P_{160}K_{120}Si_{14}$	105.0 e	108.3 cd	7566.82 b	68.67
9 $N_0P_0K_0Si_0$	35.0 a	59.0 a	2370.85 a	
Average	89.72	100.14	6437.06	
Std. dev.	21.25	18.05	1941.02	
Std. error	3.22	3.19	972.0	
LSD ≥ 95%	10.32	8.96	3109.6	

A regression analysis of wheat yield data was carried out and the following multiple regression model was used, which included the four macroelements and three two-factor interactions. The results of the analysis are presented in Table 4.

$$Y = b_0 + b_1 N + b_2 P + b_3 K + b_4 Si + b_5 NP + b_6 PK + b_7 KSi$$

Table 4. Regression analysis of wheat yield

Coef.	b	st. err.	t	p
b 0	434.870	75.7483	5.7410	0.0002
b1 N	6.355	10.1627	0.6253	0.5458
b2 P	2.945	17.8042	0.1654	0.8719
b3 K	45.059	23.7389	1.8981	0.0869
b4 Si	-133.103	101.7380	-1.3083	0.2200
b5 NP	0.577	1.1513	0.5008	0.6274
b6 PK	-3.098	1.5351	-2.0184	0.0712
b7 KSi	8.230	6.5791	1.2510	0.2394

The results showed that the element potassium (K) and phosphorus-potassium (PK) interaction had a significant effect on yield, while interaction nitrogen and silicon had virtually no significant effect on yield. Silicon fertilization of plants has been shown to improve N use efficiency and agronomic parameters of crops (Pavlovic et al., 2021). In our study silicon fertilization with SiO_2 did not prove to be very efficient in increasing plants yields, probably due to physico-chemical interactions with soil components and lack of enough time for dissolution and translocation in the test plants.

Conclusion

As a result of the field experiment on alluvial-meadow soil with “Sadovo 1” Bulgarian variety of winter wheat, and the statistical analysis on the data on plant height within the 175 and 195 days and for the grain yields within 241 days from the beginning of the trial, the following results were obtained:

The distribution of the variants by plant height within the 175 days is in 6 homogeneous classes, while with increasing the vegetative development of wheat the differences between the variants decreased. Thus, within the 195 days there are 5 homogeneous classes, and when harvesting wheat on the 241 day, only 3 groups.

The lowest yields were in the control variant, V9, homogeneous group **a,** and they were statistically different from all the other variants, while the highest yields were obtained in V7 ($N_{100}P_{160}K_{120}Si_{28}$), followed by V3 ($N_{100}P_{160}K_{60}Si_{28}$) and V4 ($N_{200}P_{160}K_{60}Si_{14}$).

All fertilized variants fell into homogeneous class **b** and were statistically different from the control. There was only one intermediate group of **ab**, V6 ($N_{200}P_{80}K_{120}Si_{28}$) fell. The increase in yields in the variants with fertilization relative to the control was high and ranged between 56 and 71%. According to the obtained data, the smaller norm of nitrogen (N100) was more efficient than N200 in the conditions of the 2021 year. The multiple regression shows that the highest effect on yield has the element potassium and the interaction between phosphorus-potassium, while nitrogen and silicon have practically no significant effect.

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THE POTENTIAL OF LED ILLUMINATION ON THE YIELD, MORPHOLOGICAL PROPERTIES AND COLORATION OF BROCCOLI MICROGREENS

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Abstract

As microgreens are increasingly grown as functional food, their cultivation technologies require optimization of factors such as temperature, light and humidity. Quality (wavelength) and photoperiod (duration) are the most important light factors that directly affect yield and morphological properties of plants. Photosynthetically active radiation (PAR) includes wavelengths in the range of 400-800 nm, whereby specific photoreceptors of plant cells absorb blue (400-500 nm), red (600-700 nm) and dark red (700-800 nm) wavelengths. The aim of this research was to determine the effect of supplemental LED's lighting with blue (450 nm), red (620 nm) and combination of blue and red spectrum (50:50) in the photoperiod of 14 h on the yield, morphological properties, pigments and colour content of broccoli microgreens. The research was conducted in a climate chamber with controlled conditions for plant cultivation (25 °C, 60% relative air humidity). Samples of broccoli microgreens were manually cut at the base of the hypocotyl in the cotyledon phenophase at a height of 7.5 cm after eight days. Microgreens of broccoli grown under LED supplementary lighting of blue spectrum and combination of blue and red spectrum showed higher yields (1198 g/m^2) than samples grown under red spectrum (1046 g/m^2). Broccoli microgreens grown under red spectrum had a longer hypocotil (69.7 mm) than samples grown under blue and combined spectrum. Samples grown under blue light treatment had larger leaf lenght (9 mm) than samples grown under red light treatment and combined light treatment. Most clorophylls were acumulated under blue light (0.920 mg/l) as well as carotenoids (0.410 mg/l) and most colors were accumulated under combined light. Based on the results, it can be emphasised that growing microgreens under supplemental lighting (LED) has a positive effect on morphological parameters, yield and pigment content.

Keywords: *Brassica oleracea* L. *var. italica* Plenck, *supplemental lighting, microgreens morphology, yield, clorophyll content.*

Introduction

Key factors in plant cultivation in protected areas that need to be optimized for adequate growth and development are temperature, light and humidity. Light is a limiting factor for cultivation that affects germination, growth, morphological changes and synthesis of specialized metabolites in plant (Vaštakaite et al., 2015; Lobiuc et al., 2017). The effects of light on plant growth and development depend on the wavelengths of the light spectrum that plant absorbs. Wavelength, radiation intensity and photoperiod are the most important factors of light that affect yield and morphological properties of plants. Photosynthetically active radiation (PAR) includes wavelengths in the range of 400-800 nm. Plant cells absorb ultraviolet (UV-A) (280-400 nm), blue (400-500 nm), red (600-700 nm) and dark red (700-800 nm), wavelengths (Hasan et al., 2017; Zhang et al., 2020). Lamp types usually used as additional lightning in greenhouses and climate chambers are high-pressure sodium lamps (HPS), metal halide lamps (MH), xenon and fluorescent lamps (Mitchell et al., 2012). Due to numerous shortcomings of these light sources as supplementary lighting, the usage of light emitting diodes (LED) is increasing due to a number of advantages, from overheating, high energy efficiency and durability (Zhang et al., 2019, Ma et al., 2021) to a number other technical advantages (Margit and Viršile, 2013; Turner et al., 2020). Radiation intensity and photoperiod of LED's are the most important factors that need to be optimized in plant cultivation in controlled conditions (Negri et al., 2021). The additional illumination with LED's has an positive effect on the morphological characteristics of plants. The combination of red and blue wavelengths stimulates growth (increases yield) and positively affect on the synthesis of specialized metabolites. Microgreens are incompletely developed plants consumed in the cotyledon phenophase with or without the first true leaves (Di Gioia and Santamaria, 2015; Opačić et al., 2020). Microgreens develop from vegetable seeds, herbs, cereals and wild plant of many plant families: *Brassicaceae, Asteraceae, Chenopodiaceae,...* (Kyriacou et al., 2016). They are harvested and consumed fresh 7-14 days after germination (Xiao et al., 2015; Turner et al., 2020). Microgreens are specific and popular for cultivation because of simple and short cultivation cycle (Ebert et al., 2012), easy germination (Kyriacou, 2016) and can be grown outdoors or in greenhouses and climate chambers with additional lighting (Brazaitytė et al., 2015). Because of easy and successful cultivation in ecological conditions that do not require large cultivation aeras, can be easily produced in urban i suburban aeras where the land is limiting factor (van Iersel et al., 2017). Another significant advantage of their cultivation is short growth cycle without substrate, fertilizers and plant protection products. The aim of this research was to determine the effect of different supplemental LED's lighting in the photoperiod of 14 h on the yield, morphological properties, pigments and colour content of broccoli microgreens.

Material and Methods

The research was conducted in climate chamber of the company Vesela motika in Zagreb, Croatia. The experiment was placed in climate chamber under controlled conditions of temperature 25 ˚C and 60% relative humidity in three repetitions under supplementary lighting by LED's. Tempereture deviations were 25 ± 2° C and relative humidity deviations were 60 ± 5%. Plants were treated with three different spectra of supplementary illumination blue (450 nm), red (620 nm) and combination of red and blue spectrum of wavelenghts in the ratio (50:50) in the photoperiod of 14 hours.

Broccoli microgreens seeds (Lokvina - Varaždin, Croatia) were sown in plastic PVC trays dimensions 0,37 x 0,23 x 0,05 m (0.0851 m^2) in an amount of 10 g per tray without prior treatment. After germination, trays were placed on shelves away 31 cm from LED's and regulary irrigated with no added nutrients.

Yield was measured on a biomass weighing scale and the lenght of the hypocotyl and cotyledon was manually measured by taking a representative sample from each repetition. Chemical analyzes of pigment content and colour content were conducted in laboratory of Department of Agricultural Technology, Storage and Transport at Faculty of Agriculture in Zagreb. For plant pigments, clorophyll a, clorophyll b, total clorophyll and carotenoids 50 g of representative

samples was taken per repetition and determinated by the method according to Holm (1954) and Wetsttein (1957). Colors were determined from the same samples with colorimeter PCE-CSM 4 (PCE Instruments GmbH, Germany).
Statistical analysis was conducted by using analysis of variance (ANOVA). Mean values were compared by the test (LSD) at significance level 5%. For the statistical analysis procedures PROC GLM in SAS® Software version 9.3 (2010) was used. Different letters in tables indicate significant differences between mean values within each column.

Results and discussion

Results of the morphological parameters as well the yield of broccoli microgreens are shown in Table 1. Broccoli microgreens in the red spectrum achieved hypocotyl length of 69.7 mm, while treatments grown in blue light achieved lenght of 68 mm, whileunder combined light 67.3 mm. According to Jones-Baumgardt et al. (2020) elongated hypocotyls are an important attribute of growth for many commercial microgreens growers because plants facilitate mechanical harvesting process, but can reduce robustness of harvested tissues. Blue spectrum of supplemetnary light has an effect on the size of the leaf, e.i. lenght of microgreens cotyledons. According to Jamal Uddin et al. (2017) blue LED light exposure causes leaf expansion in plants like lettuce and these may have attributed to the higher fresh weight of leaves compared to others. Also blue light enhances moisture absorption in plants that might also contribute to it. Broccoli mocrogreens treatment under blue spectrum has a cotyledon length of 9 mm, while red light spectrum treatment has a cotyledon lenght 5.7 mm and combined light spectrum treatment 7.3 mm. Yield is the most important in plant production as a functional food and producers are trying to get the highest and quality yield. One of the main factors that affect yield is light. In climate chamber when choosing a source of additional lighting it is important to choose light source and combination of wavelenghts that will give the highest yield. In this experiment highest yield achieved treatment under combined wavelenghts and blue wavelenghts (1198 g/m^2). The lowest yield was achieved by the treatment of red wavelenghts (1046 g/m^2). Many reports demonstrated the positive influence of the red:blue light on plant growth and photosynthesis (Kamal et al., 2020). In case of no. of leaves, length of stem, diameter of stem and fresh weight of leaves blue LED supplementation treatment showed superiority to others (Jamal Uddin et al., 2017).
Pigment content is shown in Table 2. Samples under blue illumination acumulated the most chlorophyll a (0.613 mg/l), chlorophyll b (0.307 mg/l), total chlorophyll (0.920 mg/l) and carotenoids (0.410 mg/l). According to Kamal (2020) it is reported that broccoli microgreens grown under blue light produced higher and more nutrient dense microgreens. Least chlorophyll a (0.527 mg/l) and carotenoids (0.347 mg/l) accumulated under red light. Studies conducted in Kennedy space center also support this finding and showed that in absence of blue light wheat seedlings failed chlorophyll synthesis (Jamal Uddin et al., 2017). Chlorophyll b (0.263 mg/l) and total chlorrophyll (0.810 mg/l) accumulated least under combined light.
Color content accumulated the most under combined lighting, but did not represent a significat difference compared to other samples (Table 3). Plant pigments have important role in removig free radicals, i.e. they have significant antioxidant properties. Due to its significant antioxidant activity, plant pigments show numerous beneficial affects on human health. Achieving controlled conditions in climate chamber for plant cultivation and optimized wavelenght of the supplementary light source and photoperiod there's obtained high-yield products with a high

level of specialized metabolites which due to their antioxidant properties are promoted as a functional food important for human health.

Table 1. Yeld and morphological properties (average ± standard deviation) of broccoli microgreens grown under three different types of supplementary LED lighting.

LED light	Yield, g/m^2	Hypocotyl length, mm	Cotyledon length, mm
Blue	1198^{a} ± 20.21	68.0 ± 2.00	9.0^{a} ± 0.00
Blue-red	1199^{a} ± 31.18	67.3 ± 0.58	7.3^{b} ± 0.58
Red	1046^{b} ± 31,18	69.7 ± 0.58	5.7^{c} ± 0.58
LSD $_{p=0.05}$	74.65	2.927 n.s.	1.308

Mean values followed by the same letter within each column do not differ significantly according to LSD test ($p \leq 0.05$)

Table 2. Pigment content (mg/l) of broccoli microgreens (average ± standard deviation) grown under three different types of supplementary LED lighting.

LED light	Chlorophyll A	Chlorophyll B	Total chlorophyll	Carotenoids
Blue	0.613^{a} ± 0.012	0.307^{a} ± 0.023	0.920^{a} ± 0.038	0.410^{a} ± 0.010
Blue-red	0.547^{b} ± 0.006	0.263^{b} ± 0.021	0.810^{b} ± 0.031	0.373^{b} ± 0.006
Red	0.527^{b} ± 0.015	0.287^{a} ± 0.023	0.814^{b} ± 0.038	0.347^{c} ± 0.006
LSD $_{p=0.05}$	0.0227	0.0231	0.0228	0.0227

Mean values followed by the same letter within each column do not differ significantly according to LSD test ($p \leq 0.05$)

Table 3. Color content (average ± standard deviation) of broccoli microgreens grown under three different types of supplementary LED lighting.

LED light	L	a	b	C	h
Blue	43.234 ± 6.44	-8.957^{b} ± 1.72	20.986 ± 2.90	22.821 ± 3.33	113.001^{ab} ± 1.31
Blue-red	38.784 ± 2.15	-9.725^{b} ± 1.28	20.800 ±1.40	22.976 ± 1.61	115.028^{a} ± 2.56
Red	45.286 ± 0.89	-7.549^{a} ± 2.37	18.657 ± 3.67	20.142 ± 4.26	111.661^{b} ± 2.89
LSD $_{p=0.05}$	8.334 n.s.	1.381	4.711 n.s.	4.781 n.s.	2.539

Mean values followed by the same letter within each column do not differ significantly according to LSD test ($p \leq 0.05$)

Conclusion

As microgreens are increasingly grown as functional food, it is necessary to optimize key factors for their cultivation in order to obtain quality products. Supplemental lighting by LED's is a new technology for growing microgreens in greenhouses and climate chambers. LED's emitt photosynthetically active wavelenghts which affect on nutrient composition, but also on the morphological characteristic of microgreens. The combination of red and blue wavelenghts stimulates growth (increases yield), positive affects the lenght of hypocotyl, size of cotyledon and pigment and color composition of broccoli microgreens. Pigments and coloration play important role in the nutritional composition of microgreens as antioxidants that are important for human health. This results defined the conditions, quality and photoperiod to grow microgreens of broccoli rich in nutrients with adequate yield by increasing biomass. The goal of every cultivation is to get a quality nutritionally rich product in the highest possible yield that can be obtained by optimizing the factors for cultivation.

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EVALUATION OF A MEDICINAL PLANT *ECHINACEA PURPUREA* IN TERMS OF STEROID AND TRITERPENOID CONTENT

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Abstract

Purple coneflower (*Echinacea purpurea* (L.) Moench) is a widely known medicinal plant applied in herbal medicine. The phytochemical characterization of *E. purpurea* includes mainly the content of alkamines, phenolics, sesquiterpenoids or saponins; meanwhile, the composition of other groups of bioactive constituents, i.e., steroids and triterpenoids occurring in this plant in a free (not conjugated) form has been less studied. Therefore, the aim of the present work was the analysis of steroids and triterpenoids in the aerial parts (inflorescences and leaves) of *E. purpurea*. Diethyl ether extracts were obtained from dried and powdered samples of *E. purpurea* leaves and two separate parts of the inflorescence heads, i.e., external ray (ligulate) florets, and the internal receptacles containing disc (tubular) florets. Analysis was made by gas chromatography-mass spectrometry method (GC-MS). Apart from the typical sterols (cholesterol, campesterol, sitosterol, stigmasterol), other compounds as steroid ketones (sitostenone, tremulone, stigmastanedione) and biosynthetic intermediates of steroid pathway (cycloartanol, cycloeucalenol, 24-methylenocycloartanol) were identified. Leaves and ligulate flowers were particularly rich in steroids (approx. 0.97 and 0.95 mg/g of dry weight, respectively), however, the quantitative profile of individual compounds differed significantly, e.g., sitosterol followed by stigmasterol were the most abundant in leaves, whereas cycloeucalenol and cycloartanol were predominant in ligulate flowers. In comparison to leaves and ligulate flowers, the steroid content of tubular florets was lower (0.63 mg/g d.w.) and the composition of these compounds was relatively simpler. Triterpenoids were minor compounds in *E. purpurea* extracts, oleanane- and ursane-type alcohols and acids were found mainly in leaf extracts.

Keywords: *Echinacea purpurea, purple coneflower, steroids, triterpenoids.*

Introduction

Echinacea purpurea (L.) Moench (synonyms: purple coneflower, hedgehog coneflower, echinacea) is a North American species of herbaceous perennial plant in the Asteraceae family. Its natural habitats include dry open woods, prairies and barrens. It blooms throughout summer and autumn, depending on the local climate, forming flowering heads (capitulum) with the prominent domed central protuberance, consisting of multiple small yellow tubular florets, surrounded by a ring of pink or purple ligulate florets (Flagel *et al.*, 2008). *E. purpurea* is considered one of the most known, used and cultivated medicinal plants in the world (Manayi *et al.*, 2015; Coelho *et al.*, 2020). Herbal medicines derived from several species of the *Echinacea* genus available in North America were in use by indigenous human populations long before the introduction of European medicines, primarily as treatments for various infectious diseases and wounds (including snake bites), and also for anti-inflammatory, antioxidant and antitumor properties (Hudson, 2012). Currently, the immunomodulatory effects of *E. purpurea* are of primary concern for research, moreover, *E. purpurea* products have been licensed in Europe as agents healing infections of the upper respiratory tract (Burlou-Nagy *et al.*, 2022). Some *E. purpurea* preparations were also shown to exert antiviral, antifungal, antimicrobial, anti-inflammatory, antioxidant and psychoactive activities. The most popular preparations are infusions and tinctures available on the market in fluid forms, as well as capsules containing dried *E. purpurea.* There are also preparations for use as a topical treatment for skin and wound inflammation. As a consequence, the cultivation of *E. purpurea* as a medicinal herb crop for commerce has become economically profitable, since preparations containing this plant are among the best-selling herbal medications in Europe and the United States (Hudson, 2012; Burlou-Nagy *et al.*, 2022).

E. purpurea is considered a rich source of various bioactive phytochemicals, but only a tiny portion of them have been extensively studied (Coelho *et al.*, 2020; Burlou-Nagy *et al.*, 2022). According to numerous phytochemical reports, the most common natural compounds found in *E. purpurea* are alkamides, polysaccharides, lipoproteins, betaine, polyacetylene, sesquiterpenoids, triterpenoid saponins and phenolic compounds, e.g., flavonoids, chicoric acid, caffeic acid (Manayi *et al*., 2015; Coelho *et al.*, 2020; Burlou-Nagy *et al.*, 2022). However, due to the multiple activities of *E. purpurea* preparations, it is difficult to unambiguously identify all bioactive compounds that may contribute to exerted medicinal benefits. The “marker compounds” applied for standardization are usually caffeic acid derivatives, alkamines and polysaccharides. Nevertheless, the chemical composition differs substantially between such preparations as pressed juice obtained from fresh aerial parts or ethanol tinctures extracted either from dried aerial parts, or roots. Moreover, distinct plant parts differ in composition of the principal phytochemicals, leaves typically contain more flavonoids, whereas roots are rich in alkamines. Thus, such uncertainty in the identity of the principal bioactive compounds can make interpretation of basic and clinical studies difficult. Differences in chemical composition and resulting pharmacological properties can also come from inadequate characterization of *E. purpurea* preparations - derived with various extraction protocols, from different species and plant parts (Hudson, 2012; Coelho *et al*., 2020).

The aim of the present work was the analysis of one of the less characterized group of compounds, i.e., steroids and triterpenoids occurring in a free (not conjugated) form in the aerial parts of *E. purpurea*. Moreover, according to the complex morphology of *E. purpurea* inflorescences, their distinct parts including external ray (ligulate) florets, and the internal receptacles containing disc (tubular) florets, were analyzed separately.

Material and methods

E. purpurea seedlings were purchased from the Polish Vegetable Seed Farming and Nursery enterprise “PNOS” and cultivated in a small private plantation in Stare Bosewo, central Poland (52°460 N, 21°332 E) in an open field as a perennial plant from 2019. Aerial parts of *E. purpurea* plants for the present study were collected in August 2021. Selected not damaged inflorescences and leaves were air-dried in a room temperature. Prior to the extraction, inflorescences were separated into ligulate florets and internal receptacles. Leaves (2.64 g) and ligulate florets (1.79 g) samples were homogenized in a laboratory mortar, whereas the receptacles (7.88 g) were grinded in a laboratory mill. The ground plant material was placed in

thimbles and extracted using a Soxhlet apparatus for 8 h with diethyl ether. The obtained extracts were evaporated to dryness under reduced pressure on a rotary evaporator.
Obtained diethyl ether extracts were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Two fractions were obtained as described earlier (Woźniak *et al*. 2018): free (non-esterified) steroids and triterpenoids, and triterpenoid acids. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, whereas fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane. Nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether, and held at 2 °C for 24 h.
Analyses were performed with the use of an Agilent Technologies 7890A gas chromatograph (GC–MS) (Perlan Technologies). The system was equipped with a 5975C mass selective detector, a G4513A autosampler, and a 30 m × 0.25 mm i.d., 0.25-μm, HP-5MS UI column (Agilent Technologies, Santa Clara, CA, USA). The following temperature program was applied: the start at 160 °C (2 min), an increase to 280 °C at 5 °C/min, and the final temperature of 280 °C held for 44 min. The other employed parameters were as follows: the carrier gas (helium, 1 mL/min), inlet and FID (flame ionization detector) temperature 290 °C; quadrupole temperature 150 °C; ion source temperature 230 °C; EI ionization energy 70 eV; scan range, m/z 33–500; MS transfer line temperature 275 °C; FID gas: hydrogen 30 mL·min−1; air 400 mL/min. Wiley 9th ED. and NIST 2008 Lib. SW Version 2010 were used in GC-MS data analysis. Individual compounds were identified by comparing their mass spectra with library data, and/or their chromatographic mobility and corresponding mass spectra with those of authentic standards. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol (Rogowska *et al*., 2022).

Results and discussion

The diethyl ether extracts were obtained from the dried plant material with yield ranging from 1% to 3%, depending on the plant organ (the highest yield, 3%, was obtained from the internal receptacles; 2% from the ligulate florets, and 1% from the leaves). GC-MS analysis revealed the presence of the group of typical sterols (cholesterol, campesterol, sitosterol, stigmasterol), steroid ketones (sitostenone, tremulone, stigmastanedione) as well as cycloartanol, the saturated form of one of biosynthetic intermediates of steroid pathway, cycloartenol. These compounds were found in all analyzed plant parts. However, in leaves and ligulate flowers also additional steroids were found, i.e., stigmast-7-en-3-ol, cycloeucalenol, and various cycloartanol derivatives: 24-methylenecycloartanol, cycloartanediol and cycloartanol acetate.
The obtained results also revealed that the analyzed plant parts distinctly differed in total content of steroids and quantitative profile of individual compounds. Leaves and ligulate flowers were particularly rich in steroids, with the total content of approx. 0.97 and 0.95 mg/g of dry weight, respectively. However, despite the comparable total quantity of the steroids, the proportion among individual compounds differed significantly between leaves and ligulate flowers. In leaves, sitosterol was predominating (constituting 32% of the total steroid content), followed by

its ketone derivative tremulone (synonym: stigmasta-3,5-dien-7-one, 15%) and stigmasterol (10%). In ligulate flowers, cycloeucalenol was prevailing (27%), followed by cycloartanol (17%) and sitosterol (16%). In turn, the steroid content of the receptacles was lower (0.63 mg/g d.w.) and the quantitative profile resembled that of the leaves, with predominating sitosterol (46%) followed by stigmasterol (16%). Also the total amount of the typical basic sterols, i.e., cholesterol, campesterol, sitosterol, stigmasterol, was similar in leaves (approx. 0.54 mg/g d.w.) and receptacles (0.49 mg/g d.w.), whereas in ligulate flowers it was almost twice lower (0.26 mg/g d.w.).

Triterpenoids were found only in extracts obtained from leaves and ligulate flowers. In the latter, the alcohol of ursane-type carbon skeleton, α-amyrin, was found; whereas in leaves also the triterpenoid ketone, α-amyrenone, and the methyl ester of oleanolic acid (oleanane-type carbon skeleton) were detected. Nevertheless, triterpenoids were minor compounds in *E. purpurea* extracts, their total content was only 0.16 mg/g d.w. in leaves, and 0.06 mg/g d.w. in ligulate flowers. The results of quantitative determination of all identified compounds are presented in Table 1.

Table 1. The content of steroids and triterpenoids in extracts obtained from various aerial parts of *E. purpurea*: leaves, ligulate florets, and the internal receptacles.

Compound	Plant part		
	leaves	ligulate florets	internal receptacles
	Content [µg/g d.w.]		
campesterol	65.05	35.16	55.54
cholesterol	55.58	34.65	44.51
cycloartanol	42.68	165.53	37.99
cycloartanol acetate	22.14	82.01	n.d.
cycloartanediol	10.08	18.25	n.d.
cycloeucalenol	94.90	257.68	n.d.
24-methylenecycloartanol	20.44	39.04	12.56
sitosterol	315.74	152.59	292.53
sitostenone	46.72	33.95	50.79
stigmasterol	100.66	37.03	100.92
stigmast-7-en-3-ol	23.78	32.03	n.d.
tremulone	141.91	63.8	21.31
stigmastenedione	28.89	tr.	11.42
sum of steroids	**968.57**	**951.72**	**627.57**
α-amyrin	64.84	60.37	n.d.
α-amyrenone	20.26	n.d.	n.d.
oleanolic acid	62.22	270.79	22.28
oleanolic acid methyl ester	75.95	n.d.	n.d.
sum of triterpenoids	**223.27**	**331.16**	**22.28**

Abbreviations: d.w., dry weight; tr., traces; n.d., not detected.

The presented results were obtained from the analysis of a sample of *E. purpurea* composed of several randomly selected distinct plants collected from one plantation, therefore, it should be treated as an initial study. Nevertheless, the obtained data complete the existing list of phytochemicals occurring in *E. purpurea*, and provide new insights into their distribution in the plant. The profile of main sterols (cholesterol, campesterol, sitosterol and stigmasterol) has been described previously (Chen *et al*., 2013; Coelho *et al*., 2020), however, the detailed steroid and triterpenoid composition including the differences among the distinct *E. purpurea* plant parts is reported in the present study for the first time. Such features as the prevalence of cycloeucalenol and cycloartanol in the extract obtained from ligulate flowers are rare and rather unique, therefore, after their confirmation in further studies on more variable and statistically larger samples, they might be proposed as valuable metabolic markers for the evaluation of *E. purpurea* aerial parts used for herbal preparations. It could facilitate the standardization of the extracts for more reliable research, since the proportions among leaves and inflorescences can be significantly changeable in the collected plant material, influencing the composition of the obtained extracts and their pharmacological properties.

It was reported previously that non-polar extracts from *E. purpurea* plant, including those obtained with the use of acetone or even n-hexane, exerted such effects as antimicrobial properties or cytotoxic activity against some human cancer cell lines (Coelho *et al*., 2020). Although it is not likely that the compounds described in the present study are solely responsible for *E. purpurea* pharmacological properties, they constitute a substantial portion of non-polar extracts, and might play a role in a synergistic action with other bioactive constituents.

Conclusions

This report complements existing data on the steroid and triterpenoid composition in the aerial part of *E. purpurea*, and significantly contributes to phytochemical characterization of this widely applied medicinal plant. Some described features might be proposed as metabolic markers of distinct *E. purpurea* plant parts to facilitate the standardization of extracts.

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EFFECTS OF BIOSTIMULATORS ON GROWTH OF TOMATO PLANTS CULTIVATED UNDER PROTECTED AND FIELD CONDITIONS

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Abstract

Biostimulators can be used in order to stabilise plant growth in even unfavourable climate conditions or fluctuating conditions in the rhizosphere and they stimulate the process of the formation of plant-organs making the plants resistant to diseases and viruses. In our investigations with tomato plants, liquid substances based on different organic compounds such as lignite coal (Humates), waste of food production (Lactate), algeas (Megafol), plant-based substances (Čudomiks,) and microorganisms such as *Bacillus subtilis* were used as biostimulators. The effects of biostimulators were investigated in tomato soilless cultivation, cv. Ferrari RZ, in the greenhouse of the experimental station in Berlin. Humates, lactates, and *B. subtilis* were added to the nutrient solution, single or mixed and well sprayed on the leaves. Humates improved plant growth even when the EC was very high. In the case of extreme pH values of 4.5 or 7.5 better growth parameters were recorded when lactate had been added to the nutrient solution. In some cases, the development of tomato plants was also enhanced by combinations of humates, lactates, and B. subtilis. The field experiment was carried out on grey-red soil in the valley of the river Raša (Istria). Biostimulators were applied on indeterminate, high beef tomato variety ‘Signora F1’ with a training system on one or two branches. PE mulch foil, fertigation, and trellis were used on the plantation. The influence of the triple treatment on fruit number, individual fruit weight, yield distribution, time of disease onset, and branch length was examined. The results of the study show that growing with two side shoots and treating with Megafol is the superior combination in terms of yield. On the other hand, the cultivation form with one side shoot in combination with Megafol leads to a better distribution of yields.

Keywords: *Solanum lycopersicon L., Humates, Lactates, Bacillus subtilis, Megafol.*

Introduction

Plant biostimulators contain plant substances and/or microorganisms, whose function when applied to a plant or rhizosphere is to stimulate the natural process of absorption and effectiveness of nutrients, tolerance to abiotic stresses and crop quality. They are often used for foliar application, but also for the rhizosphere in addition to the standard fertilization treatment. In this way, they stimulate and strengthen plant metabolism through the roots (Mešić et al., 2022). Du Jardin (2015) mentioned different categories of biostimulators classified by composition: (1) humic and fulvic acids, (2) amino acids, protein hydrolysates and other nitrogen compounds, (3) extracts of seaweed and algae, (4) inorganic biostimulators, (5) beneficial (mycorrhizal) fungi, (6) beneficial bacteria.

The application of bioregulators was studied with the aim to improve both the nutrient balance and plant growth on tomato cultivation in a greenhouse and on the field. According to previous investigations, humates, lactates and Bacillus subtilis as well as algae, seem to be particularly suitable in this respect. The special function of humus and the humic acid in the rhizosphere of plants is known, but the roots have to develop in hydroponic (soilless) systems with a few amount in ‘substrate culture’ or without the influence of humic acid, in ‘water culture’. Humic acids can accelerate the plant-growth, stimulates the process of the formation of plant organs, increases the unspecific resistance of the plants against stress conditions like high temperature, frost, drought, strong radiation (Faust, 1999). Humates have an influence on the nutrient uptake and the respiration process, the amount of sugar and amino acids, further reduce the accumulation of nitrate and makes the plants resistant to diseases and viruses (Levinsky, 1996; Böhme et al., 2005). It was found that, under certain conditions, humic acid applied to the root zone had beneficial effects on plant development (Tattini, 1990; Böhme and Hoang, 1997). According to previous investigations (Böhme et al., 2008), lactates (salts of lactic acid) seem to have also a bioregulatory effect. The application of lactates was tested as an approach to improve both nutrient balance and plant vitality. Investigations have shown that lactates have more stable bonds with several metal ions than other chelates do. Therefore lactates have been used as fertilisers and as bioregulators. Lactates are available from a Bulgarian company Ecofol (LACTOFOL®). That suspension fertiliser was designed mainly for foliar application (Pavlova and Batschvarov, 1992; Shaban et al., 1995). Hardly any information has been available so far about the effects of lactate applied to the root zone (Böhme et al., 2008). Many microorganisms from the rhizosphere can influence plant growth and plant health positively and are therefore often referred to as “plant growth-promoting rhizobacteria” (Schippers, 1992). In previous investigations, we found beneficial effects in soilless culture systems of the gram-negative rhizobacterium FZB 24® regarding the reduction of salt stress (Böhme, et al. 2005). These positive effects under salinity conditions could be confirmed by Bochow et al. (2002) in open-field research with eggplant and bell pepper in Egypt. The cultivation of different vegetables in hydroponic systems in greenhouses is quite problematic as to the proper balancing of EC and pH values. The three groups of biostimulators were therefore investigated in greenhouse experiments with the aim to stabilize in particular the chemical properties of the rhizosphere. On the field are also many stress factors for tomato plant growth caused by climate and environmental conditions, pests and diseases and unfavourable conditions in the rhizosphere with an application of biostimulators it is possible to reduce such stresses for plant growth. The field experiment was the intended to show the effects oftwo biostimulators. The first is Čudomiks an organic liquid biostimulator and plant enhancer from the "Tilurium Organic" line, a Croatian product developed by OPG Pezelj from Trilj, which is also accepted for ‘organic’ production following the EU rules. Čudomiks consists of biologically active compounds and elements extracted from plants that protect crops from fungal and bacterial diseases. The second biostimulator used is Megafol Valagro®, from Italy it promotes vegetative growth during environmental stress, stimulates plant growth and improves the effectiveness of treatments. This biostimulator contains 28% of amino acids of exclusively plant origin contains a line of vitamins, amino acids and proteins, betaines and growth factors. When applied in times of stress (frost, root asphyxia, weeding, hail), its synergistic action of betaine and amino acids allows plants to quickly and spectacularly overcome stress and improve growth (Megafol, 2022)

Material and Methods

Plant material

In both experiments indeterminate tomato (*Solanum lycopersicon* L.) was investigated. In the greenhouse experiment of the Humboldt University of Berlin, Research station Berlin-Dahlem cv. Ferrari RZ was used and in the field experiment near to Trget in Istrien, Croatia the Italian cv. Signora F1 ESASEM, a hybrid beef tomato for loose harvesting, resistant to TSWV. This cv. can be cultivated in a greenhouse and on the field.

Investigations on protected cultivation in Germany

The experiments were performed in the period from May through end of August 2011. Average temperature during the day was in average 27.5°C and during the night 23.5°C. The air humidity was between 50 and 76%. Ten tomato plants per treatment were used, cultivated in Mitcherlich pots until they had 11/12 leaves. Experimental conditions:

- The Mitcherlich pots (6L) were filled with Perlite, with an average dry density of 120 kg * m^{-3} and a grain size between 0.06mm and 1.5mm.
- K-humate with 0.01% (Fa. Humisolv) was used as the humic acid preparation.
- The Lactate (LACTOFOL ®) used in the experiments concentration: 0.08%) was added to the solution, and the lacking nutrients were supplemented
- The strain Bacillus subtilis FZB 24 of Fa. Arbitep applied as spore suspension (concentration of 10^5cfu/ ml.

The tomato plants were assessed once a week to follow up their growth and development, shoot fresh and dry matter and root fresh and dry matter as well the root length, were recorded in the final assessment. A micro irrigation was used, whereby the nutrient solution was calculated with the "Hydrofer" fertilization program in all variants (Böhme, 1993).
The stress variants consists of three levels of salt concentration EC 1.0, 3.0, and 8.0 mS cm^{-1} and pH value of 5.0, 5.8, and 7.5.

Investigations in field cultivation in Croatia

The soil of the experimental plot belongs to the characteristic type of alluvial soils in the river valleys, it is composed of clay and a lot of sand particles with a pH of 6.5-7.2 whereas the precipitation ranges from 800 to 1100 mm. Temperatures in the summer months are suitable for growing tomatoes, the difference between day and night temperatures during the summer are on average 18 ° C, and up to 30 ° C daily.
Plant density was 4.8 plants per square meter in double row, the planting distance was 90 cm between the double row beds The bed was covered with black mulch foil, the row spacing was 30 cm between rows and within the row. The effect of the two biostimulators Chudomiks and Megafol was investigated.
A different number of biostimulator treatments; 1x, 2x and 3x on tomatoes grown on one and two branches, in 14 different variants in three repetitions, a total of 42 individual plots. All variants are represented in three repetitions with a random arrangement of plots. The size of the experimental field is 5300 m^2, the size of an individual plot 126 m^2. Megafol treatment was carried out according to the instructions in a concentration of 0.33% (1 L of Megafol per 300L of water). Chudomiks was applied in the recommended dilution of 1.0%, according to the instructions on the declaration, 0.5 L of biostimulator per 50L of water. The parameters monitored in the experiment are the plant height, the number of fruits per plant, the weight of individual fruits and yield per plant.

Data evaluation

All data of the experiments were evaluated with the statistical software SPSS. Mean values and standard deviations were calculated and analysed using ANOVA - in greenhouse: LSD test, significance level $P \leq 0.05$ and in field experiment Tukey test, significance level $P \leq 0.05$.

Results and discussion

Experiments in greenhouse

The treatments showed visible effects, the results for shoot and leaf dry matter (SDM) are in line with what had been expected - highest values at EC = 3 mS $*$ cm^{-1}, lower at 1 mS $*$ cm^{-1}, and smallest at 8 mS $*$ cm^{-1} (Table 1). Compared with the control, treatments at 3 mS $*$ cm^{-1} produced only a small effect. Significantly better results were only obtained in the variant with humic acid (HA) + *Bacillus subtilis* (BS). BS and its combination with HA gave significantly better SDM also in case of nutrient deficiency, i.e. at an EC of 1 mS $*$ cm^{-1}.

The results obtained at the extremely high EC of 8 mS $*$ cm^{-1} were in line with expectations. Addition of HA, BS and HA + BS caused a significant enhancement of tomato plant development. The effect of HA and BS on root development (Table 1) is similar to that on SDM. Significantly higher root dry matter (RDM) was recorded above all with EC values of 3 and 8 mS $*$ cm^{-1}, respectively. Results are somewhat different for root length (RL). In the nutrient deficiency variant (EC = 1 mS $*$ cm^{-1}) all plants had longer roots than the control, but only slightly different root lengths were recorded if EC was higher.

Table 1. Parameters of tomato plants treated with Humic acid, Lactate and/or Bacillus subtilis at EC 1, 3 and 8 mS cm^{-1} respectively

Variants	Shoot dry Matter [g/plant]			Root dry Matter [g/plant]			Root length [m]		
	EC 1	EC 3	EC 8	EC 1	EC3	EC8	EC 1	EC 3	EC8
Control	16.67b	25.27b	5.97c	3.02a	2.94ab	0.86b	56.89b	136.81a	32.89b
Humic Acid (HA)	16.92b	23.43b	16.15a	2.72b	3.45a	1.74a	115.25ab	109.47b	47.43a
Lactate (LA)	13.63c	22.01c	5.54c	2.37b	2.91ab	0.62b	114.75ab	68.52c	35.82b
Bacillus subtilis (BS)	18.83a	23.98b	14.43b	3.27a	3.48a	1.61a	135.44a	138.21a	41.63ab
HA+BS	18.05a	27.08a	16.07a	2.9b	3.63a	1.84a	131.69a	127.78ab	52.96a
LA+BS	13.29c	15.69d	5.39c	1.93c	2.2b	0.66b	86.94b	47.04c	31.75b
HA+LA+BS	13.26c	17.11bd	5.39c	1.96c	2.74b	0.62b	90.17b	66.76c	35.65b

Different letters indicate significant differences (LSD, P=0.05).

If pH was low (pH 5.0), all treatments showed higher SDM than the control, a fact that was particularly obvious in the combination HA + LA + BS (Table 2). Particularly noticeable are the significantly better result at pH 5.8 (which level is considered optimal) and with BS.

Table 2. Parameters of tomato plants treated with Humic Acid, Lactate and/or Bacillus subtilis at pH 5, 5.8 and 7.5, respectively

Variants	Shoot dry Matter [g/plant]			Root dry Matter [g/plant]			Root length [m]		
	pH5	pH 5.8	pH7.5	PH 5	pH 5.8	pH 7.5	pH 4.5	pH 5.8	pH7.5
Control	11.0b	8.1c	2.0c	1.6a	0.7c	0.1c	31.3a	48.00b	5.7c
Humic Acid	11.2b	9.5c	4.2b	1.6a	1.0b	0.4b	41.3a	50.88ab	14.2b
LACTOFOL	9.6c	10.2b	8.7a	1.5a	1.4a	1.9a	41.9a	68.70a	24.8a
Bacillus subtilis	12.8a	9.5c	2.2c	1.6a	0.8	0.09c	32.3a	55.90ab	4.6c
HA+BS	12.7a	11.1b	2.9c	1.5a	1.2a	0.5b	37.9a	49.73b	10.2b
LA+BS	9.4c	10.8b	9.4a	1.3b	1.3a	1.9a	36.9a	47.15b	26.7a
HA+LA+BS	10.3b	15.1a	10.1a	1.7a	1.5a	1.4a	34.5a	64.35a	25.9a

Different letters indicate significant differences (LSD, P=0.05).

The results at pH 7.5, a value that is extremely high for tomato, are unambiguous.
It can be conclude, humic acid encourages the longitudial growth of plant roots, a fact that has been established at the various ECs used in the experiments. These results are in line with the findings of Tattini et al. (1990) who found that humic acid led to greater length of the root system and a larger number of lateral and hair roots. This might also explain why at an EC of 1 mS * cm^{-1} root dry matter did not increase in the same way as the root length. The beneficial effect of HA at 8 mS * cm^{-1} may also be due to its high sorptive capacity. All variants with LACTOFOL® developed significantly better than the control and also better than the other treatments. Root dry matter revealed even more obvious effects of the LA variants. This experiments, too, makes it clear that it would be advisable to record not only RDM but also root length. Here again, root length was higher in the LA variants and - like for RDM - not only at pH 7.5 but also at the optimal value of pH 5.8. The positive effect of BS on the development of tomato plants with excessive or deficient nutrient supply, i.e., the variants with EC 1 and 8 mS * cm^{-1}, respectively, may be explained by the formation of enzymes that interfere with the nutrient balance or produce a general vitalizing effect. This applies also to the beneficial effects of BS at different pH values. Similar assumptions were made by Bochow et al. (1995) in his interpretation of experiments with different vegetable species. Combinations of BS and HA turned out equally effective at suboptimal EC and pH values. The interactions involved will have to be investigated in future experiments.

Experiments in the field

In the experiment, a statistically significant difference in plant height was found depending on the cultivation form. A tomato grown on one branch formed a significantly longer branch compared to a tomato grown on two stems (Figure 1). Biostimulators and the number of treatments with biostimulators did not significantly affect the height of tomatoes.

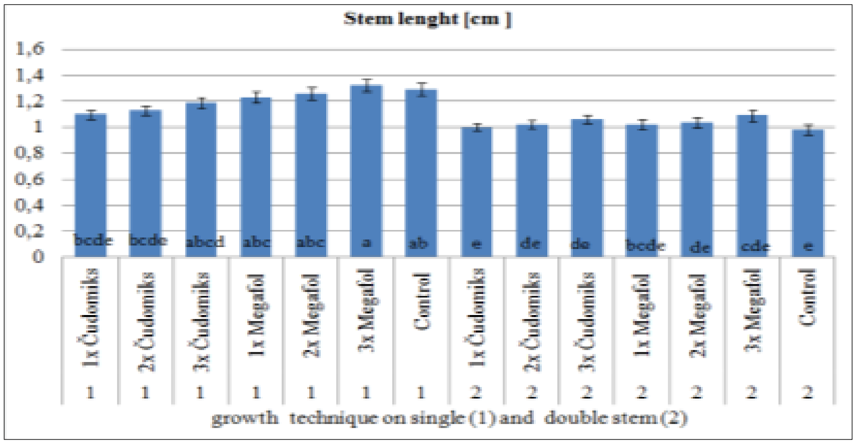

Figure 1. Influence of cultivation form, number of treatments and type of biostimulator on tomato stem height (Different letters denote significance, Tukey test, p <0.05)

The number of fruits per plant is influenced by the cultivation practice, the type of biostimulator and the number of treatments. Statistically confirmed, the largest number of fruits per plant was found in variants with double and triple treatment of Megafol in the cultivation form of tomatoes on two branches. A significant increase in the number of fruits was also statistically confirmed in comparison with the treatments with Čudomiks list (Figure 2).

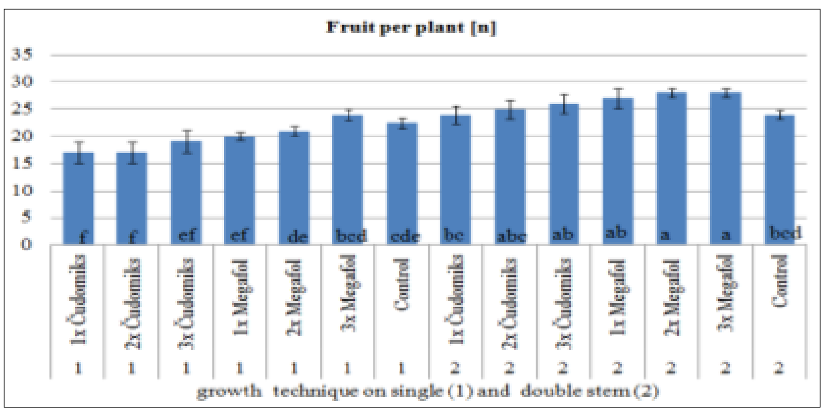

Figure 2. Influence of cultivation form, number of treatments and type of biostimulant on the number of fruits per tomato plant (Different letters denote significance, Tukey test, p <0.05)

In the cultivation form on two branches, it was found that triple treatment with biostimulators significantly affected the weight of an individual fruit compared to a smaller number of treatments. In the total yield per plant, statistically confirmed the best variant was triple treatment with Megafol in tomato cultivation on two branches, in second place in the amount of yield per plant was achieved in the variant triple treatment with Megafol in tomato cultivation on one branch (Figure 3).

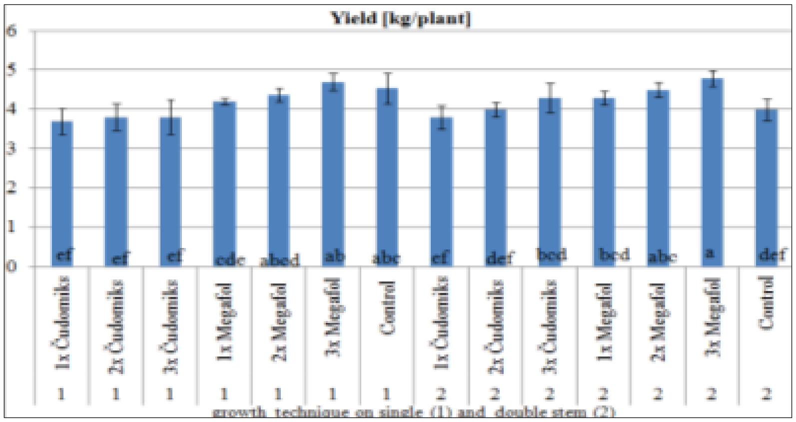

Figure 3. Influence of cultivation form, number of treatments and type of biostimulators on yield per plant (Different letters denote significance, Tukey test, p <0.05)

The results of research on the influence of biostimulators in tomato cultivation are in line with research on the application of biostimulators in nettle Radman et al. (2022) who found that biostimulators based on amino acids and humic acids did not have a justified effect on morphological indicators (plant height, weight, number of nodules and leaves), which was shown in tomato cultivation, where a significant influence on growth height had a cultivation form, neither the number of treatments nor the type of biostimulator. Research by Klokić et al. (2020) using biostimulators, Megafol and Viva, with different dominant compositions (amino acids and humic acids) confirm positively affected yield in two cultivars of semi-determinate tomato (cv. Gravity F1 and cv. Minaret F1) which is consistent with this research. Namely, proline is an amino acid, which is added to plants by the application of Megafol®. This amino acid is characterized by the ability to remove free radicals (Khedr et al. 2003), better antioxidant ability to respond to stressful growing conditions resulting in better plant condition (Kaul et al., 2008) and consequently reflected in higher plant productivity, in the formation of higher number of fruits. Parađiković et al. (2010) confirm the effects of the use of Megafol in pepper cultivation on fruit quality and the occurrence of peak rot. The effectiveness of Megafol biostimulators is also contributed by the content of tryptophan, which is a precursor in the synthesis of melatonin, which according to research by Arnao and Hernandez-Ruiz (2006) strengthens the ability to remove free radicals in metabolic processes and is helpful to improve the productivity of plant growth. Melatonin biosynthesis in plants is influenced by tryptophan and IAA (indole-3-acetic acid.

Conclusion

Tomato as one of the most famous types of vegetables in the world in cultivation is very demanding and it takes a large number of working hours to obtain the desired quality and quantity. In the experiments, the cultivation of indeterminate tomato cultivars in greenhouse and in the open field makes visible the possibility of strengthening plant growth and yield, by use of different biostimulators in particular in in stress situations.

Following the results in the greenhouse experiments can be conclude, addition of organic and/or biological agents to the substrate or nutrient solution helps to minimize stress situations and, hence, adverse effects on plant growth that are due to suboptimal EC or pH values. The humates, lactates and Bacillus subtilis have different bioregulatory effects. The combination of Humates

and BS is already produced and offered from the company Humintech under the name BioHealth® BS WSG product because the very effective bioregulatory effects.
Lactate (LACTOFOL®) was found to be also suitable as a fertilizer for making up nutrient solutions to be used in hydroponic systems. However, its stress-reducing effect is more pronounced at non-optimal pH values than at suboptimal EC.
In the field experiment was visible that the deviations in fruit quality between treatments were very small, but in quantity deviations were somewhat larger. In the variants of the cultivation form on one branch, the treatment with 3 times the biostimulator Megafol proved to be the best. In the two-branch cultivation form, the best variant was also a three-course treatment with Megafol. However, according to the fruiting schedule, cultivation on one branch was more favourable. Growing on two branches increases the number of fruits and total yields, but the potential for the occurrence of fungal diseases is increased due to the higher density of plantations. From the economic point of view, the use of Megafol biostimulants is economically viable due to the increase in yield and fruit quality.

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MORPHOLOGICAL DESCRIPTION OF *CROCUS SATIVUS* IN THE AREA OF KOZANI, GREECE

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Abstract

The aim of the present research was the investigation of the domestic genetic material of cultivated crocus and the evaluation of variability in terms ofmorphological characteristics. The experiment was established in three different areas of Kozani(Krokos, Protochori, Ano Komi) in order to evaluate the effect of the microclimate on the crocus plant. For this purpose, 250 plants from each area were identified and studied throughout their growing period, and the number of leaves, the length of the leaf, the number of flowers per corm, the number of petals, the length of petals, the number of stigma, the length and thickness of stigma, the number of stamens and the length of stamens were measured. At the same time, the variability of the plants in terms of their morphological characteristics was recorded.From the experiment it was observed that the *Crocus sativus* L. shows a special sensitivity to the microclimate of the area where it grows. It is remarkable that differences in the crocus phenotype were observed not only in the three plots with more or less different climate conditions, but also in the same plot from each region.From the characteristics measured statistically significant differences between the three experimental areas were found only in terms of leaf length and stigma thickness. The significantly longer leaf length was observed in the area of Krokos and the significantly greater thickness of the stigma in the area of Ano Komi. From the above experiment, a large percentage of plants with more stigmas was found in the area of Krokos.

Keywords: *variation, number of stigma, length of stigma, thickness of stigma.*

Introduction

The species *Crocus sativus* L. is an aromatic-medicinal plant, of the family *Iridaceae*. Saffron is the dried stigmas of the flower of the plant *Crocus sativus* L. which is cultivated mainly in countries, like Iran and India and in small areas in Greece, Italy, Morocco and Spain. In Greece, it is grown in Western Macedonia, specifically in the Kozani region, and is of great economic interest due to its use as a spice and for medicinal purposes. It is sterile with 24 chromosomes and can not be reproduced natively because of its triploidy. The only way of reproduction is through the splitting of its corms. The genetic origin of *Crocus sativus* and the nature of its triploidy have been studied by some scientists but the emerged data are not very clear. However the plants of population may be autotriploid rather than allopolyploid. The flower of the saffron has six purple petals, three yellow stamens and a red stigma which is the commercial part of the plant. However some plants have bigger flowers with more than six petals and more stigmas. It remains to be elucidated whether this variability is genetic or purely environmental since the morphological characteristics like the length and the thickness of the filament, and the double stigma are very important for the saffron economy (Mitsopoulou and Tsimidou, 2004). Therefore the presence of genetic variability in the crocus population cultivated in Greece is very

important, and the presence of genetic polymorphism is a matter of study as some morphological and agricultural differences were observed in the population.
In order to obtain new information about the genetic variability at the genetic and epigenetic level the researchers using the Amplified Fragment Length Polymorphism (AFLP) (Torricelli et al. 2019), found low genetic variability, but high epigenetic variability, which is related to some random mutation and influenced by environmental conditions, activating mechanisms that contribute to phenotypic modifications.
This study was undertaken to investigate the domestic genetic material of cultivated crocus and its evaluation of variability in terms of morphological and agricultural characteristics.

Materials and methods

The experiment was established in three different areas of the Prefecture of Kozani (Krokos, Protochori and Ano Komi),in order to evaluate the effect of the microclimate on the crocus plant, since in this case the plants were affected by different climatic conditions. A total of 250 plants were selected(150 in the area of Krokos, 50 in the area of Protochori and 50 in the area of Ano Komi) and were studied throughout its annual cycle. The selected plants were identified, and when the plant reached its final size (in May), the length and number of their leaves were measured. During the summer when the plant is dormant (June, July, August), all the cultivation practices used by farmers were applied (weed removal, surface followed, surface milling) and in September the field was prepared for the beginning of flowering. At the beginning of flowering (in the month of October), the number of flowers per corm, the number of petals, the length of petals, the number of stigma, the length and thickness of stigma, the number of stamens and the length of stamens were measured. At the same time, the variability of the plants in terms of their morphological characteristics was recorded.
The soil in the three areas Krokos, Protochori and Ano Komi was clayey, clayey and sandy respectively. No irrigation was conducted, because the rains during the year covered the plant's needs for water. The cultivation techniques (weeding, soil treatment), were done according to the traditional way of work. The data of the present study were statistically analyzed and the differences between the characteristics and the regions were evaluated using the ANOVA analysis of variance. The Tukey control criterion (HSB) was used to compare the means.

Results and Discussion

The experiment reviewed that the Crocus sativus L. plant shows a special sensitivity to the microclimate of the area where it grows, with the consequence that it directly affects its phenotype and indirectly its productivity. From the experiment it was observed that the *Crocus sativus* L. shows a special sensitivity to the microclimate of the area where it grows. It is remarkable that differences in the crocus phenotype were observed not only in the three plots with more or less different climate conditions, but also in the same plot from each region.
In the present work the length of stigma in the majority of the flowers was 4-4.5 cm and the thickness 1.5-2.5 mm. These two characteristics together with the number of stigmas are essential because the stigma is the commercial part of the crop. There were statistically significant differences in terms of stigmas thickness between the plants of the three experimental areas (in Ano Komi the stigma appeared thicker), and statistically significant difference in terms of the maximum length of stigma. Sheikh et al. (2014) studied fifty clones in terms of

morphological and physiological characteristics and found that there was a wide range of phenotypic variability for the number of flowers per stem, the plant height, the number of daughter stems/mother stem, etc. with high heredity values for all traits. The results of the present work are in agreement with those of Sheikh et al. (2014), in terms of the wide variability in the number of leaves/bulb, which ranged from 4-10 (the majority of plants formed 6-9 leaves). On the other hand, the study of Soukrat et al. (2019) with crocus bulbs from different regions of Morocco confirmed a wide range of phenotypic variability within and between populations. In addition, they reported large variations in stigmas length between different sources of bulb origin and a significant effect of bulb origin on its phenotype explained by the genotypic profile or the epigenetic effects of the different origins. In the present work, a wide variability was also observed in the length and thickness of stigma, which was similar in the three regions in terms of the minimum stigma length, in terms of the total number of leaves, of the number of petals, the number of stamens, etc., which can be attributed to epigenetic phenomena, caused by the effect of the microclimate of each region. The possible involvement of the epigenetic in the creation and presence of different bulb phenotypes was supported also by Busconi et al. (2018).

Plants from the Krokos area brought fewer leaves and more flowers compared to plants from the other two areas. The flowering results are probably in line with the percentages of plants with longer leaf length recorded in the three areas. These percentages were 67% for the area of Krokos, 34% for the area of Protochori and 42% for the area of Ano Komi. It is obvious that there are differences between plants of the three areas, as a result of the different climatic conditions of three area. The same observation has been taken by Molina et al. (2004), Fernandez (2004), Mollafilabi (2004), Sheikh et al. (2014).

The results of the present work showed differences in induction of flowering, number of flowers per bulb and stigma not only in the three different areas of the experiment but also in the same plot in each area. The same result was noticed by Soukrat et al. (2019) who found a wide range of phenotypic variability within and between populations. In the present work, differences were identified in the phenotype of the plants, within the same plot of the three areas, which were exposed to the same environmental influences, in the same soil and climatic conditions. Therefore these differences can not be attributed to environmental conditions due to the fact that all plants (from each plot) grew in the same environment and the phenotypic behavior of many plants was different. For example, many plants produced three flowers per corm, while others produced one or no flowers per corm. The size of the stigma in some plants was larger while in others it was much smaller, and this raises questions about the existence of genetic or not genetic basis of this variability. Some studies (Nemati et al. 2019) identified genetic polymorphism in the crocus plant, while other studies (Brighton 1977, Grilli Caiola 2004) did not detect genetic differences. Torricelli et al. (2019) reported that although significant morphological differences were observed in propagating material from Italy and Iran, molecular markers (AFLP) revealed limited genetic differences (with genetic similarity = 0.985). Also Busconi et al. (2018) supported the possible involvement of the epigenetic in the identification of alternative crocus phenotypes. Therefore there is a need to investigate whether there is genetic polymorphism in the crocus plant and to study the possibilities of exploiting this polymorphism and the selection of individual plants from different populations from each region separately.

Finally, as it emerged from the results of the present work, in soils with a high percentage of organic matter or in soils with a high percentage of clay, a large number of plants had flowers with more than three stigmas, which means higher productivity. Fernandez (2004), and

Sampathu et al. (1984) considered that the loamy and the sandy soils are the best for the crocus cultivation.

Conclusion

As shown by the results of the present work, differences such as induction of flowering, number of flowers per corm, size of the stigma, were observed not only in the three different areas of the experiment, but also in the same plot in each area, exposed to the same environmental effects in identical soil conditions and in identical plant care. Therefore, these differences can not be attributed to environmental conditions, due to the fact that all plants (from each plot) grew in the same environment and the phenotypic behavior of many plants was different. It should also be noted that the percentage of flower variability that appeared in the area of Krokos was quite high and unusual.

Based on the results, a key question that arises is why some corms form an unusually large number of flowers and corms under the influence of the same environmental conditions. Therefore there is a need to investigate whether there is genetic polymorphism in the crocus plant and to study the possibilities of exploiting this polymorphism and the selection of individual plants from different crocus populations from each region separately.

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THE EFFECTS OF AN INNOVATIVE DIGESTATE AND WOOD ASH MIXTURE FERTILIZER ON POTATO YIELD QUALITY

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Abstract

Field trials with the potato variety ‘Rigonda’ were carried out in a sod stagnogley soil. Soil agrochemical parameters were: pH_{KCl} 5.9, organic matter content – 2.3%, phosphorus (P_2O_5) content – 149 mg kg^{-1}, and potassium (K_2O) content – 200 mg kg^{-1}. Potato plots were established using different treatments of a fertilizer mixture consisting of pig and cattle manure digestate and woodchip ash in different ratios (digestate to wood ash = 4:1 and 3:1). The doses of the innovative mixed fertilizer for potato were 15 and 30 t ha^{-1}. Both norms of the digestate from pure pig and cattle manure were used as control options. The experimental design was an RCB with three replications. Crude protein content in potato dry matter varied from 8.28% to 10.94%. The application of fertilizer mixtures increased the dry matter content by 1.4–2.0%, reaching an average of 21%. Fertilizer treatments which produced higher tuber yields or higher starch contents gave also higher starch yields. In our studies, the average starch content in potato tubers dry matter was 73.3% and 15.3% in a fresh potato tuber, but the average starch yield reached 3.55 t ha^{-1}. The objective of the research was to study the influence of the rates of the digestate and wood ash mixture fertilizer on the quality of potato yield.

Keywords: *digestate, wood ash, fertilizer, potato, yield quality.*

Introduction

Potato is one of the most important agricultural crops and is rightly called “the second bread”. In the world, in terms of cultivated areas, the potato crop occupies one of the first places along with rice, wheat and corn (reference). According to the literature, potato tubers contain on average 76-78% water, 13–36% dry matter, 12-15% starch, 1-3% protein, and approximately 1% minerals (reference). Potato protein has a very high biological value, because it contains an essential amino acid complex which is not synthesized in humans and animals and should be obtained from food or feed. As a result of the operation of biogas and biomass cogeneration plants, the production by-products digestate and ash are obtained. They are a good source of plant nutrients as they contain many trace elements and macroelements important for plant growth; therefore, digestate and ash can be used as an effective fertilizer for crops (Koszel and Lorencowicz, 2015; Risberg, *et al*., 2017). The physical and chemical properties of ash can vary significantly depending on plant species, plant growth conditions, parts of the plant used for combustion, parameters of the combustion process, and storage conditions (Demeyer, Nkana, Verloo, 2001). Ash contains a variety of minerals that make it a valuable source of plant nutrients. Also, they contain various macronutrients and microelements important for plant growth and development, with the exception of nitrogen which is released into the atmosphere by flue gases during combustion (Patterson *et al*., 2004; Fuzesi *et al*., 2015).

Studies have shown the positive effect of ash on soil properties, soil structure, and water regime in the soil (Demeyer *et al.*, 2001). Ash fertilizer has increased the amounts of phosphorus, potassium, calcium and magnesium used in plants in the soil (Fuzesi, *et al.*2015). A decrease in nitrogen concentration in the upper soil layers and an increase in soil pH after ash application have been observed. Ash can be used to improve soil fertility and crop yield and quality. However, the use of both products separately can cause certain environmental problems. To prevent this, at least in part, the idea arose to mix digestate and ash in certain proportions and use the obtained mixture for the fertilization of different crops. The aim of the study was to determine the effect of the digestate and wood ash fertilizer norms on potato productivity and quality.

Materials and Methods

Field trials with the potato variety ‘Rigonda’ were set up in a sod stagnogley soil. Soil agrochemical parameters: pH_{KCl} 5.9, organic matter content in soil – 2.3%, phosphorus (P_2O_5) content – 149 mg kg^{-1}, and potassium (K_2O) content – 200 mg kg^{-1}. Potato experimental plots were established using different variants of fertilizer mixtures consisting of pig (from LLC “Latvi Dan Agro”) and cattle (from JSC “Ziedi JP”) manure digestate and wood ash (from LLC “Gren Jelgava”) in different ratios (digestate to wood ash = 4:1 and 3:1). The doses of the innovative mixed fertilizer from the pure pig and cattle manure digestate were 15 and 30 t ha^{-1} for potato and were used as control options. The amounts of nutrients supplied to potatoes applying both fertilizer doses are presented in Table 1. It is seen how the addition of ash to fertilizer mix changes the nutrient input: the amount of nitrogen supplied to potatoes decreases and the amount of potassium and phosphorus increases.

Table 1. Amount of nutrients incorporated into the soil

Type of fertilizer	Fertilizer rate, t ha^{-1}	The ratio of digestate and wood ash in the mixture	Amount of nutrient elements incorporated into the soil, kg ha^{-1}		
			N	P_2O_5	K_2O
Pig manure digestate	15	1:0	98	93	35
		3:1	75	131	139
		4:1	80	128	125
	30	1:0	196	186	70
		3:1	150	262	278
		4:1	160	256	250
Cattle manure digestate	15	1:0	81	54	86
		3:1	61	101	177
		4:1	66	85	150
	30	1:0	162	108	172
		3:1	122	202	354
		4:1	132	170	300

The field was cultivated just before planting. Potatoes were winterized before planting, planted at the beginning of May. Planting of potatoes and the application of fertilizer mixtures were carried out by hand, in previously prepared furrows. The potato planting rate was 45000 tubers per ha. The variants of plots in the trial were randomized, in triplicate. The plot size was 5.6 m^2. A week after planting, and also after germination, the potatoes were hoed, but later during the vegetation period they were raked and hoed several times. The potato variety 'Rigonda' is suitable for early harvest in Latvian conditions, ensuring a sufficient proportion of large tubers as well as good taste characteristics. The dynamics of potato development and the number of potato stems were assessed during the vegetation period. For each experimental plot in all replicates at the end of the cultivation period, the total yield was recorded and also tuber size, and the quality of the tubers was determined: dry matter content, starch content (in fresh weight and in dry matter), and the contents of crude protein, fat, ash, phosphorus and potassium. For chemical composition analysis of tuber yield, the average samples of 1.0-1.2 kg were removed from each treatment. Qualitative indicators were determined in the Biotechnology Scientific Laboratory (BSL) of the Latvia University of Life Sciences and Technologies. The contents of dry matter, fat and ash were determined by gravimetric analysis, crude protein content – by Kjeldahl method, phosphorus content of the samples – by quinoline phosphomolybdate analysis, potassium content – by flame emission spectrometry, and starch content – by natural polarimetry. Data processing was performed using a three-way analysis of variance (ANOVA) "Microsoft Excel" computer program.

Results and Discussion

The development of a quality potato crop is a complex process of plant interaction with plant growing systems and environmental conditions which affect the rate of photosynthesis, and plant metabolism and growth. In the study, the two types of fertilizer had different effects on potato tuber yield. Using the mixture of pig manure digestate and wood ash in different ratios, the average yield reached 24.93 t ha^{-1}, but the use of the mixture of cattle manure digestate and wood ash gave a 22.78 t ha^{-1} average yield (Table 2). The applied fertilizer norm (F-factor> F-criterion) demonstrated a significant positive effect on the yield, but the type of fertilizer and the ash and digestate ratio did not show any significant effect on potato yield.

Table 2. Influence of the digestate and wood ash mixtures on the yield of potato variety ‘Rigonda’

Type of fertilizer (F_A)	Fertilizer rate, t ha^{-1} (F_B)	The ratio of digestate and wood ash in the mixture (F_C)	Average tuber yield, t ha^{-1}		
			(F_C) LSD $_{.05}$=2.62	(F_B) LSD $_{0.05}$ =2.70	(F_A) LSD $_{0.05}$ =2.38
Pig manure digestate		1:0	25.43		
	15	3:1	25.63	**24.66**	
		4:1	22.92		
		1:0	26.76		**24.93**
	30	3:1	25.54	**25.19**	
		4:1	23.28		
Cattle manure		1:0	25.20		
	15	3:1	20.73	**22.71**	

digestate		4:1	22.19		**22.78**
	30	1:0	25.11	**22.85**	
		3:1	22.64		
		4:1	20.81		

The highest starch yields were obtained in fertilizer variants with higher tuber yields or higher starch contents. The average starch content in potato dry matter was 73.3% and 15.3% in a natural product, but the average starch yield reached 3.55 t ha-1 (Table 3).

Table 3. Influence of the digestate and wood ash mixtures on starch production for potato variety ‘Rigonda’

Type of fertilizer (F_A)	Fertilizer rate, t ha^{-1} (F_B)	The ratio of digestate and wood ash in the mixture (F_C)	Average starch yield, t ha^{-1}		
			(F_C) LSD $_{0.05}$= 0.45	(F_B) LSD $_{0.05}$ =0.63	(F_A) LSD $_{0.05}$= 0.71
Pig manure digestate	15	1:0	4.21	**4.16**	**3.90**
		3:1	3.99		
		4:1	4.27		
	30	1:0	3.30	**3.63**	
		3:1	3.72		
		4:1	3.87		
Cattle manure digestate	15	1:0	4.15	**3.68**	**3.50**
		3:1	3.68		
		4:1	3.21		
	30	1:0	3.57	**3.32**	
		3:1	3.21		
		4:1	3.17		

Many years of research and practical experience have confirmed the fact that tubers with a low (<20%) dry matter content disintegrate faster during cooking, while tubers with a higher dry matter content have a denser texture, are less prone to mechanical damage, and are easier to use for processing. The application of fertilizer mixtures increased the dry matter content by 1.4–2.0%, reaching an average of 21% (Table 3). An increase in dry matter content was observed at lower fertilizer rates.

The nutritional value of potato depends on the content of crude protein in tubers. In the studied variants, its content in potato dry matter varied from 8.28% to 10.94%.

Table 4. Influence of the digestate and wood ash mixtures on the chemical composition of potato cultivar ‘Rigonda’ tubers

Type of fertilizer (F_A)	Fertilizer rate, t ha^{-1} (F_B)	The ratio of digestate and wood ash in the mixture (F_C)	Average content in a natural product, %		Content in dry matter, %		
			starch	dry matter	crude protein	potassium	phosphorus
Pig manure digestate	15	1:0	16.54	22.22	8.65	2.17	0.34
		3:1	16.73	22.11	8.28	2.20	0.33
		4:1	14.41	20.34	10.83	2.44	0.36
	On average (F_B)		**15.89**	**20.56**	**9.25**	**2.27**	**0.34**
	30	1:0	14.90	20.25	10.94	2.29	0.36
		3:1	14.57	20.29	10.83	2.52	0.39
		4:1	15.17	21.06	10.24	2.34	0.38
	On average (F_B)		**14.88**	**20.53**	**10.67**	**2.38**	**0.38**
On average (F_A)			**15.38**	**21.05**	**9. 96**	**2.23**	**0.36**
Cattle manure digestate	15	1:0	16.45	21.81	8.47	2.14	0.33
		3:1	15.49	20.91	9.53	2.20	0.36
		4:1	16.08	21.83	10.38	2.07	0.32
	On average (F_B)		**16.01**	**21.52**	**9.46**	**2.14**	**0.34**
	30	1:0	14.67	20.19	10.76	2.30	0.36
		3:1	15.40	20.39	9.12	2.30	0.36
		4:1	14.02	19.3	10.61	2.64	0.39
	On average (F_B)		**14.70**	**19.96**	**10.16**	**2.41**	**0.37**
On average (F_A)			**15.35**	**20.74**	**9.81**	**2.28**	**0.35**

Potassium plays a vital role in human health as it is involved in regulating muscle activity and affecting the resistance of nerve cells to stress. Its content in the dry matter of potato tubers averaged 2.28%, and the types and norms of mixed fertilizers practically had no effect on it (Table 4).

Phosphorus is involved in maintaining the acid-base balance, fat metabolism and blood circulation, it helps to restore muscles, liver and kidneys, and stabilizes the condition of teeth, hair and nails (Mitch, and Ikizler, 2012). Our research showed that the content of phosphorus in the dry matter of potato tubers averaged 0.35% and the studied factors did not change its amount. In general, the use of the mixtures of wood ash and digestate for potato fertilization and soil fertility improvement can be an efficient way to process both products and an environmentally friendly alternative to mineral fertilizers.

Conclusions

The mixtures of wood ash and biogas digestate are an innovative means of improving soil fertility. It has a positive effect on the yield of potatoes and the quality of the tuber crop.

The use of wood ash and biogas digestate mixtures for crop fertilization can be an effective way of recycling both products, and it can also be an environmentally friendly alternative to mineral fertilizers in acidic soils.

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INFLUENCES OF HIGH TEMPERATURE ON VIGOUR OF MAIZE SEEDS CULTIVATED IN THE REPUBLIC OF MOLDOVA

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Abstract

The vigour test is a sensitive indicator of seed physiological quality that provides a precise identification of important differences in physiological potential of different batches of maize seeds with similar germination percentage. Taking into account global climatic changes, the main purpose of this study was to evaluate the changes in vigour of maize seeds at supraoptimal temperatures. The study included six maize hybrids of different maturity (FAO 100 - FAO 400) obtained in 2019 from the "Porumbeni" Institute of Crop Science, the Republic of Moldova. Germination percentage, shoot length (cm) and vigour index were determined after seven days of maize seeds germination at 25^{0}C. The results revealed that the length of shoots had statistically significant differences between hybrids of early (P180) and intermediate (B203, P369) maturation ($p \leq 0.001$), as well as between hybrids of early (P180) and later maturation (P427) ($p \leq 0.01$). Initial seeds vigour of tested hybrids ranged from 402.42±43.63 to 554.20±12.84. The pre germination heat treatment of seeds at 50^{0}C during 30 min led to a significant rise in the shoots length of germinated seeds, especially in intermediate maturing maize (FAO 200-300). At the same time, a diminution in the total number of germinated seeds caused a vigour modification of experimental batches as a whole. Depending on the physiological state of the hybrid, under influence of supraoptimal temperatures, the seeds vigour decreased by 1.14-2.05 times. According to the findings, the seed vigour test could serve as a presowing technique for estimation the resistance to high temperatures of different maize hybrids.

Keywords: *Maize hybrids, Seed, Vigour, Supraoptimal temperature.*

Introduction

Maize is one of the most valuable cereal crops in the world. It occupies the second place after wheat by cultivation area and world production (FAO, 2021), providing nutrients for humans and animals and serving as a basic raw material for production of starch, oil, protein, alcoholic beverages, food sweeteners, and fuel (Ranum et al., 2014). In the Republic of Moldova (RM), the maize is also a staple agricultural crop, sown areas of which in 2020 was 546.4 thousand hectares or 35.5% of the total sown areas destined for cultivation of agricultural plants (Statistical Yearbook RM, 2021). An increase in air temperature above the norm by 2.6-3.7ºC over the past 10 years and irregular precipitations have been a negative aspect of the weather for crops in RM. Because of climate changes the yields of maize for grain modified in RM in large interval from 13,4 to 58,5 quintals per hectare, that reflected in volumes of gross harvest. For example, in 2019 the gross harvest of maize in RM was 2129,9, but in 2020 – 785,2 thousand tonnes (Statistical Yearbook RM, 2021).

Laboratory determination of the sowing qualities of seeds by evaluation the energy and total germination of seed under optimal conditions often led to a discrepancy between the values of laboratory and field germination. Laboratory test for germination is not predicted the seeds emergence, what the seedlings resist to unfavorable weather conditions, develop into productive plants and produce high yield. Therefore, there is a need to applicate of more informative indicators and assessment methods of sowing qualities of seeds. Vigour index have a closer positive correlation relationship with field bio-morphological features compared to laboratory germination (Marcos-Filho, 2015; Mondo et al., 2015). The application of vigour index as indicator of maize seeds and plants resistance to various temperatures and water potentials was shown (El-Abady, 2015; Shi et al., 2020; Shah et al., 2021; Khaeim et al., 2022; Khan et al., 2022). High vigour seeds may improve crop yield in two ways: first because seedling emergence from the seedbed is rapid and uniform, leading to the production of vigorous plants, and second because percentage seedling emergence is high, so optimum plant population density could be achieved under a wide range of environmental conditions (Ghassemi-Golezani et al, 2015). The differences and possible advantages of the initial development of seedlings (vigour) and total germination under various environmental temperature regimes were reported (Tabakovic et al., 2020). Testing the modification of seeds vigour of maize supposed to supraoptimal temperatures could serve as the preliminary evaluation of heat resistance of different maize hybrids. Thus, the purpose of this study was to determine the seeds vigour of different hybrids of maize cultivated in the Republic of Moldova and its changes influenced by supraoptimal temperature.

Materials and methods

The experiments were carried out in 2021-2022 years in laboratory conditions in the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova.

Seed materials. Maize seeds of fife hybrids Porumbeni 180 (P180), Porumbeni 369 (P369), Porumbeni 383 (P383), Porumbeni 374 (P374), Porumbeni 427 (P427) and one BEMO 203 (B203), which differs by maturation period (FAO 100-400), size of seeds (tab. 1) and resistance to supraoptimal temperature, were generously contributed by the „Porumbeni” Institute of Crop Science. The seeds were collected in 2019.

Table 1. The weight of 1000 seeds

Hybrid	P180	B203	P369	P374	P383	P427
Weight, g	271.55±4.64	231.69±3.38	355.12±4.81	305.35±5.39	323.84±4.20	270.05±4.84

Laboratory testing. Each experiment consisted of 100 seeds (25 seeds on 4 replicates). Vigour determination procedure included following steps:

a) soaking seeds of maize in water for 24 hours;
b) seed treatment at 50ºC, 30 min in water bath;
c) germination of intact and treated seeds in optimal conditions. Index of total germination was determined on the seventh day as prescribed by the standard method of International Seed Testing Association (ISTA) (ISTA, 2019);
d) measure of roots and shoots length of germinated seeds;
e) calculation of vigour index as it was described early (Kerecki et al., 2021; Khan et al., 2022). Vigour = Shoot length (cm) x Seed Germination (%)

Statistical analysis. The obtained experimental data were processed by the statistical methods using the software package Statgraphics Plus 5.0. The ANOVA test was applied for variance analysis of morpho-physiological attributing characters, Student test in assessment of statistically significant differences between plots (Raudonius, 2017).

Results and discussion

The seeds of maize taken for testing had the different germination capacity (tab. 2), and index of total germination varied from 80% (P3830) to 100% (B203). Vigour estimated after 168 h germination was high in all observed batches, and with the exception of hybrid P369, was not significant differences between tested hybrids. Tabakovic et al. (2020) affirmed that the classification of seeds by fractions, contributes to the more uniform weight of the endosperm; and that this weight is important for the uniform seed vigour and germination. We did not find any correlation between seed weight and its vigour of different hybrids. Pearson correlation coefficient was equal -0.1407. In our experiments, the seeds of hybrid P369 had the highest weight for 1000 seeds, but its vigour was significantly lowest ($p \leq 0.001$). Seeds weight uniformity of single maize hybrid, probably, plays an important role in the uniformity of seedling emergence, plants development and crop maturation, but "seed properties that determine the potential for rapid, uniform emergence and development of seedlings under a wide range of field conditions" are the seed vigour, not seeds weight (Egli & Rucker, 2012).
However, the shoot/root lengths of different hybrids had the significant differences in comparison with P180 at different probability levels (tab. 2). The length of shoots had statistically significant differences between hybrids of early (P180) and intermediate (B203, P369) maturation ($p \leq 0.001$), as well as between hybrids of early (P180) and later maturation (P427) ($p \leq 0.01$).

Table 2. Morpho-physiological characters of intact maize seeds (control batches)

Hybrid	Shoot length, cm	Root length, cm	Vigour	Germination, %
P180	6.07±0.18	10.76±0.36	529.64±12.28	88
B203	5.13±0.25***	12.12±0.47**	554.20±12.84	100
P369	4.22±0.25***	9.13±0.41**	402.42±13.63***	96
P374	5.25±0.19**	12.36±0.52*	484.75±15.64	92
P383	6.33±0.25	12.02±0.48*	506.35±14.53	80
P427	5.36±0.25**	8.57±0.27***	485.21±18.62	90

*; **; *** - statistically significant difference with P180 at $p \leq 0.05$; $p \leq 0.01$ and $p \leq 0.001$, respectivelly

The maize seeds supposed to treatment by temperature at 50^0C during 30 min had the different physiological response in dependence on traits of hybrids (tab. 3). Take into account the shoot/root length and vigour of seeds germinated after thermotreatment, the hybrids can be separated, conditionally, into three groups. First, the heat resistant hybrids were B203 and P369, the shoot lengths of which were longer in comparison with control and its vigour diminished only by 3-10%.

Table 3. Morpho-physiological characters of treated maize seeds

Hybrid	Shoot length, cm	Root length, cm	Vigour
P180	5.38±0.18*	8.94±0.38***	346.29±16.25***
B203	6.66±0.30***	14.45±0.55**	538.08±15.77
P369	5.15±0.28*	9.04±0.46	354.62±16.17*
P374	5.17±0.24	9.96±0.66**	329.03±24.13***
P383	4.57±0.41***	5.83±0.72***	247.96±22.64***
P427	5.32±0.28	7.88±0.42	397.73±21.78***

*; **; *** - statistically significant difference with control batches at $p \le 0.05$; $p \le 0.01$ and $p \le 0.001$, respectively

Another group included the hybrids P374 and P427, the vigour of which was significantly affected ($p \le 0.001$) and reduced by 20-30%. The hybrids P180 and P383 were no heat resistant, the shoot and root lengths were significantly shorter and index of vigour decreased by 40-50% in comparison with intact seeds (tab. 3). Their vigour index had statistically significant difference at $p \le 0.001$ compared with other hybrids. Conform of seed vigour decreasing by temperature the hybrids can be presented in followed sequence: P203 > P369 > P427 > P374 > P180 > P383. Thus, the best heat resistance was showed by hybrid B203 (tab. 3). Differential responses of maize seeds to heat stress predetermined by individual features of hybrids.

Olasoji and Ajayi (2020) studying the changes in physical and physiological quality of different maturity group of maize seed during the storage reported that seeds of early genotypes had significantly higher quality parameters. We have not noted the direct dependences of hybrid heat resistance on the seeds weight and belonging to the FAO classification. Previously (Ivanova et al., 2021), it was demonstrated that the supraoptimal temperature (50^0C) provoked the changes in mobilization of reserve substances from endosperm during seed germination and modified the metabolic efficiency. Heat effect was more pronounced in the P180 hybrid, less in the P374 and even less in the P427 hybrid, which correlated with a decrease in vigour of the same hybrids, data of which are presented in current study. The decreasing of vigour can be related to the delay in metabolic processes, namely in mobilization of reserve substances (Medeiros et al., 2019). Using accelerated aging for modeling the vigour of maize seeds, it was shown that the high vigour seeds suffered minimal changes in biochemical composition during stress, and lower tolerance of low vigour seeds was associated with reduced lipid and protein content and increased amino acids, carbohydrates and phosphorus compounds in the embryo (Andrade et al., 2020). The absolute content of protein in seeds correlate with their vigour and could be used for rapid screening of maize (Wen et al., 2018).

Conclusion

Changes in vigour of maize seeds supposed to supraoptimal temperature allowed us to distribute the tested hybrids by their resistance to heat. These results obtained in laboratory condition needs to be confirmed by field experiments, but they could serve as a presowing estimation of tolerance capacity of different maize hybrids to abiotic stress.

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CHARACTERISTICS OF THE CONTENT OF LIPOPHILIC COMPOUNDS IN PROPOLIS AND SELECTED TYPES OF HONEY

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Abstract

Honey was one of the first foods of humans originating directly from nature. Nowadays, many studies are devoted to the composition and medicinal properties of honey. In this work, the analysis of lipophilic compounds, including sterols and triterpenoids, in four types of honey: buckwheat, linden flower, colza, honeydew honeys, and propolis was performed. The analyses were made by gas chromatography-mass spectrometry method (GC-MS) in diethyl ether extracts obtained from the samples of honeys and propolis. Aliphatic compounds (fatty acids, long-chain alkanes); phytosterols (campesterol, sitosterol, stigmasterol); steroid ketones (sitostenone, tremulone); neutral triterpenoids, e.g., alcohols as amyrins and betulin, ketone friedelin (only in linden flower honey); triterpenoid acids, e.g., oleanolic, ursolic, maslinic, corosolic and pomolic acids; and vitamin E were identified. The highest total content of triterpenoids and steroids was noticed in propolis (1115 µg/g), linden flower honey (68 µg/g), followed by buckwheat (58 µg/g) and colza (39 µg/g) honeys, whereas the lowest content was found in honeydew honey (22 µg/g). Sterols and triterpenoids can be considered as bioactive compounds, however, they occur in honey in too small amounts to exert directly health-promoting or therapeutic activity, although they can act synergistically with other compounds, as phenolics. Moreover, phytosterols and triterpenoids, as compounds synthesized by the plants, can potentially be applied as the markers of the origin of honey. The comparison of antioxidative potential of tested honeys and propolis with the use of DPPH test showed that propolis has the highest antioxidant properties, followed by the buckwheat honey.

Keywords: *honey, steroids, triterpenoids, propolis.*

Introduction

Honey was one of the first foods of humans originating directly from nature. It has been used as important source of carbohydrates in the human diet, as well as the only widely available natural sweetener to improve the taste of various food products. Nowadays, due to the growing interest in health-promoting properties of honey, many studies are devoted to its composition and biological activities (Zhou *et al*., 2012; Abeshu and Geleta, 2016). The most well known component of honey is sugar (mainly fructose and glucose, but honey contains also much less amounts of disaccharides as sucrose or maltose); in simplification, honey can be characterized as an impure, supersaturated sugar solution. However, the composition of honey is much more than just sugar, hence the variability of different honeys in such features as color, aroma, flavor. Important known ingredients of honeys are various organic acids, volatiles, phenolic compounds, mineral salts, amino acids, proteins, enzymes and even vitamins found in trace amounts (Zhou *et al*., 2002; Ball, 2007; Wilczyńska, 2010). Honey has been used as medicine in many cultures for a long time. It is one of naturally existing remedies that has been applied in the treatment of wounds and skin infections. Moreover, honey has been demonstrated to exert anti-inflammatory and immune-boosting properties, a broad spectrum of antibacterial activity, and antioxidant potential. Some reports indicate that honey prevents and treats gastrointestinal disorders such as peptic ulcers, gastritis and gastroenteritis. It also poses prebiotic effects and promotes health of gastrointestinal tract (Abeshu and Geleta, 2016). Antioxidant potential of honey is mainly attributed to the occurrence of phenolic compounds (e.g., phenolic acids and flavonoids as quercitin and hesperetin), ascorbic acid, carotenoid derivatives, organic acids, amino acids and enzymes as glucose oxidase and catalase (Abeshu and Geleta, 2016). Various types of honey can significantly differ in the content of these compounds (Wilczyńska, 2010; Deng *et al.*, 2018; Pentoś *et al*., 2020). Other biological properties, as antibacterial activity, can also vary according to the honey composition and origin (Weston, 2000, Różańska, 2011). Despite the growing number of reports on honey as medicinal agent, various studies indicated high variability in chemical composition as well as in potential medicinal uses among honeys of different floral origin. Therefore, if honey is considered to be used as therapeutic agent, standardization and quality control is highly recommended (Simova *et al*.; 2012; Abeshu and Geleta, 2016; Yelin and Kuntadi, 2019). The aim of the present was the analysis of less characterized honey ingredients, i.e., lipophilic compounds, including sterols and triterpenoids. These phytochemicals can be potentially considered as markers of the honey origin. Four types of honey: buckwheat, linden flower, colza, honeydew honeys, and one propolis sample were selected for investigation. Although propolis is not actually honey, it is an important honeybee product used in traditional medicine, known for its pharmacological properties, including antibacterial, antiseptic, anti-inflammatory, and anesthetic activities (Anjum *et al*., 2019).

Material and methods

Buckwheat honey and linden flower honey were obtained in 2019 from the apiary in Rogoża (Warmian-Mazurian voivodeship in Poland); colza honey and propolis from the apiary in Niedźwiada Duża (Lublin voivodeship); honeydew honey from the BeeBee apiary in Pruszyn (Podlaskie voivodeship). The analyses were made in 2019 and 2020 in Department of Plant Biochemistry, Faculty of Biology, University of Warsaw. Samples of 10 g were prepared from each type of honey, and dissolved in 20 mL of water. The solutions were extracted 3 times with 50 mL portions of diethyl ether in a glass funnel, afterwards the diethyl ether was evaporated under reduced pressure in a rotary evaporator. The solution of propolis was prepared by dissolving a 1 g sample in 10 mL of diethyl ether.

Obtained diethyl ether extracts were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Two fractions were obtained as described earlier (Woźniak *et al*., 2018): free (non-esterified) steroids and triterpenoids, and triterpenoid acids. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, whereas fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane. Nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether, and held at 2 °C for 24 h.

Analyses were performed with the use of an Agilent Technologies 7890A gas chromatograph (GC–MS) (Perlan Technologies). The system was equipped with a 5975C mass selective detector, a G4513A autosampler, and a 30 m × 0.25 mm i.d., 0.25-μm, HP-5MS UI column (Agilent Technologies, Santa Clara, CA, USA). The following temperature program was applied: the start at 160 °C (2 min), an increase to 280 °C at 5 °C/min, and the final temperature of 280 °C held for 44 min. The other employed parameters were as follows: the carrier gas (helium, 1 mL/min), inlet and FID (flame ionization detector) temperature 290 °C; quadrupole temperature 150 °C; ion source temperature 230 °C; EI ionization energy 70 eV; scan range, m/z 33–500; MS transfer line temperature 275 °C; FID gas: hydrogen 30 mL•min−1; air 400 mL/min. Wiley 9th ED. and NIST 2008 Lib. SW Version 2010 were used in GC-MS data analysis. Individual compounds were identified by comparing their mass spectra with library data, and/or their chromatographic mobility and corresponding mass spectra with those of authentic standards. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol (Rogowska et al., 2022).
The 10 mg/ml etanolic solution of propolis and honey samples were prepared to measure free radical-scavenging activity with the use of the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Styczyński *et al*., 2020). 2 ml of 0.1 mM DPPH in ethanol was added to 2 ml of ethanol containing different amounts of the analyzed propolis or honey solution to produce final concentrations of 0 (control), 20, 40, 60,120 and 200 µg/ml. The absorbance at 517 nm was measured after 10, 20 and 30 min. The antioxidant properties were compared according to IC_{50} values, calculated as the concentration of antioxidant solution needed to decrease the initial DPPH concentration by 50%.

Results and discussion

GC-MS analysis of diethyl ether extracts obtained from honey and propolis samples revealed the presence of several types of compounds: aliphatic hydrocarbons, fatty acids, long-chain alcohols; fragments of molecules of phenolics (probably derived from the natural polyphenolic compounds as a result of electron bombardment during mass spectrometry analysis); steroids, including phytosterols; neutral triterpenoids (alcohols and ketones); triterpenoid acids; tocopherols.
In all analyzed samples, a group of the most common phytosterols typical for the higher plants, i.e., campesterol, sitosterol and stigmasterol, were identified. Moreover, honey samples contained also another sterol, cholesterol (occurring in both plants and animals); cycloartanol (the saturated form of sterol precursor, cycloartenol; detected in all samples except for honeydew honey); and two steroid ketones, sitostenone (stigmast-4-en-3-one) and tremulone (stigmasta-3,5-dien-7-one). In the propolis sample, small amounts of another phytosterol, avenasterol (stigmasta-5,24(28)-dien-3-ol), and less typical steroids (22,23-dihydroxystigmasterol and 3-hydroxyspirost-8-en-11-one) were also detected.
The occurrence of triterpenoids was more diverse than steroids. Among the neutral triterpenoids, only the ursane-type alcohol α-amyrin, accompanied by its ketone form, α-amyrenone, were found in all analyzed samples. In contrast, oleanane-type alcohol, β-amyrin, was detected only in propolis, whereas its ketone form, β-amyrenone, in colza honey. Lupane-type alcohol betulin (with two hydroxyl groups), was found in propolis; whereas friedooleanane-type ketone, friedelin, in linden flower honey.

All analyzed samples contained triterpenoid acids belonging to lupane-, oleanane- and ursane-types, i.e., betulinic acid, oleanolic acid and ursolic acid, respectively. Both oleanolic and ursolic acids were accompanied by their ketone derivatives, 3-oxo-oleanolic and 3-oxo-ursolic acids. The occurrence of acids with additional hydroxyl groups, i.e., maslinic acid, was detected in all samples except for colza honey; pomolic acid was found in propolis as well as in linden flower and honeydew honeys; whereas corosolic acid exclusively in propolis. Oleanolic and ursolic acids with additional double bond (olean-2,12-dien-28-oic acid and ursa-2,12-dien-28-oic acid) were detected only in the propolis sample. Apart from free forms of triterpenoid acids, they were also detected as naturally occurring methyl esters; oleanolic acid methyl ester in samples of colza, linden flower and honeydew honeys, ursolic acid methyl ester in linden flower and honeydew honeys, whereas betulinic acid methyl ester only in the propolis sample.
The results of quantitative determination of all identified compounds are presented in Table 1.

Table 1. The content of steroids and triterpenoids detected in diethyl ether extracts obtained from propolis and honey samples.

Compound	Propolis	Buckwheat honey	Colza honey	Honeydew honey	Linden flower honey
	Content (μg/g)				
Steroids:					
cholesterol	37.55	6.85	1.45	0.74	3.15
campesterol	37.84	3.56	2.16	0.46	4.66
stigmasterol	96.00	1.42	0.31	0.58	3.55
sitosterol	242.28	25.04	17.89	3.62	17.75
cycloartanol	23.41	0.76	2.10	n.d.	2.67
avenasterol	11.22	n.d.	n.d.	n.d.	n.d.
tremulone	15.89	6.91	2.42	0.92	4.44
sitostenone	13.98	1.74	1.10	0.77	2.69
Sum of steroids	*478.17*	*46.28*	*27.43*	*7.09*	*38.91*
Triterpenoids:					
α-amyrenone	15.24	0.50	0.46	0.67	1.82
α-amyrin	32.89	2.24	1.79	0.62	3.11
β-amyrenone	n.d.	n.d.	2.10	n.d.	n.d.
β-amyrin	45.14	n.d.	n.d.	n.d.	n.d.
betulin	2.20	n.d.	n.d.	n.d.	n.d.
friedelin	n.d.	n.d.	n.d.	n.d.	1.69
oleanolic acid	62.86	1.88	1.82	8.82	11.99
betulinie acid	11.64	n.d.	n.d.	n.d.	n.d.
ursolic acid	59.00	1.69	0.78	0.88	1.19
maslinic acid	2.03	2.54	n.d.	2.58	0.88
corosolic acid	2.44	n.d.	n.d.	n.d.	n.d.
pomolic acid	225.25	n.d.	n.d.	0.34	0.34
3-oxo-olean-12-en-28-oic acid	51.21	2.15	1.12	0.31	0.57
3-oxo-urs-12-en-28-oic acid	23.58	1.02	0.30	0.50	0.37
olean-2,12-dien-28-oic acid	15.44	n.d.	n.d.	n.d.	n.d.

ursa-2,12-dien-28-oic acid	63.21	n.d.	n.d.	n.d.	n.d.
betulinic acid methyl ester	25.05	n.d.	n.d.	n.d.	n.d.
oleanolic acid methyl ester	n.d.	n.d.	3.51	0.22	2.71
ursolic acid methyl ester	n.d.	n.d.	n.d.	0.46	3.81
Sum of triterpenoids	*637.18*	*12.02*	*11.88*	*15.4*	*28.48*
Total	*1115.35*	*58.3*	*39.31*	*22.49*	*67.39*

n.d. –not detected

The most complex composition and the highest content of both steroids and triterpenoids were noticed for propolis. Steroids constituted 43% of all identified compounds in the propolis sample, with predominating sitosterol (50% of all steroids). The total content of triterpenoid acids was almost 6-fold higher than the amount of the neutral triterpenoids.
The occurrence of significant amounts of triterpenoids in propolis is not surprising according to the previous reports (Yam-Puc *et al.*, 2019). In contrast, the data concerning the content of triterpenoids and steroids in honey are scarce and not precise (Yelin and Kuntadi, 2019).
The total contents of steroids and triterpenoids in the samples of honey analyzed in the present study were relatively low as compared with results obtained for propolis. The highest steroid and triterpenoid content was determined for linden flower honey, followed by buckwheat honey, whereas the lowest content was noticed for honeydew honey (Table 1). The proportion between the amount of steroids and triterpenoids was different in the analyzed honey samples, steroids constituted 80% of the total content of the identified compounds in buckwheat honey, 70% in colza honey, 58% in linden flower honey, and only 31% in honeydew honey. Sitosterol was the predominating compound among steroids, whereas oleanolic acid among triterpenoids in all analyzed honey samples.
The comparison of antioxidative activity of tested honeys and propolis determined with the use of DPPH test showed that propolis has the highest antioxidant properties. Among the honey solutions, the highest scavenging activity was noticed for the buckwheat honey, followed by the honeydew honey. The IC_{50} values calculated for the analyzed samples are presented in Table 2 (the highest antioxidant activity is connected with the lowest IC_{50} value).

Table 2. The values of IC_{50} parameter of DPPH-scavenging test calculated for the analyzed propolis and honey solutions.

Sample solution	IC_{50} [mg/ml]
Propolis	0.76
Buckwheat honey	4.21
Colza honey	7.07
Honeydew honey	5.31
Linden flower honey	8.05

The obtained results confirmed earlier findings on significant variability of biological activities of various types of honey (Deng *et al.*, 2018; Pentoś *et al.*, 2020). Regarding IC_{50} values, the buckwheat honey has almost twice higher antioxidant potential than linden flower honey.

Conclusions

The present study revealed the occurrence of low-polar lipophilic compounds, including steroids and triterpenoids, in analyzed samples of propolis and selected types of honey. Due to the relatively low content of these compounds, they do not seem to be solely responsible for the main bioactivities and health-promoting properties attributed to honey and honey-related products, however, they might act synergistically with other compounds, e.g. phenolics. The composition of plant-derived phytosterols and triterpenoids occurring in honey can potentially be applied as the markers of the origin of honey, however, their significance as marker compounds requires further studies.

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RESULTS OF TESTING THE QUALITY OF WORK OF DIFFERENT TYPES OF CROP SPRAYER NOZZLES

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Abstract

The quality of the work of the nozzles of the pesticide application machine is very important having in mind their function. In order to perform successfully, accurately, and efficiently protection of crops from biological agents, it is necessary that the sprinklers perform quality and uniform leakage of working fluid with a jet of appropriate shape, and droplet size with deposition on plant parts. According to ISO 10625, at an operating pressure of 2 bar, the nozzles must have a flow rate of 1.3 [l min^{-1}], and at an operating pressure of 3 bar a flow rate of 1.6 [l min^{-1}]. Bearing in mind the fact that rarely do sprayers achieve these flow values according to EN 13790 is a deviation of up to 5% for new sprayers in relation to the nominal capacities. The goal of our research was to determine the quality of work of different types of nozzles depending on the magnitude of the working pressure and the height of the nozzle. In the tests, the workflow determined liquids on several types of sprinklers, at an operating pressure of 2 and 3 bar. To test the flow AAMS-Salvarani measuring equipment was used in accordance with European standards according to EN 13790. The use of this measuring equipment provides the possibility for precision in determining the quality of work and irregularities in work. The obtained results indicate that it exists a significant influence of the value of working pressure and height on the quality of sprayer operation. Based on the obtained results, it was concluded that the lowest deviation of the working fluid flow of 1.35 was % measured at an operating pressure of 2 bar for type B nozzles, while the largest deviation from 34.61% was measured at a working pressure of 3 bar in type C sprinklers.

Keywords: *Nozzle, Flow, Quality of work, Working pressure, Distribution.*

Introduction

The quality of a spray application is directly influenced by the components of the sprayer (Ferguson *et al.*, 2015 and 2016; Massola *et al.*, 2018), application parameters (Forney *et al.*, 2017; Hassen *et al.*, 2013), and climate conditions (De Cock *et al.*, 2017). In this scenario, pesticide application technology plays a key role in determining the best parameters for each situation, which may vary according to the target as well as the pesticide being used (Cooper *et al.*, 2007; Srivastava, 2014; Nuyttens *et al.*, 2017). Nozzles play a key role in defining droplet size, shape and angle of outlet jet, liquid quantity, and uniformity of distribution (Faqiri *et al.*, 2005; Sedlar *et al.*, 2013; Tadic *et al.*, 2014; Višacki *et al.*, 2014; Griesang *et al.*, 2021). According to the ISO 10625 standard, nozzles at an operating pressure of 2 bar must achieve a flow of 1.3 [L min^{-1}], and at 3 bar a flow of 1.6 [L min^{-1}] (Wolf *et al.*, 2006). As it is rare for a sprayer to achieve such a flow according to the European standard EN 13790, the permissible deviation of new nozzles is up to 5% in relation to the nominal capacity. Uniformity of liquid distribution is expressed by the coefficient of variation (CV) of the spray flow so that from 1-10% - excellent distribution from 10-12% - good, from 12-15% - satisfactory, and over 15% - bad (Urošević *et al.*, 2010). For the "Mlaz" sprayer, the average flow was 1.58 [L min^{-1}], the coefficient of variation was 1.99%, and for the "Kovin" sprayer, 1.61 [L min^{-1}] with a coefficient of variation of all 0.75% (Banaj *et al.*, 2014). At an operating pressure of 2 bar, the flow rate of the "Andrić" sprayer was 0.81 [L min^{-1}] with a coefficient of variation of 7.38%, and the sprayer "Kovin" was 1.33 [L min^{-1}]-coefficient variations of 1.01%, while at a pressure of 3 bar the flow was 1.00 [L min^{-1}] and 1.61 [L min^{-1}], respectively (Tadić *et al.*, 2010). The average flow rates ranged from a minimum of 0.788 [L min^{-1}] sprayer D to a maximum of 1.384 [L min^{-1}] for sprayer E. The average operating fluid flow rates of sprayers A, B and C, were 1.332 [L min^{-1}], 1.005 [L min^{-1}] and 1,045 [L min^{-1}], respectively, while the coefficient of variation ranged from 12.94 [%] sprayer C to 27.42 [%] - sprayer E (Đokić *et al.*, 2018). The smallest deviations according to the ISO standard at a pressure of 2 and 3 bar were achieved by sprayers manufactured by Mlaz (+ 1.81% at a pressure of 2 bar and + 0.81% at a pressure of 3 bar) and Laznik (-2.93% at a pressure of 2 bar and - 1.01% at a pressure of 3 bar). The largest deviations according to the ISO standard at an operating pressure of 2 and 3 bar were achieved by the Andrić sprayers (-58.83% at a pressure of 2 bar and -64.44% at a pressure of 3 bar) and AG (+ 9.95% at a pressure of 2 bar and + 3.70% at a pressure of 3 bar) (Tadić *et al.*, 2012). The aim of the research was to determine the flow of nozzles from different manufacturers depending on the defined parameters, how much their deviation is in relation to the defined standards and to recommend their use.

Material and Methods

In the production conditions of central Serbia, in the vicinity of Kragujevac during 2021. tests were carried out on the quality of work of different types of field sprayers. Flow tests of various nozzles were performed with AAMS-Salvarani measuring equipment (flow meter S001), in accordance with the inspection control according to the European standard EN 13790 which is part of the European directive 2009/128/ EC, 2006/42 / EC. These standards prescribe methods and equipment for testing machines and apparatus for pesticide application. Sprayers from four different manufacturers with the designation 110 04 made of plastic were tested: Hardi - type A and ASJ SF - type B, or brass: Andrić type C and Kovin type D. The measurement included 16 nozzles of each type. The pressure was measured with a manometer with a diameter of 100 mm with a division accuracy of 0.6, and before the start of the measurement, the manometer was checked on a control-tester. The liquid flow of different nozzles was determined at operating pressures of 2 bar and 3 bar, respectively, and the flow values were read in 5 replicates. The air temperature during the measurement was 20^0 C, relative humidity 87%, and the wind speed was in the range of 0.3 - 2.9 [m s^{-1}]. The obtained results were processed using Microsoft Office Excel 2007.

Results and Discussion

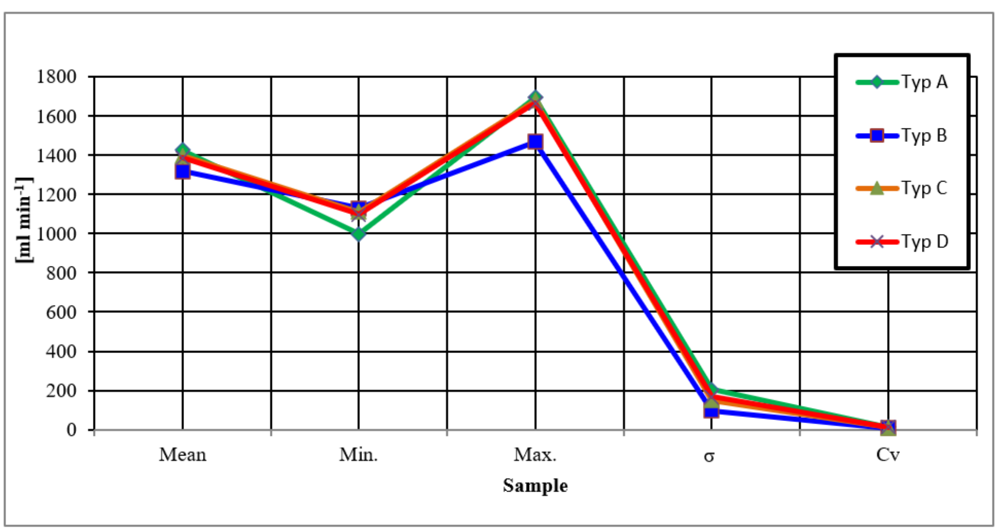
Tables 1-2 and Graphs 1-2 show the results of the research. Based on the obtained results, it is noticed that the liquid flow on the tested nozzles was uneven, with a significant deviation from the declared values.

The average flow values of the sprayer at an operating pressure of 2 bar varied from 1317.51 [ml min^{-1}] - sprayer type B to 1424.94 [ml min^{-1}] sprayer type A, which is also the highest average value fluid flow of the tested nozzles. For type C sprayers, the liquid flow was 1387.51 [ml min^{-1}] and for type D sprayers 1397.8 1 [ml min^{-1}]. The coefficient of variation ranged from 7.14 for Type B sprinklers to 14.13 for Type A sprinklers (Table 1).
Assessing the realized differences in the obtained values of the working fluid flow on the tested nozzles at an operating pressure of 2 bar, it can be noticed that the measured flow values on the type A sprinkler were significantly higher than other types of nozzles, while between nozzles type B, C and D statistically significant differences in working fluid flow (Table 1).

Table 1. Fluid flow of the tested nozzles at an operating pressure of 2 bar

Parameters	Nozzle type Type A	Type B	Type C	Type D
	Average flow on nozzles [ml min^{-1}]			
Mean	1424.94*	1317.51	1387.06	1397.81
σ	204.97	96.25	167.96	150.57
Cv	14.13	7.14	12.23	11.10
Min	998.02	1129.12	1098.24	1111.33
Max	1693.12	1468.15	1664.17	1679.21
Variation ± [%]	+9.61	+1.35%	+6.70%	+7.52%
LSD *	5%	100.59		
	1%	132.30		

Bearing in mind that according to the ISO 10625 standard at an operating pressure of 2 bar, the nozzles should achieve a working fluid flow of 1300 [ml min^{-1}], which rarely happens, a deviation of 5% is allowed for new nozzles compared to the declared values - standard EN 13790. Analyzing the values of measured working fluid flows on the tested nozzles at an operating pressure of 2 bar, it is noticed that the largest deviation according to the standard was achieved with type A nozzles +9.61%, and the lowest with type B nozzles +1.35%. For type C nozzles, the deviation was +6.70%, and +7.52% for type D nozzles (Table 1).



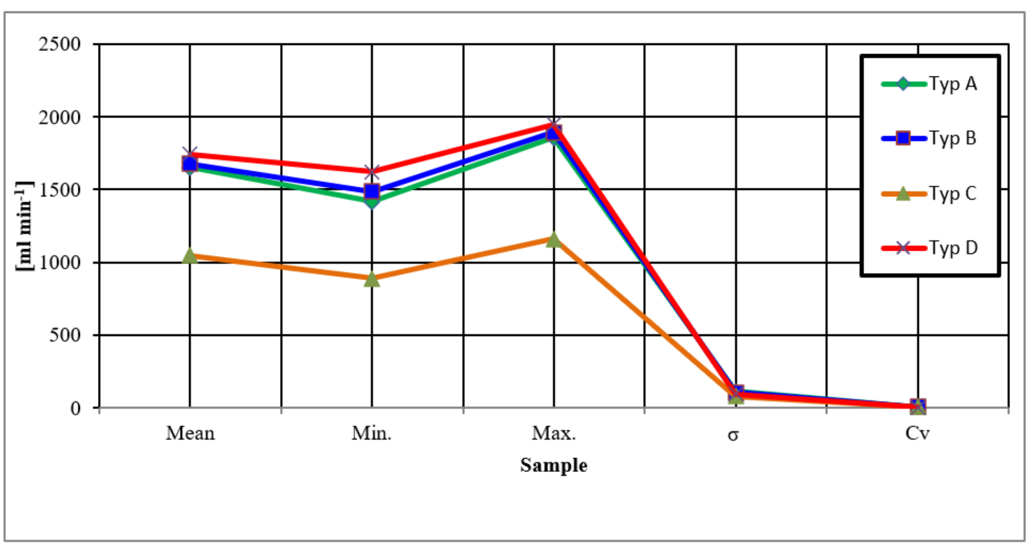
Graph 1. Statistical indicators of flow of tested nozzles at a pressure of 2 bar

Table 2 shows the flow values of the tested nozzles at an operating pressure of 3 bar. The obtained results show that the average values of liquid flow varied in the range from 1046.25 [ml min^{-1}] for type C nozzles to 1741.75 [ml min^{-1}] for type D nozzles. For type A nozzles, the liquid flow was 1653.13 [ml min^{-1}] and for type B nozzles 1678.31 [ml min^{-1}]. The coefficient of variation ranged from 5.19 for type D nozzles to 8.17 for type C nozzles (Table 2). Analyzing the obtained differences in the flow of working fluid on the tested nozzles at an operating pressure of 3 bar, it can be seen that statistically significantly lower average values of the flow of working liquid were measured on the type C nozzle compared to other tested nozzles. There were no statistically significant differences in working fluid flow between type A, B, and D nozzles (Table 2). According to the ISO 10625 standard, at an operating pressure of 3 bar, the nozzles should achieve a working fluid flow of 1600 [ml min^{-1}], which is rare, so a deviation of 5% is allowed for new nozzles in relation to the declared values - standard EN 13790.

Table 2. Fluid flow of the tested nozzles at an operating pressure of 3 bar

Parameters	Nozzle type Typ A	Typ B	Typ C	Typ D
	Average flow on nozzles [ml min^{-1}]			
Mean	1653.13	1678.31	1046.25**	1741.75
σ	112.9	106.28	81.14	95.81
Cv	7.10	6.13	8.17	5.19
Min	1417.15	1488.14	890.03	1623.24
Max	1855.13	1894.10	1162.18	1947.14
Variation ± [%]	+3.32	+4.89	-34.61	+8.86
LSD *	5%	125.80		
	1%	165.40		

Based on the obtained results, it can be stated that the largest deviation according to the above standard was achieved on the type C nozzle, -34.61% in relation to the declared values, and the smallest on the type A sprayer + 3.32%. The deviation was +4.89 for type B sprinklers and 8.86% for type C nozzle (Table 2).



Graph 2. Statistical indicators of flow of tested nozzles at a pressure of 3 bar

Similar results on the influence of defined parameters on the flow of working fluid on nozzles in their research are given by other authors (Tadic *et al.*, 2012 and 2914; Sedlar *et al.*, 2013; Višacki *et al.*, 2014; Banaj *et al*, 2014; Tadic *et al.*, 2014; Ferguson *et al.*, 2015 and 2016; Massola *et al.*, 2018; Đokić *et al.*, 2018; Griesang *et al.*, 2021).

Conclusions

Based on the obtained results, it can be concluded that different values of liquid flow were measured on the nozzles that deviated from the declared values. Average flow values at an operating pressure of 2 bar ranged from 1317.51 [ml min^{-1}] - type B sprayer to 1424.94 [ml min^{-1}] type A sprayer. The measured values of flow on the type A nozzle were significantly higher compared to other types of sprinklers, while there were no statistically significant differences between type B, C, and D nozzles. The highest deviation of the liquid flow at a working pressure of 2 bar according to the ISO 10625 standard was achieved with the nozzle type A + 9.61%, and the smallest with the nozzle type B + 1.35%. Average fluid flow values at an operating pressure of 3 bar ranged from 1046.25 [ml min^{-1}] for type C nozzle to 1741.75 [ml min^{-1}] for type D nozzles. Statistically significantly lower average values of working fluid flow were measured on the type C sprayer compared to other tested nozzles. There were no statistically significant differences in working fluid flow between type A, B, and D nozzles. The highest deviation according to the ISO 10625 standard was achieved on the nozzle type C, -34.61% in relation to the declared values, and the lowest deviation on the sprayer type A + 3.32%.

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EFFICIENCY OF CORN HYBRIDS GROWING TECHNOLOGIES DEPENDING ON THE KINDS OF FERTILIZER APPLICATION

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Abstract

Substantiation of efficiency of corn cultivation technologies using different types and methods of fertilizer application are presented in the paper. Field research was established in the field crop rotation of FE "Bogatyrivske" Romensky District of Sumy Region, Ukraine during 2019-2021 on dark gray podzolic soil. Statistical data processing was performed using the software package SAS 9.4. Hybrids are quite flexible to growing conditions and respond positively to nutrition optimization. The yield changes on average for 2019–2021 from 6.07 to 8.50 t/ha. Application of $N_{22}P_{57}K_{57}$ (active substance) in the form of diamophos provided a yield increase by 0.99 - 1.01 t/ha or 16.4%, and the application of nitrogen fertilizers against this background provided an additional yield increase by 0.57 - 1.33 t/ha or 7.95 - 18.6%. With the introduction of KAC 32 , the yield increase of the hybrid EC Concord amounted to 2.19 t/ha or 36.1%; hybrid EC Asteroid 2.44 t/ha or 38.0% compared to the control version. The reaction of hybrids to the application of ammonia water and urea, which was found due to increased yields, was lower compared to the application of KAC 32. The profitability of corn grain production is extremely high – 44 - 80%. In combination of Humilin Stimul with all types of fertilizers, the highest effect was obtained with a single extra-feeding in the microstage BBCH 15-17. The energy efficiency coefficient for fertilizer application is reduced to 4.81 - 5.49, depending on the forms of fertilizers.

Keywords: *thermal units, yield, yield index.*

Introduction

Research on the impact of climate change on agro-climatic conditions for growing crops, their yields, and gross output in a particular region and in the Left Bank Forest-Steppe, in particular, is relevant. Corn, in terms of production and consumption, came out on top in the world among cereals (FAO, 2020). Corn is an intensive type of crop that requires significant production costs to form a high yield, which often exceeds other cereals. Corn with C4-type photosynthesis is able to effectively use environmental factors even in stressful conditions. Advances in corn breeding contribute to the production of high-yielding, innovative hybrids, which in turn are extremely demanding on growing conditions and technologies. (Shafi et al, 2012; Sharifi et al, 2009). Under changing climatic conditions and growing technologies, the adaptability of corn hybrids plays an important role (Ruiz, B. M., et al, 2019; Kalenska, S. et al, 2020; Ross et al, 2020). Corn responds effectively to optimizing plant nutrition through productivity growth (Chassot et al, 2001; Lopushniak et al, 2011). Genetic progress in grain yield has been achieved mainly through an increase in the yield index or harvest index. The harvest index is also an important indicator of varieties and hybrids' adaptation to local conditions (Egli, 2022).

Corn needs increased mineral nutrition, primarily due to the long growing season and the ability of plants to absorb nutrients almost to the end of grain ripening (Pierson & Warren, 2013). The use of new generation fertilizers with macro- and microelement composition, nanofertilizers, and long-acting fertilizers provides targeted use of nutrients by plants (Batsmanova et al, 2020; Balawejder et al, 2019; Novytska et al., 2020). Optimization of plant nutrition is closely related to the preservation of soil fertility, microbiological biodiversity, and environmental safety (Yang et al, 2022).

The increase in corn yield is closely correlated with the rate of nitrogen fertilizers and their effectiveness increases with the combined use of nitrogen fertilizers (Paponov et al, 2003; Rossini et al, 2018; Sajid et al, 2015). The efficiency of corn nutrients is closely related to soil conditions, moisture supply, heat resources, and root system development (Casali et al, 2022; Zhoua et al, 2019; Yan et al, 2011; Smetanska et al, 2021; Duvick & Cassman, 1999). In favorable weather conditions, weather and nitrogen factors combine to provide high yields (Trachsel et al, 2016). The largest intake of macronutrients (42–81%) occurs during the period of active growth of vegetative mass (Neilson et.al., 2015). The formation of crop yields depends significantly on the supply of nitrogen, while the efficiency of its use is determined by meteorological conditions (Fernández & Rubio, 2015). Thus, if from the introduction of relatively small doses of nitrogen on average in a few years the yield of basic products can increase by only 10%, in years with favorable moisture conditions the yield increase can be 50% or more (Khalili et.al., 2013). Corn initially needs only 25% of the nitrogen it needs (Pierson & Warren, 2013). It was studied that in the initial phases of growth nitrogen uptake is insignificant (3-9%). Nitrogen enters the plant more intensively starting from the phase of 6-8 leaves. Thus, if before the phase of 8 leaves only 2-3% of nitrogen is absorbed, then from the phase of 8 leaves to the phase of drying of flower columns on the cobs about 85% of the total amount of nitrogen is absorbed (Paponov & Engels 2003). The need for this nutrient increases rapidly after the formation of 10 leaves. According to research results, prolonged nitrogen nutrition of corn provides high efficiency (Balawejder et al, 2020; Shafi et.al., 2012). The most intensive absorption of nitrogen occurs in the period from 10-12 leaves to the milky ripeness of the grain. Maximum potassium occurs in the first half of the growing season. In the early stages of the growth and development of corn plants, due to undeveloped root system, suffer from a lack of phosphorus, manganese, and zinc. In the phase of intensive growth of corn plants, the need for these elements is high because they activate the enzymatic activity (Fernández & Rubio, 2015). To achieve high corn yields, it is critical to maintaining adequate nitrogen levels throughout all phases of crop growth and development. Nitrogen application in several ways optimizes plant nutrition and reduces unproductive losses, ensuring an increase in individual productivity and grain yield.

Material and methods

In 2019-2021, two field experiments were established on dark gray podzolic soils (Andriyashivka village, Romenskyi district, Sumy region). Experiment 1 - two-factor: *factor A* "hybrid": EC Concord and EC Asteroid; *factor B*: "fertilizers": 1) without fertilizers - control; 2) $N_{22}P_{57}K_{57}$ (diamophos) - background; 3) background + N_{120} (ammonia water); 4) background +

N_{120} (KAS 32); 5) background + N_{120} (urea). Field studies were performed with medium-ripe hybrids (factor C) EC Concord (FAO 250) and EC Asteroid (FAO 290).
The sowing of corn was carried out in the period from 15 to 23 April. Harvesting was carried out in sections. Prior to harvest, control samples were taken to determine crop structure and grain quality. Repetition - quadruple. Location of plots - randomized. The area of accounting area is 50 m^2. Variational and statistical data processing was performed by methods of correlation and variance analysis using software "MS Office 2010", "Statistica 6". Statistical data processing was performed with using software package SAS 9.4.

Results and discussion

The years of research differed significantly in terms of hydrothermal parameters, which allowed us to objectively assess the yield of the studied hybrids and identify their response to different types of nitrogen fertilizers in the weather conditions of the research years. In all years of research, April and May had a significant amount of precipitation that significantly exceeded the long-term amount of precipitation in the study region (Table 1).
Significant moisture reserves contributed to the emergence of friendly seedlings. The total amount of precipitation during the active growing season (June - October) was lower compared to long-term data. The only exception was August 2021, during which 61.3 mm fell compared to 58.0 mm of average long-term precipitation. Moisture supply was particularly critical, also in all years, in August, September, and October. Lack of moisture had a negative effect on the reduced weight of 1000 seeds.

Table 1. Average monthly precipitation and coefficients of deviation materiality of average monthly precipitation from long-term data

Month	Perennial average monthly precipitation, mm 2008-2018	Average monthly precipitation, mm				Hydrothermal coefficient[1]		
		2019	2020	2021	2019-2021	2019	2020	2021
April	36,0	33,1	32,3	67,2	44,2	2,0	3,6	9,7
May	57,0	67,5	147,2	83,4	99,3	1,4	4,6	2,1
June	73,0	42,0	54,0	73,1	56,4	0,6	0,8	1,2
July	72,0	56,2	60,4	51,5	56,0	1,0	0,9	0,7
August	58,0	9,7	29,6	61,3	33,5	0,1	0,5	0,9
September	44,0	40,3	33,5	6,8	26,9	1,0	0,7	0,1
October	40,0	22,4	30,1	0,01	17,5	1,0	1,0	0,01

Note[1] HTC: <0.4 - very severe drought; 0.4 - 0.5 - severe drought; 0.6 - 0.7 - average drought; 0.8 - 0.9 - mild drought; 1.0 - 1.5 - enough moisture; > 1.5 - excessively humid.

In general, the temperature regime of the research years was favourable for the growth and development of corn hybrids. Average daily temperatures differed little from perennial temperatures, with some exceptions. Compliance of thermal resources with the requirements of corn hybrids is a critical condition for hybrids selection for precocity, a realization of genetic potential, yield formation, and grain quality. It is important for the study region to establish the optimal sowing dates and the end of the growing season, which is related to the temperature regime. In order to assess the growing conditions of corn hybrids, we calculated the accumulation of thermal units (CHU) during the growing season of plant growth and development according to the method of Brown & Bootsma, 2014. The peculiarity of the CHU calculation is that the calculation is carried out for each day and the minimum and maximum temperatures are taken into account. The sum of CHU more objectively characterizes the thermal resources of a particular area compared to the sum of active and effective temperatures. The years differed in terms of heat input - both in absolute terms and the length of the period of receipt. A significant difference between the years was actually in the period before sowing and emergence. Thus, 2021 was characterized by a fairly long period of "entering" the active stage of CHU accumulation in the spring. The stable transition to CHU accumulation took place only on April 10, but further CHU accumulation took place mainly due to daytime heat units and lasted until the end of the 1st decade of May.

During the active vegetation of corn plants (May - October) 2021 was accumulated - 3602 CHU (tabl.3). The difference compared to the period May - September was 276 CHU the period May - November - only 46 CHU. It should be noted that in 2021 there was a maximum supply of thermal resources compared to 2020 and 2019 - respectively 3511 and 3438 CHU. The amount of CHU before May and after October has no significant effect on the total. In October, in all years there was still an accumulation of thermal units, which indicates that during this period there may be a full-fledged filling of grains and an increase in the weight of 1000 seeds.

Table 2. Sum of thermal units for periods (CHU)

Period, month	Year			Average 2019-2021
	2019	2020	2021	
IV-XI	3615	3686	3891	3731
IV-X	3550	3686	3845	3694
IV-IX	3342	3323	3570	3412
V-XI	3503	3523	3648	3558
V-X	3438	3511	3602	3517
V-IX	3230	3148	3326	3235

Hybrids are plastic to growing conditions and with a high response to fertilizers. The range of yield changes for 2019–2021, EC Concord hybrid ranged from 6.07 (control) to 8.26 t/ha with the application of KAS 32 (N_{120} kg/ha a. s.) against the background of $N_{22}P_{57}K_{57}$ (diamophos); EC Asteroid - from 6.16 to 8.50 t/ha, respectively (Table 3).

Table 3. Yields of corn hybrids depending on the application rate and type of fertilizer

Fertilizer, *factor B*		Year			Average	Yield growth	
Fertilizer rate, kg/ha a.s..	Type of fertilizer	2019	2020	2021		t/ha	%
Concord(*factor A*)							
Control, without fertilizer		$5,31^a$	$6,55^a$	$6,34^a$	$6,07^a$	-	-
$N_{22}P_{57}K_{57}$ – background	Diamophos	$6,23^b$	$7,61^b$	$7,35^b$	$7,06^b$	1,00	14,1
background + N_{120}	Ammonia water	$6,81^c$	$8,18^c$	$8,02^c$	$7,67^c$	1,60	20,9
background + N_{120}	KAS	$7,37^d$	$8,93^d$	$8,48^d$	$8,26^d$	2,19	26,6
background + N_{120}	Urea	$6,45^b$	$8,59^{cd}$	$8,17^c$	$7,74^c$	1,67	21,6

Asteroid (*factor A*)							
Control, without fertilizer		5,18[a]	6,80[a]	6,49[a]	6,16[a]	-	-
$N_{22}P_{57}K_{57}$ – background	Diamophos	6,38[b]	7,69[b]	7,44[b]	7,17[b]	1,01	14,1
background + N_{120}	Ammonia water	6,71[b]	8,30[c]	8,22[c]	7,74[c]	1,59	20,5
background + N_{120}	KAS	7,34[c]	9,20[d]	8,96[d]	8,50[d]	2,34	27,6
background + N_{120}	Urea	6,97[bc]	8,49[c]	8,41[c]	7,96[c]	1,80	22,6

The yield index (*YI*) differs significantly for corn hybrids, with different fertilizer systems and weather conditions - 0.31 - 0.51. With the introduction of N_{120} on the background of $N_{22}P_{57}K_{57}$, a much higher corn hybrids yield is formed, and the vegetative mass is also formed more intensively. Under favourable weather conditions, the *YI* has a significant range of variation. The total mass of plants and grain yield, even for a certain hybrid, varies significantly depending on the fertilizer in wet years, and in dry years the variation is insignificant. Due to the fact that the *YI* for the application of additional nitrogen was lower in all years and for all hybrids, with some exceptions. There is a tendency to change the *YI* depending on the type of fertilizer - during the introduction of KAS, the *YI* in all years was stable and was in the range of 0.48 - 0.51, depending on weather conditions.

Conclusions

Optimization of corn hybrids nutrition with the use of different types of fertilizers, and evaluation of economic and energy efficiency of cultivation technologies allowed to establish their high economic and practical feasibility. Corn hybrids are quite malleable and respond positively to nutrition optimization - yields vary from 6.07 to 8.50 t/ha. Application of $N_{22}R_{57}K_{57}$ in the form of diamophos provides an increase in yield by 0.99 - 1.01 t/ha or 16.4%. Additional application of nitrogen fertilizers on the background of diamophos provides an increase in yield from 0.57 to 1.33 t/ha or 7.95 - 18.6%. With the introduction of KAS 32, the increase in yield of the EC Concord was 2.19 t/ha; EC Asteroid - 2.44 t/ha compared to the control. The yield index differs for the cultivation of corn hybrids, with different fertilizer systems and weather conditions - 0.31 - 0.50. Under favourable weather conditions, the yield index has a significant range of variation and depending on the rate and type of fertilizer in wet years, and in dry years the variation is negligible.

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CROP PRODUCTION: FOOD SECURITY AND SOLUTIONS IN UKRAINE

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Abstract

Identifying the most important factors that make it possible to produce more food in the world and ensuring their efficient and rational use is crucial. Changes in the structure of plant use (food, feed, bioenergy) are exacerbating the problem of food security in the world. Nowadays, in order to solve food security problems, it is necessary to preserve and restore plant biodiversity. Climate change is causing the increasing use of plants with C4 type photosynthesis. Protection of natural ecosystems will ensure the biodiversity conservation. Stabilization of crop production, land use efficiency and innovative technologies for growing crops are interdependent. Field research was conducted in LLC "Biotech LTD", which is located in the central part of Boryspil district, Kyiv region. The farm is located in the Left Bank Forest-Steppe of Ukraine. The field experiment was conducted on dark gray podzolic soil. Growing sorghum hybrids in line with the principles of adaptive crop production must be carried out in accordance with the requirements of sustainable development of agriculture and environmental protection. The latest forms of fertilizers, the use of which involves the targeted use of nutrients, prolonged action of fertilizers underlie the plant nutrition system. The latest technologies for growing crops require an increase in energy consumption and resources per unit of output, leads to a growing impact on the environment, which requires a constant balanced assessment of technologies for energy efficiency.

Keywords: *food security, climate change, grain sorghum*.

Introduction

The global food and energy crises, land use management and environmental protection are pressing issues in the world and the success of their solution requires significant changes and innovative decisions. The expected increase in the world's population requires an increase in crop yields of more than 70% over the next 30 years to meet human needs (FAO, 2020; Alexandratos and Bruinsma, 2012). This growth should be achieved without increasing arable land and while limiting the use of fertilizers and pesticides. Addressing this impending agricultural crisis will be one of our biggest scientific challenges in the coming decades, and success will need significant improvements on many levels. Improving the efficiency and productivity of photosynthesis in agricultural plants will be important to address this major problem (Baslam et al, 2020; Ort et al., 2015; Lawlor, 1995; Novytska et al, 2020). Due to selection it is necessary to increase the yield by + 2.4% per year, while the current figure is only + 1.3% (FAO, 2020). Areas of breeders

activity in recent decades have been associated with increasing yields and quality, improving the response of plants to abiotic stresses, and the issue of photosynthesis has not been given enough attention (Leng and Hall, 2019; Long et al., 2015). At the same time, abiotic stresses such as heat, drought, and flooding will tend to reduce crop yields by up to 50% by 2050 unless specific management practices are introduced to shape crop productivity. Droughts are projected to increase in intensity and severity, increasing the risk of crop losses by 24% for soybeans, 21% for corn, 18% for rice, and 20% for wheat (Leng and Hall, 2019). Drought is one of the main abiotic stresses that limits crop production due to limitations of photosynthesis (Adotey, 2021). Droughts have already caused the loss of 1,820 million tons of grain over the past 4 decades (Lesk et al., 2016). Currently, a number of researches are being conducted with plant systems that aim to increase crop yields through photosynthetic efficiency and productivity, which have an important impact on the global issue of crop productivity and bioenergy production (Honchar et al., 2021; Eremenko et al., 2019; Batsmanova et al., 2020; Kalenska et al., 2021; Long et al., 2006). On the other hand, the predicted increase in the level of CO_2 in the atmosphere as a substrate for photosynthesis can contribute to an increase in yield up to 30% depending on the plant species and environmental conditions (Liu et al., 2022; Long et al., 2006; Sanz-Saez et al., 2017). Photosynthesis is highly sensitive to abiotic stresses (Chiluwal et al., 2018). Increased temperature and drought can offset the positive effects of increased CO_2 on yields (Ruiz-Vera et al., 2013; Gray et al., 2016). Advances in genomics, genetics, and modelling tools have paved the way for increased photosynthetic efficiency resulting in increased yields under climate change scenarios (Zhu et al., 2010; Long et al., 2015; Ort et al., 2015).
Increasing the share in agricultural production of photosynthetic plants that fix carbon on the C4 type of photosynthesis is one of the ways to reduce the greenhouse effect or biosequestration. C4 plants make up about 5% of plant biomass and 1% of known plant species, but at the same time they account for about 30% of terrestrial carbon fixation. Among cultivated plants, C4 plants are important - their productivity is from 33 to 38% of the total productivity of crops. Plants with C4 type photosynthesis have a rapid rate of growth, development, synthesis of organic matter. At high temperatures and relatively low concentrations of CO_2, plants most effectively capture carbon to form carbohydrates through C4 photosynthesis rather than through the more common C3 pathway (Vicentini et al., 2008). With optimal supply of moisture and nutrients, crops of corn and sugar cane are the most productive of the known agrocenoses. C4 type photosynthesis plants, in addition to food, are widely used for biofuel production (Sendžikienė et al., 2012). C4 plants are resistant to arid conditions, high temperatures, able to grow in salinity and lack of CO_2. Photosynthesis changes significantly under the influence of abiotic factors (Dusenge et al., 2019).
Grain sorghum is a valuable high-yielding crop with C4 type photosynthesis, which ranks fifth among cereals in terms of world production (Smith & Frederiksen, 2000; Cousins et al, 2003; Adotey et al.2021). With climate change and uses, culture is becoming increasingly popular in Ukraine. The last decades were characterized by periodic arid conditions during the growing season of spring cereals, which led to a significant decrease in yield. Sorghum is an alternative crop under such conditions. If earlier this crop was treated as a source of green mass needed to meet the needs of livestock, now it is interesting for grain producers (Smith & Frederiksen, 2000). Grain sorghum has significant economic and agro-technical value, advantages in terms of versatility of use - raw materials for the production of food, biofuels, feed and more (Ostemeyer, 2022; Sendžikienė et al., 2012).

The main aim of the study is to establish the patterns of growth and development of grain sorghum plants, photosynthetic activity, features of yield formation and grain quality depending on nutrition and inter-row spacing in north part of Ukraine.

Material and Methods

Field research was conducted during 2015-2017 in LLC "Biotech LTD", which is located in the central part of Boryspil district, Kyiv region. The farm is located in the Left Bank Forest-Steppe of Ukraine. The field experiment was conducted on dark gray podzolic soil. The humus content in the soil - 2.8%, pH - 6.0. In general, the soil is highly supplied by mobile compounds of phosphorus, and potassium and is very lowly provided by easily hydrolyzed nitrogen. Soil quality is 59 point. The study area is not typical for grain sorghum cultivation in Ukraine. Traditionally, grain sorghum is grown in the south - in the Steppe zone. However, climate change has led to the promotion of this culture in the northern regions of Ukraine. The climate of the study area is moderately warm and moderately humid. The sum of active temperatures during the growing season is 2500 - 2600°C. About 75% of precipitation falls during the growing season, which is favorable for productivity formation. However, rainy periods lasting 18-20 days, and in some cases 35-45 days, are also typical. The temperature regime of the vegetation period in 2015 was close to the average long-term values, and in 2016 and 2017 these parameters had slight deviations in some months. In 2015 and 2017, precipitation was significantly lower during the period of active sorghum vegetation than the average long-term data. Only in May and October 2015, 13.7 and 3.6 mm more precipitation fell than the long-term dose, and in July the amount of precipitation was close to the long-term average. In May 2016, 126.7 mm more precipitation fell, in June - 82.9 and in October - 81.9 mm, but the lack of precipitation was observed in August and September. In 2017, during the active part of the growing season, there was a constant lack of precipitation (8.2-44.9 mm below normal) and only in October, there were 44.6 mm more than long-term indicators. In general, climatic conditions were favorable for growing grain sorghum. Harvesting was carried out in sections. Prior to harvest, control samples were taken to determine crop structure and grain quality. Repetition - quadruple. Location of plots were randomized. The accounting area is 50 m^2.Statistical data processing was performed with using software package SAS 9.4.

Results and Discussion

Photosynthetic activity of grain sorghum crops

Photosynthetic activity of crops is an important factor in grain sorghum yield formation because C4 type photosynthesis plants are much more demanding to provide solar energy and its absorption by the photosynthetic system than C3 type photosynthesis plants (Dembele et al., 2021; Bollam et al, 2021; Cousins et al, 2003). The formation of crops with optimal overall and optical density, and large contact surfaces of the leaf surface with intense sunlight is relevant because the yield depends on leaf area and productivity of photosynthesis (Smith & Frederiksen, 2000). The formation of the leaf surface area depends on plant nutrition and weather conditions. It was found that the maximum leaf surface area of crops was formed by applying $N_{60}P_{60}K_{60}+N_{60}$ and inter-row spacing of 70 cm. The dry matter accumulation in plants and the accumulation of vegetative mass by crops of grain sorghum were formed under the influence of weather, fertiliszers. Optimal for the accumulation of dry matter were options with the inter-row spacing of 50 cm and of fertilizer application $N_{60}P_{60}K_{60}+N_{40-60}$. In the period after the end of flowering to full maturity of plants, the increase in dry matter per unit area was mainly due to the formation and maturation of seeds. The maximum accumulation of dry matter on average in 2015-2017 was characteristic of the ‘Brigga F1’ hybrid with application $N_{60}P_{60}K_{60}+N_{40-60}$ and inter-row spacing of 50 cm and in the phase of full maturity was 16.2 and 16.3 t/ha, respectively. The photosynthetic potential of sorghum crops in the period of "flowering-full maturity" was maximum. Hybrids differed in the intensity of photosynthetic potential - ‘Lan 59’ ranged from 1450 to 1832; ‘Brigga F1’ - 1856 - 2397; ‘Burggo F1’ - 1854 - 2398 thousand m^2/ha × day. With increasing rates of nitrogen fertilizers, photosynthetic potential increased. The net productivity of photosynthesis during the growing season reached its highest in the period of "exit into the tube-ejection of the panicle". During the period of "flowering-full maturity" net productivity of photosynthesis decreases. The net productivity of photosynthesis in the growing with the inter-row spacing of 50 cm in all studied hybrids was the most effective.

Yield structure and yield

Productive tillering depends on the hybrid, the uniformity of plant placement in crops and fertilizers. The hybrid ‘Lan 59’ plants formed 1.06–1.54; 'Briggo F1'–2.76 - 3.24; ‘Burggo F1’ - 2.16–2.64 productive stems/plant. Productive tillering of plants of all hybrids was greater with the inter-row spacing of 35 cm and application $N_{60}P_{60}K_{60}+N_{60}$. As the row spacing increased, the productive tillering of plants of all hybrids increased. In the panicle of grain sorghum was laid - 493 - 1013 pieces. The yield of grain sorghum hybrids on average during the years of the study varies from 4.89 to 8.69 t/ha. Fluctuations in yield in terms of years and the studied factors ranged from 3.98 to 9.14: 'Lan 59' - 3.98-6.03; ‘Brigga F1’ - 6.49–9.14; ‘Burggo F1’ - 6.45–8.49 t/ha (Table 1). Variety 'Lan 59' provided, on average over the years of research, the maximum yield with the inter-row spacing of 50 cm - 5.73 t/ha. Hybrids reacted differently to changes in inter-row spacing. ‘Brigga F1’ with the inter-row spacing of 35 cm formed a yield of 7.78 t/ha; 50 cm - 8.48; 70 cm - 7.93 t/ha. The ‘Burggo F1’ hybrid generated the highest yield with the inter-row spacing of 50 cm - 7.86 t/ha. With the pre-sowing application of N_{60} compared to N_{20}, the yield of hybrid ‘Lan 59’ increased by 0.41–0.51 t/ha; ‘Brigga F1’ - 0.27–0.40; ‘Burggo F1’ - 0.22–0.29 t/ha.

Table 1. The yield of grain sorghum hybrids depending on inter-row spacing and nitrogen fertilizer rates, t/ha

Fertilizing rate, kg/ha *factor C*	Year								
	2015			2016			2017		
	Inter-row spacing, cm, *factor B*								
	35	50	70	35	50	70	35	50	70
	‘Lan 59’, *factor A*								
$N_{60}P_{60}K_{60}$	$^{e}5.20^{a}$	5.21^{a}	4.70^{a}	4.49^{a}	4.50^{a}	3.98^{a}	4.99^{a}	5.16^{a}	4.50^{a}
$N_{60}P_{60}K_{60} + N_{20}$	5.36^{a}	5.49^{a}	4.85^{a}	4.66^{a}	4.78^{a}	4.22^{a}	5.26^{a}	5.39^{a}	4.76^{a}
$N_{60}P_{60}K_{60} + N_{40}$	5.76^{b}	6.00^{b}	5.32^{b}	5.01^{b}	5.21^{b}	4.62^{b}	5.65^{b}	5.89^{b}	5.23^{b}
$N_{60}P_{60}K_{60} + N_{60}$	5.79^{b}	6.03^{b}	5.35^{b}	5.03^{b}	5.24^{b}	4.65^{b}	5.69^{b}	5.92^{b}	5.25^{b}
	Briggo F1								
$N_{60}P_{60}K_{60}$	7.82^{c}	8.60^{d}	8.02^{c}	6.49^{c}	7.52^{d}	6.98^{c}	7.68^{cd}	8.37^{cd}	7.85^{c}
$N_{60}P_{60}K_{60} + N_{20}$	8.08^{c}	8.83^{d}	8.28^{c}	7.02^{cd}	7.68^{d}	7.20^{c}	7.93^{d}	8.67^{d}	8.14^{d}

$N_{60}P_{60}K_{60} + N_{40}$	8.46^{d}	9.11^{e}	8.53^{d}	7.36^{d}	7.92^{e}	7.41^{e}	8.31^{e}	8.94^{d}	8.37^{d}
$N_{60}P_{60}K_{60} + N_{60}$	8.50^{d}	9.14^{e}	8.56^{d}	7.39^{d}	7.95^{e}	7.45^{e}	8.35^{e}	8.98^{d}	8.41^{d}
	Burggo F1								
$N_{60}P_{60}K_{60}$	7.52^{c}	7.95^{c}	7.51^{d}	6.45^{c}	6.87^{c}	6.55^{c}	7.39^{c}	7.84^{c}	7.23^{b}
$N_{60}P_{60}K_{60} + N_{20}$	7.74^{c}	8.19^{c}	7.77^{d}	6.73^{cd}	7.12^{c}	6.76^{d}	7.60^{cd}	8.04^{c}	7.64^{c}
$N_{60}P_{60}K_{60} + N_{40}$	7.97^{c}	8.45^{d}	7.99^{c}	6.93^{cd}	7.35^{d}	6.95^{d}	7.83^{d}	8.30^{cd}	7.85^{c}
$N_{60}P_{60}K_{60} + N_{60}$	8.00^{c}	8.49^{d}	8.01^{c}	6.96^{cd}	7.38^{d}	6.96^{d}	7.86^{d}	8.33^{cd}	7.87^{c}

Note: Values in a row followed by the same letter are not significantly different at $P \leq 0.05$.

With the pre-sowing application of N_{60} compared to N_{20}, the yield of hybrid ‘Lan 59’ increased by 0.41–0.51 t/ha; ‘Brigga F1’ – 0.27–0.40; Burggo F1’ - 0.22–0.29 t/ha. There is a positive correlation between yield and nitrogen application rate (r = 0.49). The studied factors had a different share of participation in the sorghum yield formation: "hybrid" – 43%; "conditions of the year" – 21; "fertilizer rate" – 20; "inter-row spacing" – 10%. Grain sorghum, when grown on soils with low nitrogen content, responds positively to the introduction of nitrogen, which is manifested through increased yields.

Grain quality of grain sorghum

The protein content in sorghum grain variety ‘Lan 59’ is 10.5 –11.7%; ‘Brigga F1’ –10.8–11.7; ‘Burggo F1’ – 10.7 – 11.9% (Table 2).

Table 2. The content of protein and starch in grain sorghum depending on the elements of growing technology, %, the average for 2015 - 2017.

Hybrid (factor A)	Fertilizer rate, kg/ha (factor C)	Protein			Starch			Oil		
		Inter-row spacing, cm (factor B)								
		35	50	70	35	50	70	35	50	70
‘Lan 59’	$N_{60}P_{60}K_{60}$	10.5	10.6	11.0	75.5	75.4	75.5	3.22	3.35	3.39
	$N_{60}P_{60}K_{60} + N_{20}$	10.6	10.7	11.1	75.5	75.3	75.4	3.22	3.33	3.40
	$N_{60}P_{60}K_{60} + N_{40}$	10.9	11.2	11.5	75.4	75.1	74.8	3.27	3.42	3.42
	$N_{60}P_{60}K_{60} + N_{60}$	11.0	11.4	11.7	75.2	74.7	74.3	3.34	3.43	3.50
‘Brigga F1’	$N_{60}P_{60}K_{60}$	10.8	10.8	11.0	73.6	73.6	73.0	1.93	2.38	2.09
	$N_{60}P_{60}K_{60} + N_{20}$	10.8	10.9	11.0	73.5	73.5	72.9	2.02	2.37	2.08
	$N_{60}P_{60}K_{60} + N_{40}$	11.0	11.2	11.4	73.4	73.3	73.2	2.06	2.31	1.85
	$N_{60}P_{60}K_{60} + N_{60}$	11.2	11.6	11.7	73.1	73.2	72.6	2.08	2.48	2.18
‘Burggo F1’	$N_{60}P_{60}K_{60}$	10.7	10.9	11.1	73.6	73.3	73.0	2.16	2.72	2.45
	$N_{60}P_{60}K_{60} + N_{20}$	10.8	11.0	11.5	73.5	73.2	73.0	2.16	2.72	2.39
	$N_{60}P_{60}K_{60} + N_{40}$	11.2	11.4	11.9	73.5	73.1	72.7	2.13	2.61	2.84
	$N_{60}P_{60}K_{60} + N_{60}$	11.3	11.6	11.8	73.2	72.6	72.6	2.47	2.73	2.68

The share of influence of factors on the protein accumulation in sorghum grain is: "fertilizer rate" – 52%; "inter-row spacing" - 32%; "hybrid" – 9%. The starch content in the grain of sorghum hybrids varies from 72.6 to 75.5%. With the introduction of nitrogen for pre-sowing tillage, there is a tendency to reduce the starch content, as there is an inverse correlation with the protein content. The starch content in grain sorghum is determined by 44% of the properties of the hybrid; 21% –the rate of nitrogen fertilizers; 20% – inter-row spacing.

Sorghum grain has a high-fat content compared to other cereals. The fat content in the grain of the hybrid ‘Lan 59’ is higher compared to other hybrids - 3.22 - 3.50%; ‘Brigga F1’ - 1.85 - 2.48; ‘Burggo F1’ - 2.13 - 2.72%.

Conclusions

Preservation of plant biodiversity, efficient use of photosynthetically active radiation, expansion of crops with different types of photosynthesis, and innovations in cultivation technologies are the basis for crop production to ensure food security and raw materials for industrial processing. Grain sorghum is a promising grain crop of universal use for growing in the Ukraine. Yields of hybrids on average over the years of the study ranged from 4.89 to 8.69 t/ha. Fluctuations in yield over the years and depending on fertilizers and inter-row spacing were 3.98 - 9.14, including hybrids:‘Lan 59’ - 3.98–6.03; ‘Brigga F1’ - 6.49–9.14; ‘Burggo F1’ - 6.45–8.49 t/ha. The maximum yield of all hybrids was formed at the inter-row spacing of 50 cm: ‘Lan 59’ - 5.40; ‘Brigga F1’ - 8.48; ‘Burggo F1’ - 7.86 t/ha. With an increase in the rate of pre-sowing nitrogen to N_{60} compared to N_{20}, the yield increased by 0.22–0.51 t/ha; With the change of nitrogen from N_{20} to N_{60}, the protein content in the grain of the Burggo hybrid increased to 11.8%. The fat content in sorghum grain was - 1.85 - 3.79%; while that of starch was 72.6 - 75.5%. The chemical composition of grain sorghum grain indicates that the directions of grain use are multi-vector.

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MODERN BIOTEHNOLOGICAL APPROACHES TO THE BERRY CROPS STUDY (*VACCINIUM CORYMBOSUM* L.)

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Abstract

The biotechnology development promotes the new approaches to the study of economically valuable crops – the biochemical and molecular markers usage (proteins and nucleic acids). Modern methods allow solving the problem of genotype identification, as well as a number of issues related to the property rights protection, plant material safety control, the appreciable varieties conservation, etc. For the first time the highbush blueberry *Vaccinium corymbosum* L. molecular genetic identification was carried out and the genetic passports were elaborated using the SCoT marker system. This marking system provides the possibility of checking the variety conformity of planting material. The obtaining data will also can be used for copyright protection and in marker-associated plant breeding of the *Ericaceae* JUSS. family. Proteomics makes it possible to characterize plant species and to identify putative molecular markers of species and varieties and to reveal protein markers of the functional state of the plant organism. For the first time the total proteome investigation of *Vaccinium corymbosum* L. by 2D - electrophoresis were performed using the Protean i12 IEF Cell automatic station (Bio-Rad, USA) and the proteomic maps of five high bush blueberry varieties has been obtained. The proteomic maps (biochemical passports) will make it possible to develop methods for plant biological productivity and to carry out a quick selection of crops that are promising for biotechnological production. The proteomic maps as well will be useful as the test systems of the plant organism state at different stages of its growth and development or to identify the targets of regulatory impact. The obtained results develop the biology of valuable berry crops as well as the scientific approaches to their usage in the economics and biotechnology.

Keywords: *Vaccinium corymbosum* L., *proteomic map, genetic passport, biotechnology, copyright protection.*

Introduction

To meet the high demand in a high-quality healthy food products and medicinal raw materials it is necessary to introduce new sources of vitamin products. The Central Botanical Garden of the National Academy of Sciences of Belarus is carrying out the *Ericaceae* JUSS. family introduction since 1980. It made it possible to develop in our republic the new direction – the comercial berry growing. To increase the quality of planting material it is necessary to study the fundamental processes of plant growth and development in order to establish the possible ways of their regulation. It is also of current interest to confirm the planting material conformity to the declared variety (the genetic passports elaboration). Biotechnology development promotes a new approaches and technologies to study and analyze a plant organism genome and proteome. Modern analytical methods to study the biological macromolecules specificity make it possible to solve the problem of genotype identifying as well as a number of issues related to the property rights protection, the valuable varieties conservation and a plant material safety control (presence of pathogens), etc. The molecular markers usage based on the protein and DNA fragments polymorphism helps to solve a number of fundamental and applied problems, including the new test systems creation. These test systems will be used for the genetic polymorphism analysis in plant breeding. It is known that the key stage in hereditary information implementation is the biosynthesis of proteins. Consequently, during the life cycle some protein groups and the individual proteins may appear or disappear. Proteins are functionally, quantitatively and qualitatively responsible for the certain stage of plant development and/or for the response on the external factors influence. The determination of such proteins makes it possible to use them as a test for the plant state and as a target for regulatory impact. The purpose of the work was to carry out the DNA identification (certification) and to assess the proteomic status of the highbush blueberry plants – *Vaccinium corymbosum* L. For the first time the molecular genetic identification for *Vaccinium corymbosum* L. varieties were carried out by Start Codon Targeted (SCoT) system. This method of DNA certification allows checking the cultivar’s conformity to the original variety. For the first time by 2-D electrophoresis method the proteomic maps of the leaf tissue have been obtained. The proteomic status of highbush blueberry varieties has been estimated. The proteins that claim to be the *Vaccinium* specie markers and highbush blueberry varieties markers were identified for the first time.

Materials and Methods

The plant material

The study included 5 varieties of highbush blueberry (*Vaccinium corymbosum* L.) from the Central Botanical Garden collection (‘Atlantic’, ‘Concord’, ‘Bluecrop’, ‘Elizabeth’, ‘Weymouth’). For each variety we used the leaf tissue from three different plants (three plants per cultivar).

Analytical Methods

Total DNA preparations were obtained by CTAB extraction (Dempster, 1999) in our modification. Qualitative and quantitative analysis of the obtained DNA preparations was carried out by the spectrophotometric method. The resulting total DNA preparations met the quality requirements: $R_{260/280}$ – 1.7–1.9; $R_{260/230}$ – 1.7–2.2.

Molecular genetic certification of highbush blueberry (*Vaccinium corymbosum* L.) was carried out on the basis of total DNA multilocus marking. To carry out DNA labeling the multilocus marker system SCoT (Start Codon Targeted) was chosen (Collard, Mackill, 2009). We used a basic set of 36 SCoT primers that were being tested to obtain a highly polymorphic, reproducible markers. For the molecular genetic certification of the studied taxa 7 SCoT primers were selected (Table 1.). All primers were characterized by sufficient polymorphism and reproducible amplification activity.

Table 1. SCoT primers for *V. corymbosum* cultivars genotyping

Primer	5′→3′	Nn	GC %	T_m °C
SCoT-18	ACCATGGCTACCACCGCC	18	67	60.8
SCoT-19	ACCATGGCTACCACCGGC	18	67	60.8
SCoT-22	AACCATGGCTACCACCAC	18	56	56.3
SCoT-28	CCATGGCTACCACCGCCA	18	67	60.8
SCoT-29	CCATGGCTACCACCGGCC	18	72	62.9
SCoT-31	CCATGGCTACCACCGCCT	18	67	60.8
SCoT-35	CATGGCTACCACCGGCCC	18	72	62.9

The reaction mixture for PCR analyses (25 µl) composition: 1 × reaction buffer containing 2 mM $MgCl_2$ (Evrogen), 0.2 mM dNTPs (Evrogen), 20 mM SCoT primer (PrimeTech), 1 U of HS Taq polymerase and 30 ng of genomic DNA. PCR was performed in a SureCycler 8800 thermal cycler (Agilent). PCR protocol: initial denaturation at 95 ºC for 3 min (30 cycles, sequentially including denaturation at 95 ºC for 30 s, primer annealing (T_m) for 30 s, elongation at 72 ºC for 2 min) and final elongation at 72 ºC for 10 min. Amplification products were separated in agarose gels (1.2% Agarose, 1×TAE) prestained with ethidium bromide. Fragment lengths (bp) were calculated using Quantity One software (Bio-Rad).
The total fraction of cellular proteins from leaf tissue was obtained by TCA/acetone precipitation according to Amme (Amme S., 2005) with our modification. Proteins were purified using 2-D Clean-Up Kit (GE Healthcare). The protein pellet was dissolved in ReadyPrep Rehydration/Sample buffer (Bio-Rad). The protein amount was determined using the RC DC Protein Assay kit (Bio-Rad).
Isoelectrofocusing (IEF) was performed on an automatic station Protean i12 IEF Cell (Bio-Rad) on the immobilized dry strips (ReadyStrip™ IPG Strips, pI 3-10, 11 cm, Bio-Rad). The second direction of 2D electrophoresis (SDS electrophoresis) was carried out according to the Laemmli method (Laemmli U. K., 1970) on prepared Criterion TGX Precast Midi Protein Gel, 13.3 × 8.7 cm, 4–15%, 11 cm (Bio-Rad). Precision Plus Protein Unstained Standards (Bio-Rad) was used as a MW marker. Gels were stained with Oriole Fluorescent Gel Stain (Bio-Rad) and photographed using the VersaDoc system (Bio-Rad). The proteomic maps analysis was carried out using specialized software PDQuest 2-D Analysis Software (Bio-Rad), taking into account only a clearly visible spots. Proteins MW (kDa) were identified by comparing the experimentally calculated molecular weights with the data from UniProt database (https://www.uniprot.org/).

Results and Discussion

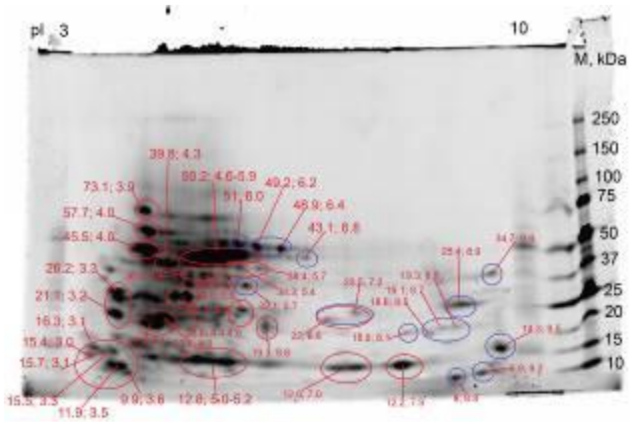
All selected primers generated a clear, reproducible markers and the set of markers was unique for each blueberry taxa. In virtue of 71 DNA markers the genetic distances were calculated and the studied genotypes of *Vaccinium corymbosum* L. varieties were clustered using the Neighbor joining (NJ) method. Based on these data the separate dendrograms were constructed for each primer as well as a complex dendrogram. The multilocus marking of the *V. corymbosum* L. genotypes by the SCoT marker system made it possible to differentiate all the studied genotypes, to develop and compose the unique profiles for each of them and to calculate the genetic distances of relationship/distance. Based on the obtained multilocus DNA spectra, the genetic passports for all the studied samples were composed (Table 2).

Table 2. Molecular genetic passports of *V. corymbosum* varieties

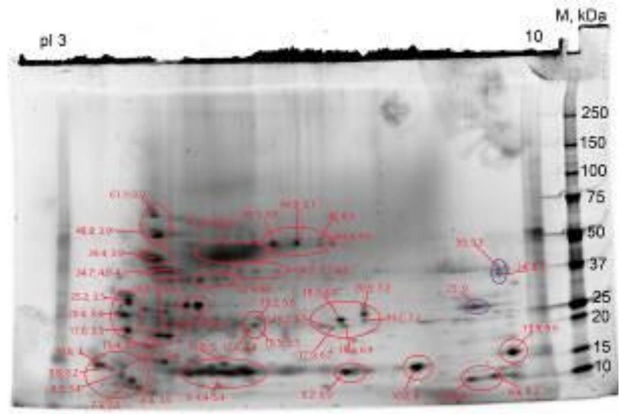
Primer	DNA marker
‘Bluecrop’	
SCoT-18	$SCoT18_{627}$, $SCoT18_{538}$, $SCoT18_{395}$, $SCoT18_{320}$, $SCoT18_{284}$, $SCoT18_{211}$, $SCoT18_{175}$
SCoT-19	$SCoT19_{614}$, $SCoT19_{568}$, $SCoT19_{479}$, $SCoT19_{413}$, $SCoT19_{338}$, $SCoT19_{226}$, $SCoT19_{175}$
SCoT-28	$SCoT28_{611}$, $SCoT28_{529}$, $SCoT28_{415}$, $SCoT28_{371}$, $SCoT28_{248}$
SCoT-31	$SCoT31_{1055}$, $SCoT31_{978}$, $SCoT31_{612}$, $SCoT31_{571}$, $SCoT31_{449}$, $SCoT31_{414}$, $SCoT31_{381}$, $SCoT31_{342}$
SCoT-29	$SCoT29_{1017}$, $SCoT29_{697}$, $SCoT29_{562}$, $SCoT29_{516}$, $SCoT29_{461}$, $SCoT29_{360}$, $SCoT29_{322}$, $SCoT29_{240}$, $SCoT29_{210}$
SCoT-35	$SCoT35_{983}$, $SCoT35_{779}$, $SCoT35_{657}$, $SCoT35_{539}$, $SCoT35_{453}$, $SCoT35_{314}$, $SCoT35_{286}$, $SCoT35_{220}$
SCoT-22	$SCoT22_{777}$, $SCoT22_{613}$, $SCoT22_{564}$, $SCoT22_{464}$, $SCoT22_{433}$, $SCoT22_{306}$, $SCoT22_{269}$, $SCoT22_{224}$
‘Atlantic’	
SCoT-18	$SCoT18_{538}$, $SCoT18_{395}$, $SCoT18_{320}$, $SCoT18_{284}$, $SCoT18_{211}$
SCoT-19	$SCoT19_{840}$, $SCoT19_{662}$, $SCoT19_{614}$, $SCoT19_{568}$, $SCoT19_{280}$, $SCoT19_{226}$, $SCoT19_{175}$
SCoT-28	$SCoT28_{529}$, $SCoT28_{424}$, $SCoT28_{371}$, $SCoT28_{248}$, $SCoT28_{168}$
SCoT-31	$SCoT31_{612}$, $SCoT31_{571}$, $SCoT31_{449}$, $SCoT31_{381}$, $SCoT31_{342}$
SCoT-29	$SCoT29_{697}$, $SCoT29_{322}$, $SCoT29_{240}$, $SCoT29_{210}$
SCoT-35	$SCoT35_{779}$, $SCoT35_{657}$, $SCoT35_{539}$, $SCoT35_{453}$, $SCoT35_{314}$, $SCoT35_{286}$, $SCoT35_{220}$, $SCoT35_{185}$
SCoT-22	$SCoT22_{645}$, $SCoT22_{564}$, $SCoT22_{464}$, $SCoT22_{433}$, $SCoT22_{286}$, $SCoT22_{224}$
‘Elizabeth’	
SCoT-18	$SCoT18_{335}$, $SCoT18_{320}$, $SCoT18_{284}$, $SCoT18_{211}$
SCoT-19	$SCoT19_{840}$, $SCoT19_{662}$, $SCoT19_{614}$, $SCoT19_{568}$, $SCoT19_{226}$, $SCoT19_{175}$
SCoT-28	$SCoT28_{581}$, $SCoT28_{529}$, $SCoT28_{424}$, $SCoT28_{371}$, $SCoT28_{248}$, $SCoT28_{168}$
SCoT-31	$SCoT31_{571}$, $SCoT31_{414}$, $SCoT31_{381}$, $SCoT31_{342}$
SCoT-29	$SCoT29_{360}$, $SCoT29_{322}$, $SCoT29_{240}$, $SCoT29_{210}$
SCoT-35	$SCoT35_{983}$, $SCoT35_{779}$, $SCoT35_{657}$, $SCoT35_{539}$, $SCoT35_{314}$, $SCoT35_{286}$, $SCoT35_{220}$, $SCoT35_{185}$
SCoT-22	$SCoT22_{645}$, $SCoT22_{564}$, $SCoT22_{464}$, $SCoT22_{433}$, $SCoT22_{286}$, $SCoT22_{224}$

Elaborated system of genetic SCoT markers makes it possible to register the genotypes of blueberry varieties in the form of molecular genetic passports which will be used in marker-associated breeding.

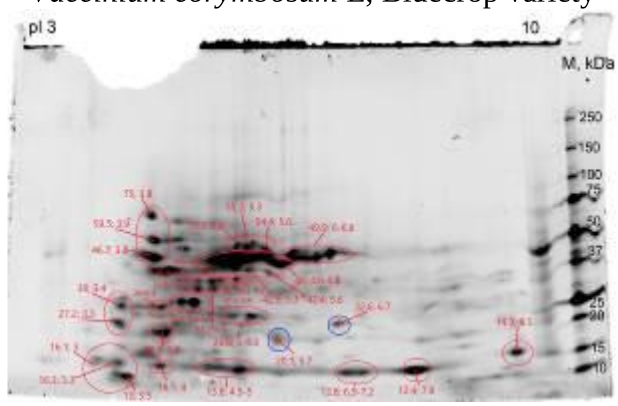
Proteomics is a promising approach that can complement genomics, transcriptomics and metabolomics. It should be emphasized that at the present time there is no a detailed study of the blueberry proteomic status. The blueberry total proteome investigation by 2-D electrophoresis using an automatic station Protean i12 IEF Cell (Bio-Rad, USA) has been performed in our republic for the first time. For the first time the total proteome screening of introduced highbush blueberry varieties, promising for breeding and industrial cultivation, was carried out.



Vaccinium corymbosum L, Weymouth variety



Vaccinium corymbosum L, Bluecrop variety



Vaccinium corymbosum L, Atlantic variety

Figure 1 – Proteomic maps of *Vaccinium corymbosum* L varieties

It was determined that the basic mass of proteins is in the range of molecular masses from 10 to 80 kDa and the pI range of 3.5 – 7.5. Protein profiles are characterized by a high degree of identity. In all blueberry samples 9 similar groups of proteins were observed, which, in our opinion, can be a potential markers of *Vaccinium corymbosum* L. specie. Besides, the differentially expressed proteins were marked on the proteomic maps. Protein bands similar for all samples were in the range of Mm 12–60 kDa, pI 3–5 and were characterized by the following parameters: 1) Mm 57.7–60.0 kDa, pI 5.2–5.5; 2) Mm 49.5–50.0 kDa, pI 5.2–5.5; 3) Mm 49.5–50.0 kDa, pI 5.2–5.5; 4) Mm 38.2–40.0 kDa, pI 4.1–6.4; 5) Mm 23.6 kDa, pI 4.4–4.6; 6) Mm

19.3–23.5 kDa, pI 4.1–7.2; 7) Mm 21.1–29.8 kDa, pI 3.4–3.7; 8) Mm 11.9–15.5 kDa, pI 3.0–3.5; 9) Mm 12.2–12.6 kDa pI 7.0–7.9.

Differentially expressed acidic proteins similar to all blueberry samples had the following characteristics: Mm 73.1 kDa, pI 3.9; Mm 57.7 kDa, pI 4.0; Mm 45.5 kDa, pI 4.0. Presumably, these proteins are the hydroxylase enzymes and the transcription factors. These proteins can claim to be the marker proteins of the *Vaccinium corymbosum* L. specie. Differentially expressed proteins can claim to be the markers of variety. On the proteomic maps some proteins with low molecular weights (predominantly a protective PR proteins and proteins (subunits) of pathogens) were revealed. Based on this data it can be concluded that the electrophoresis method is useful for pathogens testing.

Further identification of the lueberry proteins will be continued by mass spectrometric analysis.

Conclusions

Our research is both fundamental and applied. For the first time the DNA identification using the SCoT marker system was carried out. The SCoT marker system can be used to create the *Vaccinium corymbosum* L. genetic passports which will be used in copyright protection and in marker-associated plant breeding of the Ericaceae Juss family., as well as in agriculture to product the high-quality planting material the international standards corresponding.

For the first time the proteomic status of *Vaccinium corymbosum* L. was assessed and the potential biochemical markers of specie and cultivar belonging of *V. corymbosum* L. taxa were revealed.

The proteomic maps will make it possible to develop methods for plants bioproductivity determining and will allow to select promising crops for biotechnological production. In addition, the proteomic maps can be used as test systems for an organism state at different stages of growth and development or as a target for regulatory action.

The obtained results develop the biology of valuable berry crops, as well as the scientific approaches to their usage in the national economy.

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INFLUENCE OF INTERCROPPING SWEET SORGHUM WITH CLIMBING BEAN ON FORAGE YIELD AND QUALITY

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Abstract

Cereals are highly important in feeding ruminant animals for their high dry matter production and low cost. Sweet sorghum is an important silage crop and has an increasing popularity because of the need for relatively smaller quantities of water per unit dry matter production compared to maize. Regarding to high feed costs of protein supplementations, legumes can be used in livestock nutrition for their high protein content and, thus, reducing production costs. Since legumes have low dry matter yield, acceptable forage yield and quality can be obtained from intercropping cereals and legumes compared with their sole crops. Sweet sorghum (*Sorghum bicolor* L.) and climbing bean (*Phaseolus vulgaris* L.) intercropped in different sowing densities and pure sweet sorghum crop were evaluated to determine the best intercropping system with respect to forage yield and quality. The highest dry matter yield was produced by sorghum population and climbing bean (SBPV3) 22.1 t ha^{-1}, and the lowest by solo sweet sorghum (18.4 t ha^{-1}). All intercrops had higher crude protein values in dry matter 95 g kg^{-1} for the SBPV1, 105 g kg^{-1} for the SBPV2 and 115 g kg^{-1} for the SBPV3, than the monocrop sweet sorghum (80 g kg^{-1} DM). Intercropping of sweet sorghum with climbing bean reduced neutral detergent fiber, resulting in increased forage digestibility. Therefore, sweet sorghum intercropping with climbing bean could substantially increase forage quantity and quality, and decrease requirements for protein supplements as compared with sole sweet sorghum.

Keywords: *Intercropping, Sweet Sorghum, Climbing Bean, Yield, Quality.*

Introduction

Intercropping is the practice of cultivating two or more crops simultaneously on the same piece of land during the same time span (Guleria and Kumar, 2016). It is characterized by rotation and diversification in time and space dimensions (Biabania *et al.*, 2008). Intercropping systems help farmers to exploit the principle of diversity (Ghosh, 2004). They help avoid reliance on a single crop and result in different products of different nature such as fodder, oil and pulses (Iqbal *et al.*, 2018a). Another key benefit associated with intercropping is its potential to increase soil productivity per unit area and efficient utilization of farm resources (Mucheru-Muna *et al.*, 2010). Cereals intercropping with legumes result in increased resource capture by component crops and improve soil microbial activity along with improved conversion resource efficiency that drives higher biomass production (Alvey *et al.*, 2003). In addition, soil fertility improves when legumes are intercropped with cereal forages (Iqbal *et al.*, 2018b). According to Ghosh *et al.* 2006 and 2007, different crops have different root lengths and so nutrients are absorbed from different soil horizons. Intercropping of cereals with legumes also increases productivity per unit of land area due to atmospheric biological nitrogen fixation that takes place in the root nodules of legumes (Pal and Sheshu, 2001). Sorghum (*Sorghum bicolor* L.) is an important silage crop and has an increasing popularity because of the need for relatively smaller quantities of water per unit dry matter production compared to maize (Bean *et al.,* 2013). Dry bean (*Phaseolus vulgaris* L.) is a common legume cultivated for its edible seeds all over the world. It has a well-developed root system and the stems are many branched. The bean is a fast growing, warm season legume, and, it can grow in a diverse range of environmental conditions worldwide because of its adaptability. There are many types and varieties of beans grown in all the regions. However, selecting a high yielding (seed and herbage), disease resistant variety is the most important factor for successful cultivation. In addition, bean serves as an adequate source of protein. Furthermore, it can be planted alone or intercropped with other crops such as corn and sorghums. Legumes contain more than twice crude protein than forage sorghum, therefore, sorghum-legume intercropping has the potential to increase the biomass and quality of forage per area compare to sole sorghum (Eskendari *et al.,* 2009). Forage sorghum can be intercropped with forage legumes such as cowpea, cluster bean, soybean etc. which are totally compatible with sorghum in terms of sowing time and irrigation (Iqbal *et al.,* 2015). This study was designed to determine the influence of different sowing densities of sweet sorghum-climbing bean intercropping on forage yield and quality.

Material and Methods

A field experiment was carried out during the 2016 and 2017 growing seasons at he experimental fields in Daruvar (45°35'34″N, 17°13'25″E), Croatia. Meteorological data of the experimental site is presented in Table 1.

Table 1. Monthly meteorological data during the growing seasons 2016 and 2017

Year	Meteorological data	Month April	May	June	July	August	September
2016	Mean air temperature (°C)	12.3	15.5	20.4	22.3	19.4	17.0
	Rainfall (mm)	35.3	83.7	99.6	152.6	66.2	48.5
2017	Mean air temperature (°C)	10.9	16.5	21.8	22.9	22.4	14.7
	Rainfall (mm)	62.8	45.0	70.3	71.9	29.0	121.7

The experimental design was a randomized complete block system with three replications. Sweet sorghum hybrid seed "Zerberus" was obtained from Seed Company KWS. Seed of the climbing bean determinate cultivar "Meraviglia Di Venezia" was obtained from "Green Garden" company. The individual plot size was 5 m × 2.8 m for each treatment. The sweet sorghum population included 250 000 plants ha^{-1} (SB) that were spaced at 70 cm × 5.7 cm and climbing bean population had 50 000 (PV1), 75 000 (PV2) and 100 000 plants ha^{-1} (PV3) that were spaced at 70 cm × 28.6 cm, 70 cm x 19 cm and 70 x 14.3 cm, respectively, in rows alternating with sweet sorghum. Tillage was carried out in autumn by ploughing to 30 cm depth. Presowing seedbed preparation was done using a tractor-mounted rototiller. All plots were fertilized with the same amount of fertilizer before sowing, containing 150 kg of N ha^{-1}, 100 kg P_2O_5 ha^{-1} and

200 kg of K_2O ha^{-1}. Sweet sorghum and climbing bean were sown to a depth of approximately 5 cm by maize drill on May 5, 2016 and on May 3, 2017. Herbicide Dual Gold 960 EC (active substances 960 g/L S-metolachlor) was applied pre-emergence at a dose of 1 L ha^{-1} and herbicide Basagran 480 (active substances 480 g/L bentazon) was applied post-emergence in intercropping sweet sorghum with climbing bean at a dose of 2 L ha^{-1}. The soil of the experimfield ental had a pH 4.4 (M-KCl), 3.3% organic matter, poorly supplied with physiologically active phosphorous (7.6 mg P_2O_5/100 g soil), medium supplied with physiologically active potassium (22.3 mg K_2O/100 g soil), while the total nitrogen content averaged 0.15% in topsoil. The crops were hand harvested when the sweet sorghum reached at soft dough stage (give dates of harvesting in both years) and climbing bean at R7 stage and then chopped into 10 mm size pieces with a chaff cutter. The dry matter content was determined by drying in an oven at a temperature of 65°C to a constant mass. Crude protein content was measured according to Kjeldahl (AOAC, 2000) and neutral detergent fibre according to Van Soest *et al.* (1991). Analyses of variance were made for dry matter yield and forage quality parameters ($P<0.05$), and the Tukey test was used for comparing means ($P<0.05$). Data were analyzed using SAS statistical software (SAS Inst., 2013).

Results and Discussion

The differences in the yield of dry matter (Table 2) were statistically significant ($P<0.05$). Dry matter yields ranged from 21.2 t ha^{-1} (SBPV3) to 17.9 t ha^{-1} (SB) in 2016. In the following growing season of 2017, the yield of dry matter ranged from 23.0 t ha^{-1} (SBPV3) to 18.8 t ha^{-1} (SB). Consequently, dry matter yields were higher in 2017 than in 2016. This could be due to the impact of more favorable environmental factors (effect of the year) such as solar radiation, water and temperature during plant vegetation. The average yield of dry matter over the two years showed that SBPV3 (22.1 t ha^{-1}) was the best intercropping production system (Table 2).

Table 2. Yield of dry matter and yield of crude proteins of sweet sorghum and sweet sorghum-climbing bean intercropped

Treatments	Yield of dry matter in t ha^{-1}			Yield of crude proteins in t ha^{-1}		
	2016	2017	Mean	2016	2017	Mean
SB	17.9d	18.8d	18.4d	1.38d	1.56d	1.47d
SBPV1	18.5c	19.9c	19.2c	1.68c	1.97c	1.83c
SBPV2	19.7b	21.3b	20.5b	1.97b	2.34b	2.16b
SBPV3	21.2a	23.0a	22.1a	2.31a	2.78a	2.55a
Mean	**19.3b**	**20.8a**		**1.84b**	**2.16a**	

Different letters in the column indicate significant difference ($P<0.05$)

According to the results, when climbing bean had higher populations intercrop, dry matter yields on the plots increased. One of the possible explanations for higher yields for the intercrops is their ability to exploit different layers of soil without mutual competition. Besides, higher consumption of environmental resources, agronomic practices, crop genotypes, photosynthetic active radiation and soil moisture during the rainy season may affect yield and potential use of the intercropping system (Anil *et al.,* 1998; Lithourgidis et al., 2006). Basaran *et al.,* (2017) indicated that, legumes contribution to sweet sorghum in mixtures was significant and increased the dry matter yield. The intercropped maize with cowpea (*Vigna unguiculata* (L.) Walp.) and bean (*Phaseolus vulgaris* L.) produced higher dry matter yield than monocrop maize (Geren *et al.,* 2008; Uher *et al.,* 2019a, Uher *et al.,* 2019b). In this study it was found that the yield of crude protein of intercropped fodder SBPV1, SBPV2 and SBPV3 was significantly (P<0.05) higher than SB (sole crop sweet sorghum) during a two year study (Table 2). Treatment SBPV3 had the highest yield of crude protein (2.31 t ha^{-1}) in 2016 and (2.78 t ha^{-1}) in 2017 in comparison to other fodder mixtures (Table 2). According to the results, when climbinb bean had increased seed number in intercrop, crude proteins yields on parcels increased. Basaran *et al.,* (2017) indicated that, legumes contribution to sweet sorghum in mixtures was significant and increased the yield of crude proteins. One of the main reasons of intercropping sweet sorghum and climbing bean is the increase crude protein level in silage. Crude proteins are very important in cattle feed and, silage containing more crude proteins is desirable. In this study it was found that the value of crude protein of intercropped fodder SBPV1, SBPV2 and SBPV3 was significantly (P<0.05) higher compared to SB (sole crop sweet sorghum) during a two year research (Table 3).

Table 3. Content of crude protein and neutral detergent fiber (NDF) of sweet sorghum and sweet sorghum-climbing bean intercropped

Treatments	Crude protein in g kg^{-1} dry matter			Neutral detergent fiber in g kg^{-1} dry matter		
	2016	2017	Mean	2016	2017	Mean
SB	77d	83d	80d	550a	532a	541a
SBPV1	91c	99c	95c	511b	493b	502b
SBPV2	100b	110b	105b	488c	462c	475c
SBPV3	109a	121a	115a	465d	445d	455d
Mean	**94b**	**103a**		**504a**	**483b**	

Different letters in the column indicate significant difference (P<0.05)

According to the results, when climbing bean had higher populations in intercrop, the content of crude protein in the mixture increased. Armstrong *et al.* (2008) found that climbing bean intercropped with corn had the greatest potential among the climbing beans to increase crude protein concentration compared with monoculture corn. The intercropping of maize (*Zea mays* L.) with climbing bean (*Phaseolus vulgaris* L.) may serve as a way to increase crude protein and improve the overall nutritive value of silage (Grobelnik *et al.,* 2005, Uher *et al.,* 2019b). The findings in this study are consistent with other research in which legumes also increased the concentration of crude proteins when grown in mixture with sweet sorghum (Basaran *et al.,* 2017). Fodder produced in sweet sorghum-climbing bean intercrops is important not only because of an increase in the content of crude protein, but also because of lower content of neutral detergent fibers. For this reason, the best option in sweet sorghum-climbing bean intercropping is the use of climbing bean genotypes that provide forage with the greatest proportion of pods at harvest. In this study it was found that the neutral detergent fibers of intercropped SBPV1, SBPV2 and SBPV3 were significantly (P<0.05) lower than SB (sole crop sweet sorghum) during two years of research (Table 3). According to the results, when climbing bean had increased seed number in intercrop, the values of neutral detergent fibers in the mixture decreased. The content of neutral detergent fiber is important in ration formulation because it reflects the amount of animal forage that animals can consume (Lithourgidis *et al.,* 2006). In general, the concentration of neutral detergent fibers is higher for grass than for legumes (Dahmardeh *et al.*, 2009). Many researchers stated that the nutritional value of cell wall components decreased with plant age related to increased lignin content (Atis *et al.* 2012; Zhao *et al.*, 2012). Since smaller amounts of fiber components are used for better digestion, the climbing bean intercropped plots were superior to monocrop sweet sorghum in terms of neutral detergent fiber. Contreras-Govea *et al.* (2011) ensiled corn and forage sorghum with different proportions of lablab bean and reported that legume must make up at least 50% of the mixture to affect fermentation and nutritive value.

Conclusion

In this study intercropping of sweet sorghum with climbing bean at various sowing densities was shown to be an effective way to affect dry matter and crude protein yield enhancing nutrient quality of the fresh forage. Intercropping of sweet sorghum with climbing bean increased the valueş of crude protein and decreased values of neutral detergent fiber in the fresh fodder mixture. Finally, the SBPV3 treatment (250 000 plants ha^{-1} of sweet sorghum and 100 000 plants ha^{-1} of climbing bean) was the most effective regarding the nutrition composition in fresh forage.

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EFFECTS OF COMPOST APPLICATION RATES AND MULCH THICKNESS ON TOMATO YIELD UNDER SALT AFFECTED SOIL OF EAST SHEWA OROMIA NATIONAL REGIONAL STATE, ETHIOPIA

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Abstract

Studies were conducted as on-farm trials at East Showa Dugda District, Girisa Kebele to evaluate the effect of different compost application rates (0, 2, 4 and 6 ton/ha) and mulch thickness (0 cm/ha, 5 cm/ha, 10 cm/ha, and 15 cm/ha) on soil physicochemical properties and crop performance in terms of yield of Solanum lycopersicom. L Production. The experiment was conducted in factorial experiment arranged in a randomized complete block design with three replications. The analysis of variance showed that, all the main effects of the growth parameters showed significant effects except mulch thickness on plant height. The result of Some yield and yield component variables (number of fruit per plan, were significantly affected by the interaction effects of mulch thickness and compost application rate. Marketable fruit yield was significantly affected by the level of compost and its interaction with mulches. Yield obtained from the treatment combination of 6 ton/ha compost rate and 10 cm mulch thickness was argonomically and economically remunerative by 15.56% for farmers**.** The optimum fruit yield was 37.23t/ha found on 6t/ha compost and 10cm mulch plot .All the yield and yield components indicated significant difference. In general, the yield obtained from the 6ton/ha compost rate and 10cm mulch thickness makes farmers' benefited weather for consumption or market purpose

Key words: *salt affected soil, soil amendment, soil properties, yield, and Solanum lycopersicomL. L*

Introduction

Soil fertility is fundamental in determining the productivity of all farming systems and is most commonly defined in terms of the ability of a soil to supply nutrients to crop (Wild, 1993). Farmers are suffering from declining soil fertility and are complaining about weak responses of their soil fertility management (Tonfack *et al*., 2013). Investigations have shown that farmers mostly rely on a single option of conventional nutrient replenishment, without taking into account the soil mineral balance (Tonfack *et al*., 2009

Salinization and low soil nutrient content are some of the major problem that hampers crop production on smallholder farmers field. About 20% of the world cultivated land and 50% of cropland generally affected by salinity (Lakhdar *et al*., 2009). Salt affected soil in Ethiopia is reported to cover over 11 million hectares of unproductive naturally salt affected wastelands (Tadele, 1993).

Maintenance of high crop yields under intensive cultivation is possible only with external fertilizer inputs (organic and in organic sources fertilizer). Although inorganic fertilizers application is the quickest and easiest way of increasing yield per unit area, the problems associated with inorganic nutrient supplementation, if not judiciously handled, it can causes pollution of ground water and does not improve soil structure and may early contribute to greenhouse gases (GHG) (Gordon *et al.*, 1993).
Tomato (*Solanum lycopersicumL.*) is one of the most widely cultivated crops in the world. It is an important source of vitamin A (30%), vitamin C (38%), calcium (2%), iron (3%) and is an important cash crop for smallholders and medium-scale commercial farmers (Naika S *et al.*, 2005). Tomato is widely cultivated in Ethiopia: both under rain fed and irrigated systems. Though there are also other favorable growing pockets in different parts of the country, the bulk of tomato production in Ethiopia is concentrated in the Central Rift Valleys (MoARD, 2009),
Salt affects tomato plant growth mainly through toxicity from excessive uptake of salt substances such as sodium, reduced water uptake, known as water stress and reduction in uptake of essential nutrients particularly potassium (FAO, 2005). This problems also observed in the central rift valley including Dugda Bora and generally, the production is low in terms of quantity and quality. This affects not only the productivity but also the quality and associated consumer’s preferences (Aleligne *et al*., 1994)). The organic fertilizers take the place of inorganic fertilizers in sustainable agriculture and the main sources of the organic fertilizers can be composted livestock manures, plant residues and industrial wastes (Aksoy, 2001 and Chowdhury, 2004). Application of organic fertilizers has been a noble and traditional practice of maintaining soil health and fertility. Tomato production by using organic fertilizer has become a current practice as it contributes to poverty alleviation of smallholders’ households by enhancing their income (World Vegetable Centre, 2007). Using with wheat straw mulch is economically more profitable than the other mulch treatment (Baye, 2011).
Dugda district is the area where many smallholder farmers are producing vegetable crop by irrigation. Among these vegetables tomato, one of the most widely grown, is severely affected by salinity (personal communication).Compost addition increased water content at both field capacity and permanent wilting point, increased shoot and root growth under stressed condition and also can decreases the effects of salinity by increase soil water availability and nutrient uptake by plants (Nguye *et al.,* 2013). Surface mulching has shown to reduce evaporation and decrease salinity hazards to improve crop production (Yang *et al*., 2006). This trial was undertaken to determine the effect of fertilizer types on the growth and yield of tomato in semi-arid environment in view to formulate and recommend fertilizer requirement for yield and profitability of *Solanum lycopersicom* Production. Objective of this paper is to evaluate effects of compost and mulch on soil physico -chemical properties under salt affected soil.

Materials and Methods

Treatments and Experimental Design

Field experiments were conducted in 2014 and 2015 as an on-farm trial at East Showa, Zone Dugda district (specific location is at 6^0 91’ and 8^0 12’ N and38^0 46’and 38^0 59’ E and an elevation of 1641-1680 m.a.s.l.). The area was semi-arid and arid climate (Mengistu, 2008). More broadly, the soil of the area is calcarious derived from mix of parent material including: basalt, ignite, lava, genesis, volcanic ash, pumice, reverie and lacustrine alluvium parent materials (EGMOA, 1975). Randomized complete block design with three replicates was utilized for the experiment. The experiment involved four treatments in a factorial combination. These were four-mulch thickness (0cm/ha, 5cm /ha, 10cm/ha and 15cm/ha) and four levels of compost (0 tons, 2 tons, 4 tons and 6 tons/ha) and one tomato variety (Roma VF). Soil samplings were done randomly form five farmers. Then, levels of soil salinity were identified from laboratory work and those that shown high salinity level was used as experimental field. The compost was prepared at Genesis farm from the mixture of animal manure, poultry manure and crop residue under plastic shade from August 2014 to December 2014. NPK fertilizer was applied one week after transplanting. The outermost rows at both sides of plots were considered as borders. A 1.5m wide-open strip separated the blocks; whereas the plots within a block were 1m apart from each other. In accordance with specifications of the design, each treatment was assigned randomly to experimental units within a block. The transplanted tomato seedlings were irrigated with furrow irrigation. The stand was thinned (at first true leaf stage) to a population of 16783plants/ha two week after transplanting during the second hoeing. From 75 kg/ha Urea, half was side dressed at second hoeing and the left half was applied at last hoeing. Stalk was stand during fruit setting time and tomato branches where tied with the stalk. Disease, insects and weeds were managed as per the recommendation of Adami Tulu research center. Harvesting was done by hand form May 16/2015 to June 01/2015.

Field trials

Land preparation was done in mid of November 2014 and ploughed with a tractor and hand leveled before planting. The seedling of the tomato (Roma VF) was raised in January 16/2015
Application was changed to furrow irrigation once a day when seedlings were about 5 to 8 cm height. The compost was applied on January 23/2015 to the prepared plots and was incorporated in to the soil before transplanting (20 days before transplanting). Healthy, vigorous, stocky and succulent seedlings were selected and transplanted with two plants per hill by hand at about 10 cm depth in the field on February 13/2015to the gross plot size of 10.5m^2 at70cm inter and 50cm intra-row spacing. Then wheat straw was applied to the transplanted field by hand per treatment on the same date of transplanting (by measuring the thickness with shtick at (0, 5, 10 and 15 cm). The stand was thinned (at first true leaf stage) to a population of 16783 plants/ha (two week after transplanting which during the second hoeing). Disease (Bloosom end rot and powdery mildew) and insects (Aphid) and management weeds were managed.

Data collection

Plant height (cm): Data were taken from five plants on plot basis when 50 % of the plants in a plot reach maturity stage by using tape meter from collar region to the apex and the mean value was determined as mean plant height.
Number of primary branches:-were taken from the same five plants on a plot basis when 50 % the plant in a plot reach maturity.
Number of clusters per plant: -were counted and recorded from the same five plants on plot at 50% maturity.
Number of fruits per plant: -Total numbers of fruits harvested from five plants were counted individually and the mean values expressed as whole number of fruit per plant
Number of fruits per cluster:-Number of fruits was counted on five plants per plot when 50 % of the plant in a plot reaches maturity and average was taken.**Fruit weight per plant (kg/pl): -** All the ripen fruits from five plants per plots were collected, weighed individually and the mean was taken to get fruit yield per plant which was expressed in kilo Grams/ plant.

Marketable fruit yield/ha: - Fruit which was cracked, damaged by insects, diseases, birds and small fruits those with sun burn were considered as unmarketable while those which were free of any feasible defect and damage was considered as marketable fruits.

Results and discussion

Growth Parameters

Plant height (cm): Compost showed significant ($P<0.01$) effect on plant height (Table 1) The highest plant height (62.75cm) and (59.07cm) were observed from the main effect of 6 and 4ton/ha compost application rates while the lowest (50.75cm) was observed from 0 ton/ha compost application rate (Table 2). Highest compost application showed significant increase in plant height by providing optimum amount of nitrogen that is the most limiting nutrient for tomato growth and is required in large amount. Similarly Brown (1995) observed that, plant growth was markedly influenced by application of poultry manure, inorganic N fertilizer and their combinations as observed from the highest plant height and number of leaves compared to the untreated.

On the other hand, the minimum plant height might be due to the result of low nutrient on the control treatment and the influence of soil salinity that decreases the rate of photosynthesis and plant growth. Salinity inhibits root elongation and influences soil - plant - water relationships and enhance the level of soluble salts within which plant growth. In agreement with this, Steppuhn (2005) reported that salinity induced reduction in crop growth, which the authors associate with reduction in water use.

Number of primary branches: The analysis of variance pointed out that the main effect of mulch and compost showed significant ($P<0.05$) effect on number of primary branches**.** Nevertheless, the interaction effects did not show significant differences. Maximum number of primary branch (5.68) was recorded from tomato plants grown in 6t/ha of compost. While the smallest number of primary branch (4.04), (4.63),(4.78) were observed from plots of zero ton/ha, 2ton and 4ton compost (Table 4). This might be due to increased total N and other organic matter by increased compost application rate and also the tomato plant root growth was stimulated by P in better utilization of water and other nutrients in the soil and promotes a sturdy growth of stem and healthy foliage. Similar with this, Tsado (2014) found that, the result of differences in the number of branches produced in avian waste compost compared to that of agricultural waste.

On the other hand, the higher number of primary branch (5.2), (5.15) and (4.80) was found on 10cm, 5cm and 15cm mulch thickness respectively. But the lower number (3.99) of primary branch was recorded from plants grown in un-mulched plot. This might be due to the fact that optimum mulch increases vegetative growth of tomato by protecting water loss from soil and facilitate mineral uptake to the plant, provide favorable condition by optimizing the soil temperature that the plant was branched than un mulched plot. This agrees with the result observed by Singh *et al*. (2007); who reported that optimum mulching improves plant growth, yield and yield quality.

Number of clusters per plants: -The interaction effects of mulch and compost showed non-significant differences on number of clusters per plants. However, number of clusters per plant varied from compost to compost and from mulch to mulch ($P<0.01$) (Table 5). The maximum number of cluster (15.45), (15.35) per plant were observed on tomato plants grown in (6 and 4 ton/ha) compost application rate respectively. Whereas, the minimum value (10.2 and 12.37) were from zero and 2ton/ha compost rate. The increased number of cluster was probably due to the ability of compost to enhance plant growth and initiate more number of flowers per branch that was become cluster per plant. This finding agrees with the findings of Curtis and Claassen (2005) and Nguyen *et al*. (2011), who reported, the positive effect of compost on plant growth by increasing nutrient availability. Similarly, maximum number of cluster (15.37 14.28 and 13.65) were recorded form tomato plants grown in 10cm.15cm and 5cm mulch thickness. While the minimum number of cluster per plant (10.12) was observed from the unmulched plot (table 1). This might be because, optimum mulch provides favorable temperature for vegetative growth, flowering, and fruit setting. It also contributed to increase number of cluster per plant that probably resulted from the increased number of primary branch per plant. Gudugi *et al*, (2012) found that, mulched plots significantly produced more number of clusters per plant, number of branch per plant and flowers when compared to no mulching.

Table 1. Influence of the main effect of compost and mulch on growth parameters, yield and yield components of tomato

Compost (ton)	PH(cm)	Npbpp	N.cpp
0	50.75^{c}	4.04^{b}	10.25^{b}
2	56.68^{b}	4.63^{b}	12.37^{b}
4	59.07^{ab}	4.78^{b}	15.35^{a}
6	62.75^{a}	5.68^{a}	15.45^{a}
SEM(±)	2.74	0.41	1.30
LSD(0.05)	5.59	0.83	2.66
Mulch (cm)			
0	56.77	3.99^{b}	10.12^{b}
5	58.88	5.15^{a}	13.65^{a}
10	58.04	5.2^{a}	15.37^{a}
15	55.57	4.80^{ab}	14.28^{a}
SEM(±)	2.74	0.41	1.30
LSD(0.05)	NS	0.83	2.66
CV	11.70	20.80	23.90

Means followed by the same letter within a column are not significantly different from each other at 5% level of significance. SEM = Standard Error of Mean, LSD=Least Significant Difference, CV= Coefficient of Variation. PH = plant height, NBPP = number of branch per plant, N CPP = number of cluster per plant

Yield and yield component

Number of fruit per cluster: - The ANOVA result (Appendix Table 2) indicated that, the interaction effects of compost and mulch resulted to significant ($P<0.01$) difference on number of fruit per cluster. The highest number of fruit per cluster (5.07) was observed at (6t/ha) compost rate with 10cm mulch thickness. On the other hand, the lower number of fruit per cluster (3.27) was found in the control plot (Table 6). The highest fruit per cluster might be from the higher amount of compost application with medium mulch thickness that improves soil fertility of the salt affected field. Cherr et al. (2006) also indicated that, the release of nutrients from decomposing mulches (rapidly and slowly decomposing) have positive effect on the soil and plant growth. Darlington, (2003) found that by increasing the organic content of the soil, biological activity can be enhanced and water and nutrient holding capacity can be improved in soils. Ogbomo, and K. (2011) found that organomineral fertilizer application is the most effective for the optimum growth yield and profitability of tomato.

Number of fruit per plant: Significant ($P<0.05$) difference was observed due to the interaction effect of both mulch and compost. The maximum fruit number (46.53, 44.54, 42.93,45.35, 42.42 and 42.42) were found from a treatment combination of 6ton and 10cm ,5cm and 6ton ,15cm and 4ton, 10cm and 4ton and 10cm mulch thickness and 2ton compost application rates. The lowest fruit number (33.83 38.8,36.22,38.8537.10,39.07 and 35.53) were recorded from 0cm and 0 ton, 10cm and 0 ton,0cm and 2ton,5cm and 2ton0 cm and 4ton 0cm and 6ton 15cm and 6 ton. The increased number of fruit might be due to the highest application of compost with mulch to facilitate plant growth and fruit development through adding organic matter that provided enough amount of nutrient to the plant. Delate et al. (2008) found that, flower, fruit number, and yield were numerically greater in the fertilized plots as compared to the untreated soil.

Fruit weight per plant in (kg): -Significant ($P<0.01$) result was observed due to the interaction effect of the main effects and the main effect of compost and mulch. The maximum weight (2.22) was obtained from (6t/ha) of compost rate and (10cm) mulch thickness. Whereas the minimum weight (1.29) was from the control (Table 2). The maximum yield might be due to the highest amount of compost rates applied which supply high plant nutrient and increase soil organic matter, reduce soil Ph, sodium concentration, increase nutrient and water uptake. And the applied mulch might be improved the fruit yield by initiating soil organic matter decomposition and increase soil moisture content on these treated plot comparing with the untreated plot. Mourao et al**.** (2010) reported that**,** tomato yield increased with the application of manure compost compared to the control treatment. The lowest yield from control plot indicated that, exposure of plants to salt stress which leads to changes in growth, morphology and physiology of the roots that will in turn change water and ion uptake that limit plant growth and fruit development. Also, the nutrient up take and amount of soil moisture might be in adequate on the control plot.

Marketable fruit yield/ha: -The result regarding marketable fruit yield/ha indicated that, significant ($P<0.01$) difference (Appendix Table 2) was observed due to the interaction effect of the main effects and the main effect of compost and mulch. The maximum fruit yield (37.23t/ha) was found on (6t/ha) compost and (10cm) mulch plot. But the lower amount (21.67 t/ha) was recorded from control plot (Table 2). The higher marketable fruit weight might be the result of soil amended with high rates of compost rate which enhance fruit yield. Because it provides proper amount of nutrient to the plant through decreasing soil Na, EC and Ph; increasing cation exchange capacity, available nutrient like phosphorus(P), nitrogen(N), other micronutrients that was important to tomato fruit development and yield. This agreed with Wallace, (2008) who observed that, organic matter application and, as a consequence, the humus soil distribution decreased soil Na, EC and Ph likely due to supplies of Ca, Mg and K. These mineral elements kept the cation-exchange sites on soil particles, minimizing adsorption of Na, so enhancing Na leaching losses during precipitation events.

Table 2. Effect of compost and mulch interaction on yield, yield components and quality parameters of tomato production

Means followed by the sat me letter within a column are not significantly different from each other at 5% level of

Treatment number	Compost rate and mulch thickness interaction	NFPC	N FPP	FWPP	MFY/ha (ton)
1	0* 0	3.27^{d}	33.83 ^{h}	1.29^{h}	21.67^{h}
2	5 * 0	4.60^{ab}	42.42^{abcde}	1.66^{cdefg}	25.16^{gh}
3	10 * 0	4.87^{a}	38.80^{defgh}	1.58^{fg}	26.43^{fg}
4	15 * 0	4.67^{ab}	41.07^{bcdef}	1.81^{bcdef}	30.29^{bcdef}
5	0 * 2	4.87^{a}	36.22^{fgh}	1.75^{bcdef}	29.50^{bcdef}
6	5 * 2	3.80^{cd}	38.85^{defgh}	1.72^{cdefg}	28.87^{cdefg}
7	10 * 2	4.60^{ab}	42.42^{abcde}	1.66^{cdefg}	27.89 ^{cdefg}
8	15 * 2	4.80^{ab}	41.00^{bcdef}	1.88^{bc}	31.62^{bc}
9	0 * 4	4.53^{ab}	37.10^{efgh}	1.63^{defg}	27.32^{defg}
10	5 * 4	4.87^{a}	39.33^{cdefg}	1.87^{bc}	31.40^{bc}
11	10 * 4	5.00^{a}	45.35^{ab}	1.97^{b}	33.03^{b}
12	15 * 4	4.93^{a}	42.93^{abcd}	1.89^{bc}	31.65^{bc}
13	0 * 6	4.67^{ab}	39.07^{defgh}	1.82^{bcde}	30.46^{bcde}
14	5 * 6	5.00^{a}	44.54^{abc}	1.85^{bcd}	30.98^{bcd}
15	10* 6	5.07^{a}	46.53^{a}	2.22^{a}	37.23^{a}
16	15 * 6	4.87^{a}	35.53^{gh}	1.61^{efg}	26.97^{efg}
SEM(±)		0.31	2.65	0.12	1.94
LSD(0.05)		0.63	5.40	0.24	3.96
CV		8.20	8.10	8.10	8.10

significance. SEM = Standard Error of Mean, LSD=Least Significant Difference, CV= Coefficient of Variation.FPC = fruit per cluster, N.FPP = Number of fruit per plant = FWPP = fruit weight per plant, MFY=Marketable fruit yield

Conclusion

Organic fertilizer needs to be rationally used in order to avoid a negative ecological impact and undesirable effects on the sustainability of agricultural production system

Mulch and compost applications are the best technology used to decrease the effects of salinity on vegetable crops and increase soil organic matter, organic carbon and essential nutrients in the

soil for the area where the majority of the income of the farmers depends on irrigation like Dugda bora district of the experimental site. Therefore the study was conducted to determine the effect of mulch and compost on tomato yield and quality under salt affected soil; to identify the optimum rate of mulch and compost; to evaluate how these improved practices can help farmers to meet consumers test and demand.
Among the yield and yield components; number of fruit per plant, fruit weight per plant, fruit polar diameter, fruit equatorial diameter, marketable fruit yield per hectare and total fruit yield per hectare revealed significant difference due to the main effects and interaction effects. Result indicated that, the main effects of compost and mulch and interaction effects of the main effects observed significant effect on thousand seed weight, seed yield per hectare and seed germination percentage. In general 6t/ha compost and 10cm mulch thickness are recommended to make farmers benefited from the system. The calculated result also confirmed that the largest net farm income and benefit cost ratio was obtained from 6t/ha compost rate and 10cm mulch thickness. Generally practicing organic fertilizers are used to maximize fruit yield and is more appropriate in terms of soil fertility improvement mainly in the off-season where irrigation is used to secure family food and cash security and salinity effects hinders the production; because, the present result indicates the yield obtained from the fertilized tomato are greater than the yield obtained from the control plot as some authors reported. So far, no research work had been made on the salinity effect by using organic compost and mulch during off-season at studied area. As a final, it is difficult to conclude and give valuable recommendation in one year experiment at one site, hence further investigation on the selection of best compatible compost rate and mulch thickness proportion for tomato production should be studied.

Future directions

Different tomato varieties must be tested rigorously with different compost rate and mulch thickness.
Plant tissue analysis must be done to evaluate the nutrient uptake of the plants
Irrigation schedule and the mineral content of the ground water and the river used for irrigation must be tested by laboratory.
Planting time must be identified with this treatments and Roma vf tomato variety
The fruit chemical analysis must be done to identify effects of mulch and compost on external quality of fruit.

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ANTIOXIDANT POTENTIAL AND PHOTOSYNTHETIC PIGMENTS IN KALE (*BRASSICA OLERACEA ACEPHALA SABELLICA*) FROM DIFFERENT SOURCES

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Abstract

Kale (*Brassica oleracea* L. *var. acephala*) is a cruciferous vegetable terms as a ‘superfood’ due to its wide range of nutrients that might help in prevention of various health problem. The nutritional and health benefits of those plants were known since antiquity but they are currently not popular vegetables in the mass diet. Kale has an unstable composition after harvesting, and moreover, very often due to its taste, it is subjected to a thermal treatment which affects its pro-health properties. Broad perspectives of plant biotechnology find application in traditional agriculture for many species. This study aimed to evaluate the antioxidant potential and content of photosynthetic pigments in kale leaves obtained from *in vitro* culture, pots cultivation and commercial source. For micropropagation shoots were transferred for 4 weeks on MS medium supplemented with benzylaminopurine. Culture was maintained at 22±1°C under 14/10 photoperiod a quantum irradiation intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Pots experiments were performed in comparable growth conditions. Antioxidant potential and pigments content was determined spectrophotometrically using DPPH reaction and methanol extraction, respectively. The results showed that kale from *in vitro* culture indicated the highest antioxidant potential in comparison to kale from pots cultivation and commercial source. That was negatively correlated with the content of photosynthetic pigments. Kale from commercial source, with the lowest antioxidant potential, indicated the highest content of chlorophyll a, chlorophyll b, and carotenoids.

Keywords: *antioxidant potential, carotenoids, chlorophylls, in vitro culture, kale.*

Introduction

In response to the increasing risk of diseases caused by improper and poor diet, it is becoming more and more popular to look for food that provides not only energy and basic nutrients, but also can have a potentially positive effect on our health. Foods that are claimed to have exceptional health benefits were defined as ‘superfood’. In terms of the plants, health-promoting properties result from the presence of i.e. feeding fibers, saponins, vitamins, but most importantly from the content of compounds with antioxidant properties (Butnariu and Sarac, 2019). Free radicals are constantly generated in the cells, nevertheless unnecessary reactive oxygen species (ROS) production may cause oxidative stress which is involved in aging process and pathogenesis of various diseases including cancer (Ikeda *et al.*, 2021).
Kale is one of the oldest cultivars of cruciferous plants known already in ancient Greece and Rome. Bioactive compounds in kale leaves might prevent many dangerous diseases, such as cardiovascular disease or cancer. Mainly, health benefits of cruciferous vegetables are related to the presence of different nutrients such as glucosinolates, polyphenols, carotenoids or terpenoid group. The main biological activity associated with kale is extraordinarily high antioxidant

activity (Šamec *et al.*, 2019). Kale extracts demonstrated a protective effect on the oxidation of very low and low density lipoproteins which may indicate a preventive role from cardiovascular diseases (Kural *et al.*, 2011).
There are a few analytical methods to assess the antioxidant potential of the various extracts, i.e. $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), $ABTS^{\bullet+}$ (2,2′-azino-bis-3ethylbenzothiazoline-6-sulfonic acid), FRAP (ferric reducing antioxidant power), ORAC (oxygen radical absorption capacity) and cupric reducing antioxidant capacity (CUPRAC) assays. $DPPH^{\bullet}$ is a free radical that accepts electrons or hydrogen radicals from donor compounds. The percentage of inhibition in $DPPH^{\bullet}$ assay could be expressed using effective concentration (EC_{50}) values, which are reported as the amount of antioxidants required to decrease the concentration of scavenging radicals by 50% (Sridhar and Charles, 2019).
The aim of this study was to compare the antioxidant properties and photosynthetic pigments content in kale extracts from different sources.

Materials and methods

To establish *in vitro* culture of the kale, seeds were sterilized with 70% ethanol for 30 seconds and then with 20% sodium hypochlorite solution for 20 minutes. After washing with sterile water, seeds were placed on MS medium with addition of 3% sucrose, pH adjusted to 5.75 and solidified with 0.8% agar. Isolated from 10-day-old seedlings shoot tips were placed on MS medium supplemented with 6-benzylaminopurine (BA) at the concentration of 2.5 $mg \cdot dm^{-3}$ (MA_{BA}). After 4 weeks of culture, the obtained axillary shoots were isolated and transferred onto the fresh MS_{BA} medium. The culture was maintained at 22±1°C under 14/10 photoperiod (quantum irradiation intensity of 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Figure 1). In greenhouse cultivation, the seeds were sown into universal soil (pH 5.5-6.5) and growth conditions were comparable to those described above, with air humidity 50-55%. Kale purchased at a grocery store came from Polish cultivation, from organic farming (PL-EKO-07 certificate).

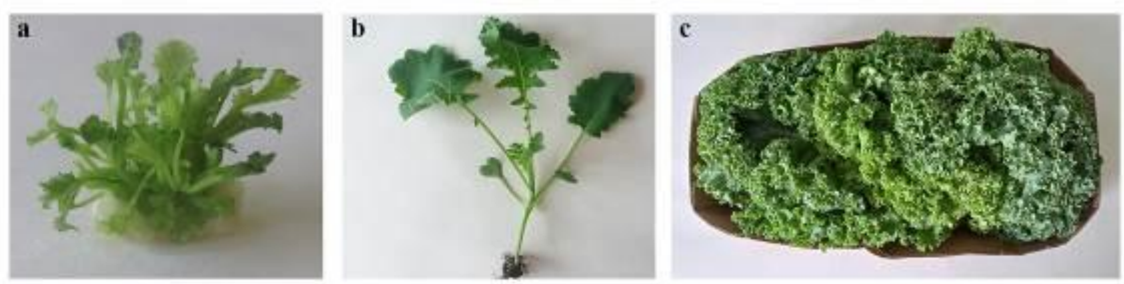

Figure 1. Kale from different sources: (a) culture *in vitro*, (b) pots cultivation, (c) commercial source.

For DPPH analyses stock solution (1 mg of plant tissue in 1 mL methanol) was prepared, and then different extract concentration (40 – 500 µg/mL) were used to calculate EC_{50} values (concentration required to obtain 50% radical scavenging activity). Briefly, 2 mL of sample or methanol (control) were added to the same volume of methanolic solution of 100 µM $DPPH^{\bullet}$. Mixtures were thoroughly mixed and left at room temperature for 10 min, then absorbance was read at 517 nm, using Shimadzu UV-1201 Spectrophotometer. The determinations were performed in triplicate. Antiradical activity was expressed as inhibition percentage (I%) and calculated using the following formula:

$$I\% = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100\%$$

Due to the color of the analyzed samples resulting from the presence of pigments (mainly chlorophylls) in the leaves, the absorbance of additional blank samples was read. To the blank samples methanol was added instead of the DPPH solution. Corrected absorbance was calculated using formula below:

$$A_{sample}^{corr} = A_{sample\ with\ DPPH} - A_{sample\ without\ DPPH}$$

The photosynthetic pigments were determined spectrophotometrically. Approximately 20 mg of plant tissue was used for analyses. Samples were placed in 10 mL of methanol for 3 days at 4ºC in the darkness. The absorbance was measured at three wavelengths: 470 nm, 652.4 nm and 665.2 nm. The determinations were made in five independent replications. The formulas published by Lichtenthaler (1987) were used to determine the content of each pigments:

$$C_{Chlorophyll\ a} = 16.72 \cdot A_{665.2} - 9.16 \cdot A_{652.4}$$
$$C_{Chlorophyll\ b} = 34.09 \cdot A_{652.4} - 8.12 \cdot A_{665.2}$$
$$C_{Carotenoids} = (1000 \cdot A_{470} - 1.63 \cdot C_{Chlorophyll\ a} - 104.96 \cdot C_{Chlorophyll\ b}) / 221$$

The results presented in this paper were expressed as the mean ± standard error. Obtained data were analyzed using Tukey’s test ($p < 0.05$).

Results and discussion

The results presented in Figure 2 show that kale from *in vitro* culture has significantly the strongest antioxidant properties. It was noted that methanol extract from kale leaves in concentration ranging from 400 µg/mL to 500 µg/mL has the highest antioxidant potential, quenching over 90% of the DPPH$^{\bullet}$ absorbance. The stronger antioxidant properties in the multiplied kale may result from the increased biosynthesis of bioactive compounds. Increased antioxidant potential was also noted for various *in vitro* cultures of *B. oleracea* var. *costata* and *Passiflora alata* Curtis, compared to *in vivo* plants. It was explained by the increased biosynthesis of phenolic compounds (Lugato *et al*, 2014; Taveira *et al.*, 2009). In *Cucumis anguria* it has also been shown that plants obtained in *in vitro* culture indicated higher content of flavonoids (Thiruvengadam and Chung, 2015).

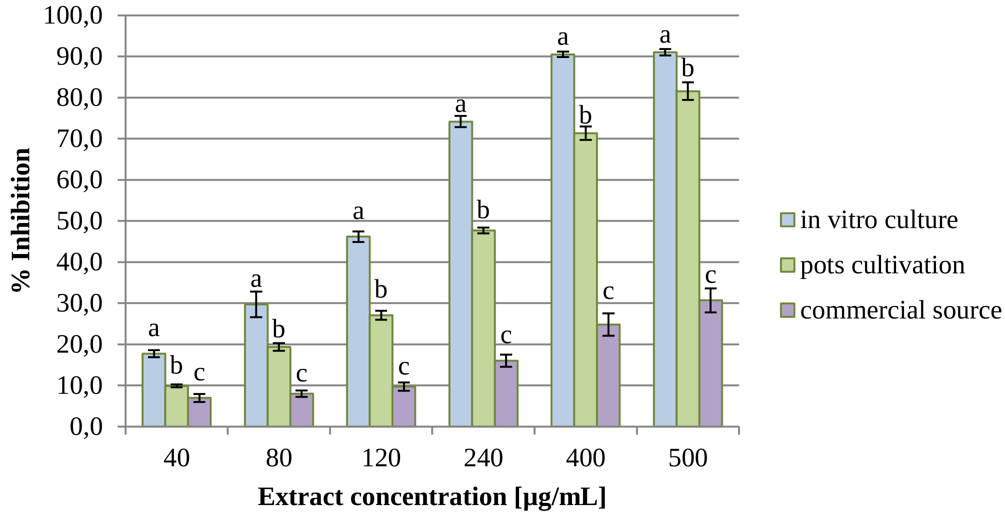

Figure 2. Antiradical activity of kale extracts using DPPH method. Different letters within the same extract concentration indicate a significant difference ($p < 0.05$) according to the t-test.

The extract from kales cultivated in a greenhouse had higher antioxidant potential than those from a commercial source. The maximum inhibition of DPPH radical was 81.5 ± 2.1% in the presence of the 500 µg/mL extract (Figure 2). In turn, the extract of the leaves from commercial source at the same concentration indicated only 30.7 ± 2.9% of inhibition. Significantly lowest ability to reduce DPPH radical in the purchased kale may result from the degradation of antioxidant compounds in the harvested leaves, stored for an indefinite period of time at room temperature. In Portulaca oleracea it was shown that only low temperature (0ºC and 5ºC) did not affect the antioxidant properties of the leaves during storage. At 10ºC a significant decrease of the antioxidant enzymes activity and the content of ascorbic acid was noted in leaves after few days in comparison to leaves freshly harvested from the field (Rinaldi et al., 2010). Calculated EC50 values for kale leaves extracts from different source strongly indicated that kale from in vitro culture is the most effective (168.3 µg/mL), while from commercial source the efficiency is more than 5 times lower (873.6 µg/mL) (Table 1).

Table 1. EC_{50} vales of the kale extract from different sources.

Source of kale leaves	EC_{50} [µg/mL]
in vitro culture	168.3
pots cultivation	873.6
commercial source	275.6

The highest level of pigments was determined in kale from commercial source and the least from the *in vitro* culture (Figure 3). The observed dependence was statistically significant. The kale purchased in the store contained 1.34 ± 0.15 µg of chlorophyll a and 1.28 ± 0.09 µg of chlorophyll b in 1 mg of fresh weight (FW) of the leaves. The kale leaves collected from the greenhouse cultivation and directly analyzed contained 0.67 ± 0.15 µg/mg of chlorophyll a and 0.58 ± 0.14 µg/mg of chlorophyll b. That was surprising result, as it could be assumed that the highest content of photosynthetic pigments will be noted in kale from pots cultivation. However, the age of the plants might have influenced those results, because the leaves from the greenhouse cultivation were younger than those purchased in the store.

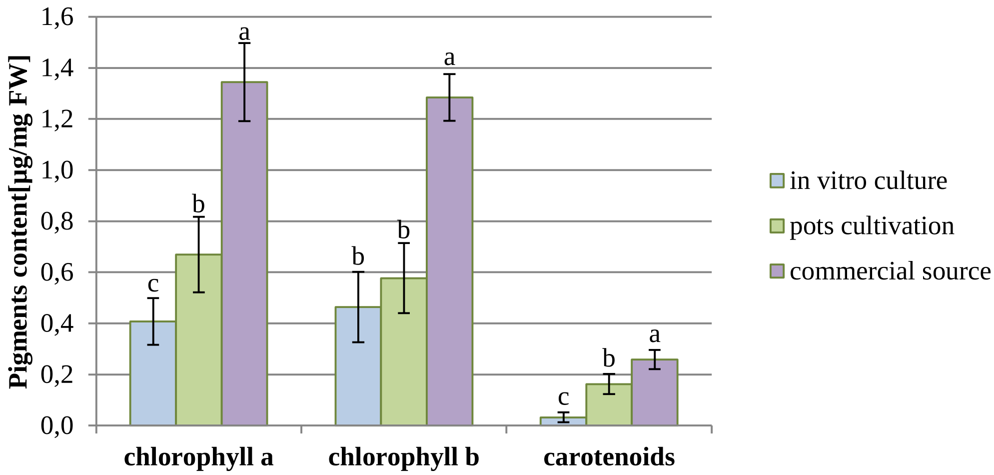

Figure 3. Pigments content (chlorophyll a, chlorophyll b, total carotenoids) in kale leaves from different sources. Different letters within the same pigment indicate significant difference ($p < 0.05$) according to the t-test.

It can be also assumed that the differences in the determined content of chlorophylls may results from a different variety of kale available in the store than those sown in this study. Nevertheless, the obtained results proved the high retention of chlorophylls in kale leaves after they have been harvested and stored in a plastic bag, as these values significantly exceeded the results obtained for kale from other sources. Kale from *in vitro* culture was characterized by the statistically significant lowest content of chlorophyll a (0.41 ± 0.09 µg/mg) and chlorophyll b (0.46 ± 0.14 µg/mg). A similar dependence was found for *Centella asiatica* (Mohapatra *et al*., 2008). Plants from *in vitro* culture were also characterized by the lowest content of carotenoids (0.03 ± 0.02 µg/mg) compared to greenhouse kale (0.16 ± 0.04 µg/mg) and those purchased in the store (0.26 ± 0.04 µg/mg). The conditions of the multiplication reduced the content of all determined pigments. The addition of sucrose (a source of carbon) into the medium and limited gas exchange (mainly the amount of available CO_2 in a closed glass vessel) might inhibit the photosynthesis process (Zenkteler and Borkowska, 2002; Martins *et al.*, 2015).

Conclusion

The results showed that kale from *in vitro* culture indicated the highest antioxidant potential in comparison to kale from pots cultivation and commercial source. That was negatively correlated with the content of photosynthetic pigments. Kale from a commercial source, with the lowest antioxidant potential, indicated the highest content of chlorophyll a, chlorophyll b and carotenoids.

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WEED CONTROL IN SUDAN GRASS CROP (*Sorghum sudanense*)

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Abstract

The paper analyzes the influence of different sowing densities and the application of herbicides with different mechanisms of action on the weediness of Sudan grass crops in subsequent sowing. At the same time, their influence on the forage yield and yield components of Sudan grass (Srem variety) was analyzed. The experiment was set up according to the split-plot system by sowing in two densities (12 and 25 cm row spacing). Three herbicide treatments were used: control - without herbicide application, Bentazone (herbicide Bentamark in the amount of 3 L ha^{-1}) and 2.4 D (herbicide Maton in the amount of 1 L ha^{-1}. The number of weeds in the mowing phase was on the variant with higher density by 72.5%, and on the variant with lower density by 46% less compared to the phase when the Sudanese grass was 20 cm high. The reduction of weediness is a consequence of the pronounced competitive relations of Sudanese grass in the later stages of growth and development. By analyzing the effectiveness of applied herbicides, it can be concluded that both active substances significantly reduced the number of weeds. Slightly higher efficiency was observed in the variant where the active substance 2.4 D was used. The general efficiency of herbicides was 89.9%. However, the best yield of green mass and hay of Sudan grass were achieved on the control variant without the application of herbicides.

Key words: *cultivation density, forage yield, herbicides, Sudan grass.*

Introduction

Sudanese grass (*Sorghum sudanense* (Piper) Staph.) is an annual plant species and one of the most important forage crops from the grass family (Poaceae). It gives high yields of green mass up to 100 t ha^{-1} and up to 20 t ha^{-1} hay of excellent quality (Đukić et al., 2001) It is characterized by high energy value, contains about 10% protein, 25-30% cellulose and about 9% minerals. In the earlier growth, the young plant contains hydrocyanic acid (HCN), and its use in younger stages is not recommended (Đukić et al., 2001; Ćupina, 2002). It has excellent drought tolerance and can be grown as a post stubble or subsequent crop (Erić et al., 2004). In relation to maize (*Zea mays* L.), Sudan grass has higher requirements for heat and significantly less for water (Đukić and Stevović, 1999). Despite the modest demands on water, it responds well to irrigation. It is characterized by high tolerance to diseases and pests and is suitable for organic production. It can be grown on different types of soil. Sudanese grass is used to feed ruminants as green mass, hay or silage (Stjepanović et al., 2011). Favourable biological characteristics, relatively modest requirements for growing conditions, high and stable yield and biomass quality, make this plant species important in solving the problem of deficiency of bulky fodder (Ćupina et al., 2002, Stevović et al., 2009).

In the Republic of Serbia, as well as in the world, Sudanese grass and its hybrids have been the subject of numerous studies that focused primarily on its value and importance in the diet of domestic animals (Ćupina, 2002; Finney et al., 2009; Stjepanović et al., 2011). On the other hand, previous knowledge, scientific results and literary sources related to its production technology, place in crop rotation, weed control and some other properties in agroecological conditions of the Republic of Serbia are very scarce (Erić et al., 2004; Mihailović et al., 2005).
Sudanese grass is known to be a good competitor. It is a very good cover crop, which is why it is increasingly used around the world as a pure cover crop or in combination with other plant species. Under optimal conditions, Sudan grass grows quickly and completely suppresses weeds. In that case, the application of herbicides is not necessary. However, in a case of the slow development of young plants due to unfavourable conditions, weed control is recommended, in order to ensure optimal and uninterrupted development. Considering the morphological characteristics of Sudanese grass, its botanical affiliation and similarity with widely row grains (Glamočlija, 2004; Vucković, 2004), it is generally accepted that in Sudanese grass crops analogous to crops of other widely row grains, active substances intended for control of broadleaf weeds are used.
The aim of this study was to analyze the impact of different cultivation densities and the effectiveness of herbicides with different mechanisms of action on weed control in the crop of Sudan grass grown as a post stubble crop.

Materials and metods

The field trial was set up in the area of Velika Plana in 2020. Sudanese grass was sown as a post stubble crop after barley harvest, on vertisol land type. Basic tillage was done by plowing to a depth of 20 cm. Immediately afterwards, fertilization was performed using NPK 16:16:16 fertilizer in the amount of 200 kg ha^{-1} and pre-sowing soil preparation by a tillage milling machine (rotary tiller cultivator).
The experiment was set up according to the split-plot system in three replicates. Two sowing densities were applied: 12 cm row spacing with a seed quantity of 30 kg ha^{-1} and 25 cm row spacing with a seed quantity of 15 kg ha^{-1}. The size of the elementary plot was 10 m^2. After germination, when the crop was 20 cm high, herbicide treatments were performed: control - without herbicide application, a.i. Bentazone (herbicide Bentamark, Agromarket, Serbia) in the amount of 3 L ha^{-1}) and a.i. 2.4 D (Maton Chemical Agrosava DOO, Serbia) in the amount of 1 L ha^{-1}). The Srem variety (Institute of Field and Vegetable Crops, Novi Sad) was planted on 15th July 2020.
The emergence of Sudanese grass was recorded on July 22th 2020, or seven days after sowing. In the phase when the Sudanese grass had a height of 20 cm, immediately before the herbicide treatment, the first weed assessment was performed (August 10th, 2020). Analyzes were performed from an area of 1 m^2 per plot. The weeds present were determined and their number was determined within each species.
The second assessment of weeds was performed in the phase of emergence of generative organs of Sudanese grass (25 days after herbicide application). The ratio of weed abundance and diversity between the first and second evaluation indicated the efficacy of the applied herbicides shown according to the Dodel formula (Dodel et al., 1963).
Sudanese grass was mowed in the emergence of the generative organs stage. The yield of forage was determined by measuring the total green mass per plot (kg ha^{-1}). After drying the samples of

1 kg at room temperature, the share of water, i.e. the share of dry matter in the forage (%) was determined, and then the yield of hay (kg ha^{-1}) was recalculated.
The average annual temperature in 2020 for Velika Plana was 12.65°C, and the annual amount of precipitation was 724 mm, which is within the multi-year average. Two drought periods were recorded, in April and September, while there was a satisfactory amount of precipitation during the Sudanese grass vegetation period (Table 1).

Table 1. Precipitation and mean monthly temperatures (T) during the growing seasons 2020

	Month						$\bar{x}$ i $\sum$
	IV	V	VI	VII	VIII	IX	
T (°C)	12.5	15.9	20.1	22.0	22.9	19	18.7
Precipitation (mm)	9.3	94.9	104.7	119.0	94.0	25.7	447.6

Results and discussion

The state of weeding, as well as the floristic composition and structure of the weed community of crops, depend on edaphic, climatic and other factors (Janjić et al., 2005). After the harvest of small grains, annual and perennial weeds often appear. The following weeds were observed on the experimental plot after harvesting barley: common blackberry (*Rubus caesius* L.), thorn apple *(Datura stramonium* L.*)*, green foxtail *(Setaria viridis* L.*)*, quack grass *(Agropyron repens* L.*)*, lamb's quarters *(Chenopodium album* L.*)*, creeping thistle *(Cirsium arvense* L. Scop.*)*, field bindweed (*Convolvulus arvensis* L.*)*. During the cultivation of the land, the mentioned weeds were mechanically controlled.

Table 2. Influence of Sudanese competition (C) on weed suppression based on I and II weed readings

Weed species	12 cm			25 cm		
	I	II	C (%)	I	II	C (%)
Green foxtail *(Setaria viridis)*	57	18	68.42%	48	35	27.08%
Flower-of-an-hour *(Hibiscus trionum)*	7	2	71.42%	5	3	40.00%
Lamb's quarters *(Chenopodium album)*	12	2	83.33%	11	9	18.18%
Velvetleaf *(Abutilon theophrasti)*	/	/	/	/	1	0.00%
Cockspur grass *(Echinochloa crus-galli)*	/	/	/	8	0	100.00%
Redroot pigweed *(Amaranthus retroflexus)*	8	0	100.00%	5	0	100.00%
Scutch grass (*Cynodon dactylon)*	/	/	/	2	0	100.00%
Johnson grass *(Sorghum halepense)*	6	0	100.00%	10	0	100.00%
Total			72.50%			46.00%

I – first evaluation; II – second evaluation

The competitive effect of Sudanese grass contributed to a significant reduction in the number of weeds in the control, as indicated by the differences between the first and second evaluation (Table 2). In the variant with higher crop density, no new weed species appeared after the second inspection. The number of developed weeds decreased by 72.5% between the two readings. In the treatment with lower crop density, more weed species were present, but the total number of weeds was similar to that in the variant with higher crop density. However, due to lower crop density and the impact of competition and allelopathy on weed reduction is less (46%). Simić (2008) states that in conditions of deficit of some of the environmental factors such as light, water, availability of minerals and heat, weeds are very often more competitive compared to many crops. However, Sudan grass is a high species of extremely fast growth, which in favourable conditions has a more intensive growth compared to most weed species. In addition, Sudan grass through its root system excretes allelopathic substances that negatively affect other plants, thus preventing the germination and growth of weeds (Wang et al., 2008; Vukadinović, 2017). These statements are confirmed by the results of our research.
The basic criteria when choosing a herbicide for weed control are mode of action, the efficiency of herbicide, physical and chemical properties, time and method of application, phytotoxicity of the preparation and action on beneficial organisms. At the same time, herbicides are combined with different modes of action with favourable selectivity properties and lower toxicity, which reduces the threat to the environment (Marković et al., 1996; Janjić, 2005). The active substance bentazone had a lower efficiency (≤75%) on the *Chenopodium album* on the variant with higher crop density, while the efficiency was satisfactory (75% -90%) on the weed species *Setaria viridis* and *Hibiscus trionum* (Table 3). In the variant with lower crop density, bentazone had lower efficiency (≤75%) on weeds *Setaria viridis*, *Hibiscus trionum* and *Abutilon theophrasti*. For all other weed species, in both crop density variants, the efficiency of bentazone was at a satisfactory level (≥90%). The overall efficiency of bentazone in the variant with higher crop density was 84.44%, and in the variant with lower crop density 51.68%. The lower efficiency of the active substance 2.4 D (≤75%) was on the species *Hibiscus trionum* in both crop densities, as well as on *Setaria viridis* in the treatment with higher crop density. Satisfactory control of 2.4 D (75-90%) was observed on the species *Chenopodium album*, at higher culture density, as well as on *Setaria viridis* at lower culture density. The efficacy of 2.4 D on other weeds was satisfactory (>90%). The general efficiency of herbicides in weed control on the variant, with higher crop density was 86.66%, and on the variant with lower crop density it was 89.88%. The efficiency of the active substances used (bentazone and 2.4 D) in this experiment confirms the results of Delchev and Barakov (2018) obtained under similar conditions in weed control in *Sorghum bicolor*.

Table 3. Efficiency (%) of applied herbicides for weed control in Sudan grass crop

Redni broj	Weed species	Bentazone		2,4 D	
		12 cm	25 cm	12 cm	25 cm
1.	Green foxtail *(Setaria viridis)*	82.5	22.9	70.2	89.6
2.	Flower-of-an-hour *(Hibiscus trionum)*	85.7	40.0	57.1	40.0
3.	Lamb’s quarters *(Chenopodium album)*	75.0	90.9	83.3	90.9
4.	Velvetleaf	100.0	0.00	-	-

	(Abutilon theophrasti)				
5.	Cockspur grass *(Echinochloa crus-galli)*	100.0	100.0	100.0	100.0
6.	Redroot pigweed *(Amaranthus retroflexus)*	100.0	100.0	100.0	100.0
7.	Scutch grass (*Cynodon dactylon)*	100.0	100.0	100.0	100.0
8.	Johnson grass *(Sorghum halepense)*	100.0	100.0	100.0	100.0
	Total efficacy	84.4	51.7	86.6	89.9

Suppresses well - 90 to 100%; satisfactory suppression - 75 to 90; poor suppression < 75%

The forage yield in both densities was higher in the control variant compared to the treatments where herbicides were applied (Table 4). The main reason for this is the higher presence of weeds in the control treatment, which are also included in the total yield. Another possible reason for the higher yield in the control variant is the absence of herbicidal stresses on the cultivated crop. Favourable agro-ecological conditions have contributed to the rapid growth of Sudanese grass. Competition and allelopathy reduced the number of weeds. The share of dry matter in the stern was similar in all variants. Hay yield followed forage yield.

Table 4. Sudanese grass yield

	12 cm			25 cm		
	Ø	Bentazone	2,4 D	Ø	Bentazone	2,4 D
Forage yield (t ha^{-1})	51.7a	43.3b	45.0b	30.4a	24.8b	23.2b
Dry matter content (%)	35.3a	32.2a	33.8a	33.5a	35.2a	30.7a
Hay yield (t ha^{-1})	18.2ab	13.9b	15.2	10.2a	8.7b	7.1b

The values denoted with different small letters within rows are significantly different at (P<0.05) in accordance with the LSD test

Conclusion

The most dominant weed species in the Sudanese grass crop were: green foxtail (*Setaria viridis*), lamb’s quarters (*Chenopodium album*) and flower-of-an-hour (*Hibiscus trionum*).

The higher density cultivation of Sudanese grass affected the lower weediness of crops. Weediness in the second reading compared to the first on the variant with higher crop density was reduced by 72.5 and on the variant with lower density by 46%. The results confirmed that Sudan grass has a strong competitive effect, thanks to which it suppresses weeds in favourable conditions and that these relations are more pronounced when the density of growing crops is higher.

The general efficiency of the active substance bentazone in the variant with a higher crop density was 84.4%, and in the variant with a lower crop density 51.7%. It had lower efficiency on the species *Chenopodium album* in the variant with higher cultivation density and on *Setaria viridis*, *Hibiscus trionum* and *Abutilon theophrasti* in the variant with lower density.

The general efficiency of the active substance 2.4 D in weed control on the variant with higher density of cultivation was 86.7%, and on the variant with lower density 89.9%. Lower efficacy was observed on *Setaria viridis* and *Hibiscus trionum* on the higher density variant and on *Hibiscus trionum* on the lower crop density variant.

The forage yield and hay yield were higher in the control variant in both densities, which is a consequence of the participation of weed mass in the total biomass and lower stress of plants under the influence of herbicides.

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DRONE - SMART TECHNOLOGY ON SMALL AND MEDIUM FARMS

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Abstract

Drones (Unmanned Aerial Vehicles) in the recent past have been used primarily for military purposes, but lately they have been implemented in civil activity as well. With the development and modernization of agricultural production drone application has become more significant, so their usage in the sphere of agriculture is in second place, right after the military, according to the currently valid data. Unmanned Aerial Vehicles (UAV) in the last few years had great interest and commercial significance in world market and the potential of their development is for them to become an element of green technologies in the near future with a stress on sustainable and smart agriculture. Agricultural drones are equipped with dedicated equipment and software which provide a wide range of specific activities. Their possibilities are multi-faceted, such as analysis of micro relief, obtaining information on moisture, soil quality and fertility levels, based on which the crop sowing strategy is developed. They have wide application in chemical treatment of soil and plants, frost protection, irrigation scheduling, as well as management of fertilizer application considering spatial variability of crop growth and field conditions. They can be used to identify present weeds in the field, in order to opportunely eradicate them from the field and stop their competition for natural resources with the main cultivated crop. With the development of smart farming and progress of sensing, robotic, information and communication technologies, drones have great potential to improve agriculture. This paper shows the benefits and possibilities of drones usage on individual small and medium farms.

Keywords: *Drones technology, UAV, small and medium farms, crop help monitoring, pesticides.*

Introduction

The world's population is constantly increasing (currently close to 8 billion people) and is predicted to reach 9.7 billion in 2050 (United Nations 2019), which is in correlation with the increased food needs. On the other hand, the total arable land of agriculture production does not increase. In order to meet these growing needs, it is necessary to modernize the traditional methods and techniques used by farmers in intensive agriculture. Intoducing new methods (Drone technology) have met the increased demand for food, and at the same time the employment of a large number of people. Until a few years ago, drones were mostly used for the military industry, but gradualy developed countries have also seen its benefits in precision agriculture (Veroustraete, 2015). According to the Association for Unmanned Vehicle Systems International - AUVSI, there are more than 2900 Unmanned Aerial Vehicles (UAV) across more than nine hundred companies providing services around the world in 2020. The Japanese were the first to successfully apply UAS technology to agricultural chemical spraying applications in the 1980s. Today, the United States is behind Japan in the application of UAV in agriculture and rest 15% by civilians for diverse applications (Puri *et al*., 2017). Especially in developing countries, the largest percentage of the rural population depends on agriculture. Agricultural fields face dramatic losses due to diseases, pests and weeds, resulting in reduced yields and crop productivity. Pesticides and fertilizers are widely used to improve their quality. In most cases, their application is done manually or with small sprayers. The WHO (World Health Organization) estimates that one million cases are seriously affected by spraying pesticides on crops that are applied manually. Drone technology has proven to be very good at saving excessive use of water, pesticides and herbicides, maintaining soil fertility, increasing productivity and improving quality. The Unmanned aerial vehicle (UAV) – aircrafts have recently become more common used for spraying pesticides to avoid the health problems of humans when they spray manually (Mogili and Deepak, 2018).

Application of drones in agriculture

Drones have long been used to recording and maping the farm and agricultural field, but their advantage in precision farming was soon seen. They can be used in agriculture for various purposes - they can spray pesticides, manure and sowing, as well as observe crop production and harvest. For livestock farms, drones can also be used to monitor animals and quickly collect useful data on animal health and population (Patel, 2016; Ren *et al*., 2020). Also, can quickly sweeps and checks to find slow-growing plants that may require remediation becouse otherwise it will take a lot of time and manpower to monitor the area where the entire crop is grown. The use of drones is becoming one of the most important aspects of managing basic farm operations. These devices can assist farmers in a wide range of operations, including analysis and planning of crop plantations, as well as field monitoring to determine crop growth and health. It is possible to monitor the nutrients available in the soil that are necessary to assess crop growth and enable appropriate fertilization in variable doses to avoid unnecessary damage to sustainable production capacity. In addition, it is possible to efficiently add N (nitrogen) fertilizer and thus avoid N stress (Del Cerro *et al*., 2021). In agricultural practice, various types of UAV are used, which have so far had mainly the function of observation and analysis of the terrain and which can be classified according to a large number of performance characteristics. Aspects such as range, speed, endurance, weight and wing load are important specifications that distinguish different types of drones and lead to useful classification systems. In addition, price, wing span, and maximum height are also characteristics that can be considered for comparing and classifying drones (Arjomandi *et al*., 2006).

Technical characteristics of UAVs

In agriculture, more than 250 models have been analyzed and summarized so far (Marinello *et al*., 2016). The newer generation of drones, including DJI T.30, Honeycomb AgDrone System, EBEE SQ-SenseFly, DJI Agras MG-1 have an additional function - treating the analyzed land with fertilizers and pesticides on small and medium farms. One of the advantages of their use is that they can be easily applied in hard-to-reach terrains. UAVs are equipped with the cameras and sensors for crop monitoring and sprayers for pesticide spraying. Thez can fly autonomously with dedicated software which allows making a flight plan and deploying the system with GPS and feed in various parameters such as altitude, ROI (Region of Interest), speed, geo-fence and

fail-safe modes (Dutta and Goswami, 2020). Some examples of currently used agricultural drones are:
- DJI T.30 - one of the newer generation mid-size drones that is often used on small and medium-sized farms. It is equipped with a smart engine (Artificial Intelligence Engine), Global Navigation System (Global navigation satellite system), high-precision omnidirectional radar system (Spherical Omnidirectional Radar System) that allows it to autonomously bypass obstacles even without visibility as and a remote control that can control up to three drones simultaneously (Single remote control for up to three drones). It shows good working conditions: wind up to 8 m/s (28.8 km/h); temperature 0 - 45℃ and humidity up to 93%. Its spray system contains a chemical tank with a built-in high-precision electromagnetic flow meter and a continuous liquid level meter. Such equipment allows the drone to be automatically interrupted at the moment when the tank is emptied and the aircraft returns to the starting point where it is refille https://www.dji.com/t30
- DJI Agras MG-1 - It is an octocopter specially designed to assist farmers to spray fertilizers, pesticides, and herbicides. It is trained to carry heavy liquid payloads up to 10 kg and can cover an area of 4-6ha in just 10–20 min. It has an incorporated divergent cooling framework to keep the air streaming to each piece of the installed electronic system. It has a Y-shaped folding design. It has foldable motor system. Its frame is made of high strength carbon fibre (https://www.dji.com/mg-1/info)
- EBEE SQ-SenseFly - It is designed to monitor crops from their initial phase of planting till their harvesting period (https://www.dronethusiast.com/agricultural-drones/). It captures crop data across four multispectral bands, plus RGB (https:// https://tecnitop.com/en). It covers a large area as compared to other quadcopters drones in a single flight.

Advantages of using UAV

Drones are creating a new agricultural revolution. It is estimated that the size of the UAV in the agricultural market will reach billions of dollars in the next few years (Ren *et al*., 2020). One of the advantages of using drones is the price, because they are cheaper than renting a small plane. Prices for flight-ready agricultural UAV systems - including hardware, sensors and software - range from $ 1,500 to over $ 25,000 (Patel, 2016). Drones operate in cloudy conditions and provide higher resolution images. The agricultural sector can benefit significantly from the implementation of drones, however, their effective application depends on some mandatory critical aspects that must be considered, including configuration, mass, payload, flight range, and cost (Marinelo, *et al*., 2016). Several key benefits of using drones in agriculture that are used for everyday agricultural tasks on small and medium farms have been recorded. For example, farmers can use them to inspect the condition of the farm at the beginning of any crop year. Drones provide higher yields in agriculture because the precise application of pesticides (Figure 1), water and the use of fertilizers that are precisely monitored by the drone will increase yields. Its spray chemicals at a faster rate as compared to other methods. It can also result in the saving of the amount of chemicals applied, which can reduce input cost and also spraying of chemicals over tall crops can be done easily by drones without any damage. They can be used to identify the weeds present in the field. These weeds could be timely rooted out from the field so that they do not compete for resources with the main crop. (Rani *et al.*, 2019). With GIS mapping integrated with drones, farmers can draw field boundaries for an accurate flight pattern. With the help of drones, it is possible to record the health status of crops using infrared, NVDI and multispectral sensors, which allows farmers to better monitor crop health, transpiration rates and rates of sunlight absorption, etc. (Puri *et al*., 2017; Zhang and Kovacs, 2012). However, despite the promising potential, the actual use of drones is still quite limited. Low costs and vehicle maintenance are an advantage in agricultural application research, however, inadequate performance continues to limit their full capability. Cost-effectiveness can be proven in cases where UAV can be. Improvements remain crucial in terms of battery life, and thus cargo and flight autonomy (Marinelo *et al*., 2016). It is estimated that dusting by drones compared to the classic can be done several tens of times faster, with significant savings in pesticides (Zhang and Kovacs, 2012). Drones, unlike airplanes, have low operating costs and are easy to move (Dutta and Goswami, 2020).

Figure 1. Sequential soil treatment (https://www.dji.com/t30)

Conclusions

Although farmers on small and medium-sized farms continue to use the traditional way of applying pesticides, it is inevitable that the use of drones will gain primacy in the near future. Drones are one of the promising potentials in precise agricultural practice and for the following reasons:

- Quick and easy installation of the necessary equipment
- Possibility of programming the work process
- Work in low visibility conditions
- The condition of the substrate does not prevent its operation
- Not damage crops during treatment
- Work on sloping terrain
- Ideal coverage of the treated surface
- Controlled pesticide application

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ASSOCIATION OF AGRONOMIC AND FORAGE QUALITY TRAITS IN RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Abstract

Red clover (*Trifolium pretense* L.) is a source of highly nutritional voluminous forage for livestock feed. The objectives of this investigation were to: I) annotate associations of agronomic and forage quality traits of red clover accessions; II) group accessions in relation to analyzed traits. The plant material used included 46 cultivars and local populations, which originated from 17 countries, representing part of the red clover collection from the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The field trial was sown during two growing seasons in Novi Sad. The agronomic traits (plant height-PH, internodes number *per* stem-IN, green mass yield-GMY, dry matter yield-DMY) were evaluated from the second cut in the nursery of the second year of life. Forage quality traits analyzed were content of neutral detergent fibers (NDF), acid detergent fiber (ADF), and crude protein (CP). In regard to very strong associations of PH, GMY and DMY, and to moderately strong associations of PH and IN, it could be anticipated that the indirect selection for higher plants with higher IN can lead to higher GMY and DMY. The breeding of red clover for NDF and ADF and elevated CP might be successful, but can reduce herbage yield (due to very weak positive and negative associations with GMY and DMY). The accessions were grouped to the six clusters which can facilitate selection and breeding for different agronomic and quality objectives.

Keywords: *Red clover, agronomic traits, forage quality, PCA biplot.*

Introduction

The red clover (*Trifolium pratense* L.) is used as animal fodder via grazing, hay making and silage (Watson and Stoddard, 2017), providing a higher N content in comparison to grasses, and elevating livestock performance via the superior nutritive value of grass plus clover forages versus grass alone (Ciaran and Ratnieks, 2021). Populations and cultivars of red clover are adapted to the soil with lower pH, less fertile with higher moisture, in comparison to alfalfa. Due to the higher altitude persistance, red clover is, besides grasses, a leading forage crop grown in a moderate climate zone (Petrović *et al*., 2014). Red clover is a perennial hermaphroditic allogamous naturally diploid (2n = 2x = 14) species, with a homomorphic gametophytic self-incompatibility, cross-fertilised in order to produce seed, and includes wild populations as well as landraces and conventional cultivars. The modern tetraploid red clover cultivars (2n = 4x = 28) have been developed using autopolyploidy from diploid genotypes through chromosome doubling (Taylor and Quesenberry, 1996). Generally, red clover breeding programs are based on mass phenotypic or recurrent selection, and therefore the cultivars produced are heterogeneous with highly heterozygous individuals (Tucak *et al*., 2013). Red clover improvements have been focused on yield and persistence (Egan *et al*., 2021). The genetic gain in red clover green mass yield varied from 0.21% to 1.39% per year (Riday, 2010). In comparison to other crops, such as maize, little yield gains in perennial forage legumes have inducements in multiyear selection cycles, lack of progeny testing, underuse of nonadditive gene actions (Riday and Krohn, 2010), breeding objectives focused on a wide range of economically important traits, such as pathogen or insect resistance, and forage quality, many of which are not correlated or may be negatively correlated with the yield of green mass (Tucak *et al*., 2013). The quality traits of perennial legumes are of a very complex nature, and their expression is influenced by, besides genetic factors, environmental factors (especially drought through direct and indirect effects on plant morphology and physiology) and by applied management (cutting frequency, plant maturity stage at cutting time, and cutting height) (Tucak *et al*., 2021). An increase in forage quality, most importantly the crude protein content is one of the main goals of red clover breeding, as red clover has a lower rate of protein degradation during ensiling (Petrauskas *et al*., 2018). Another parameters such as acid detergent fiber content, neutral detergent fiber content, digestible of dry matter, dry matter intake, relative feed value are important criteria for determining forage quality in animal feeding (Sousa *et al*., 2020). The favorable tendency of plant breeding encompasses improvement of the multiple traits at the same time, but that is difficult to achieve, because of the genetic associations among different traits (Breseghello and Coelho, 2013).

The objectives of this investigation were to: i) annotate associations of agronomic and forage quality traits of 18 red clover populations and 28 red clover cultivars based on two-year field trial; ii) group red clover germplasm accessions in relation to agronomic and forage quality traits.

Materials and methods

The red clover (*Trifolium pratense* L.) genetic material used in this investigation included 46 cultivars and local populations of diploid (2n) and tetraploid (4n) ploidy level, which originated from 17 countries, and which were preserved as the collection of red clover germplasm at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The names, type/ploidy level/country of origin of tested accessions are: NCPGRU2 (population/2n/Ukraine); NCPGRU3 (population/2n/Ukraine); NCPGRU4 (population/2n/Ukraine); NCPGRU5 (population/2n/Ukraine); Violeta (cultivar/2n/Bolivia); Nessonas (cultivar/2n/Greece); Mercury (cultivar/2n/Belgium); Lemmon (cultivar/2n/Belgium); SA1 (population/2n/Australia); SA3 (population/2n/Australia); SA4 (population/2n/Australia); BGR1 (population/2n/Romania); BGR2 (population/2n/Romania); BGR3 (population/2n/Romania); Diana (cultivar/2n/Hungary); Dicar (cultivar/4n/France); Nemaro (cultivar/4n/Germany); Una (cultivar/2n/Serbia); Avala (cultivar/2n/Serbia); Marina (cultivar/2n/Serbia); Amos (cultivar/4n/Denmark); NS-Mlava (cultivar/2n/Serbia); Italia centrale (population/2n/Italy); Bolognino (population/2n/Italy); Marino (cultivar/2n/Germany); Renova (cultivar/2n/Switzerland); Titus (cultivar/4n/Germany); Rotra (cultivar/4n/Belgium); Kora (cultivar/2n/Sweden); Vivi (cultivar/4n/Sweden); Lucrum (cultivar/2n/Germany); Noe (cultivar/2n/France); Violetta (cultivar/2n/Belgium); Britta (cultivar/2n/Sweden); Krano (cultivar/2n/Denmark); Triton (cultivar/4n/Germany); Lutea (cultivar/2n/Germany); Bjorn (cultivar/2n/Sweden); Bradlo (population/2n/Slovakia); Čortanovci (population/2n/Serbia); 89 E-0 (population/2n/Bulgaria); 91 E-44 (population/2n/Bulgaria); 91 E-63 (population/2n/Bulgaria); Sofia52 (population/2n/Bulgaria); Fertody (cultivar/2n/Hungary); Quiñequeli (cultivar/2n/Chile). The field trial was sown during 2011-2012 vegetation seasons, as randomized complete block design with three replications at the Institute of Field and Vegetable

Crops (in location Rimski Šančevi), Novi Sad, Serbia. The row spacing was 80 × 80 cm, and the row depth was 2.5 cm. The soil at the Rimski Šančevi location is chernozem with very favorable pedological attributes, providing optimal soil conditions for growing red clover. The agro-chemical features of the soil at the Rimski Šančevi location, were provided by Laboratory for soil and agroecology of the Institute of Field and Vegetable Crops. Soil samples were slightly alkaline reactions, with medium content of $CaCO_3$, poor in humus, rich in total nitrogen, with high content of easily accessible phosphorus and potassium. The assessment of the studied agronomic traits (plant height-PH, internodes number *per* stem-IN, green mass yield-GMY, dry matter yield-DMY) was done on plant material from the second cut in the nursery of the second year of red clover life. Each accession was analyzed for agronomic traits on 30 individual representative plants. Dry matter yield was determined after drying the plant samples at 105 °C to a constant weight. For the analysis of quality traits plant samples were obtained from the second cut of the second year, when about 25% of the flowers appear (7 days after three heads of a plant had begun to flower). The three average bulk green mass samples were taken and dried at 60°C during 48h for each red clover accession. Each bulk sample contained ten single plants. The determination of crude protein content in red clover accessions was done according to method AOAC (1990). Assessment of acid detergent fiber (ADF) content in red clover samples was achieved according to Van Soest (1963). The content of neutral detergent fibers (NDF) in red clover samples was determined by the method of Van Soest and Wine (1967). The Principal component analysis (PCA) was performed with the statistical program R (R Core Team, 2017).

Results and discussion

Based on the assessed agronomic and forage quality traits of tested cultivars and populations from the red clover collection, PCA biplot with explained 63.7% of total variation showed associations among traits (Figure 1).

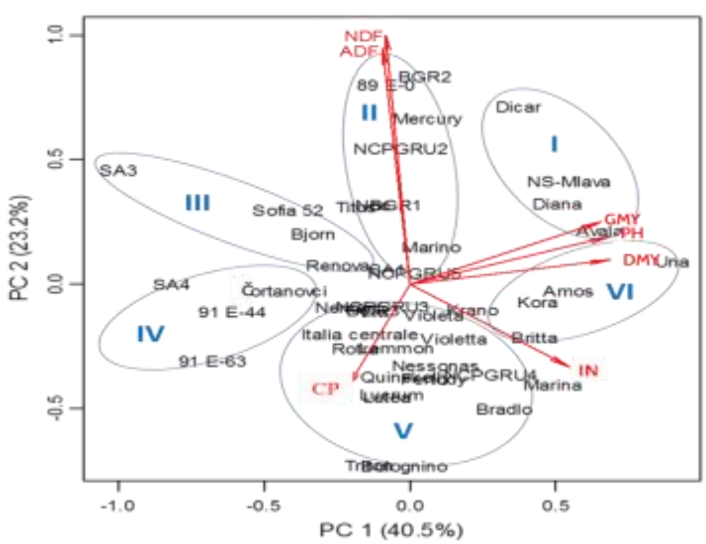

Figure 1. PCA biplot of examined agronomic and forage quality traits of red clover from the second growing season (GMY-green mass yield, DMY-dry matter yield, IN-internodes number stem, PH-plant height, NDF-neutral detergent fiber content, ADF-acid detergent fiber content, CP-crude protein content).

Green mass yield, plant height and dry matter yield were strongly positively associated. These three traits exhibited moderate strong association with internodes number *per* stem and also negative association with crude protein content. The strongest positive association was observed between neutral detergent fiber content and acid detergent fiber content. The neutral detergent fiber content and acid detergent fiber content had very weak positive association with green mass yield, plant height and dry matter yield. The moderate strong negative associations existed for neutral detergent fiber content and acid detergent fiber content in relation to crude protein content and internodes number *per* stem. PCA biplot representing red clover cultivars and populations evaluated for seven traits (GMY, PH, DMY, IN, CP, NDF, ADF) displayed six groups of accessions (Figure 1). The first group (I) of accessions had high values of green mass yield, dry matter yield, plant height, above-average values of neutral detergent fiber content and acid detergent fiber content, and below-average values of crude protein content. Red clover cultivars belonging to the first group were: Avala, Diana, NS-Mlava and Dicar. The second group (II) consisted of cultivars and populations with above-average values of neutral detergent fiber content and acid detergent fiber content, near-average values for green mass yield, plant height and dry matter yield, and below-average values for crude protein content and internodes number *per* stem. The red clover accessions in the second group were: NCPGRU5, SA1, Marino, Titus, Noe, BGR1, NCPGRU2, Mercury, 89 E-0 and BGR2. The third group (III) encompassed cultivars and populations of red clover which exhibited above-average values of neutral detergent fiber content and acid detergent fiber content, average values for crude protein content, below-average values for green mass yield, plant height, dry matter yield, and internodes number *per* stem. Red clover accessions belonging to the third group were: Renova, Bjorn, Sofia 52 and SA3. The fourth group (IV) included populations of red clover with above-average values of crude protein content, and with below average values of green mass yield, plant height, dry matter yield, and internodes number *per* stem. The red clover populations in the fourth group were: Čortanovci, SA4, 91 E-44, and 91 E-63. The fifth group (V) of cultivars and populations of red clover had above-average values for crude protein content and internodes number *per* stem, near-average values for green mass yield, plant height, dry matter yield, and below-average values of neutral detergent fiber content and acid detergent fiber content. Red clover accessions belonging to the fifth group were: NCPGRU3, Nemaro, BGR3, Vivi, Violeta, Italia centrale, Violetta, Rotra, Lemmon, Nessonas, Quinekel, Fertody, NCPGRU4, Lucrum, Lutea, Marina, Bradlo, Triton and Bolognino. The sixth group (VI) included cultivars of red clover with above-average values of evaluated agronomic traits-green mass yield, plant height, dry matter yield, internodes number *per* stem, and with below average values of neutral detergent fiber content and acid detergent fiber content. The red clover cultivars in the sixth group were: Una, Amos, Kora, Krano and Britta. Petrović *et al.* (2014) analysed 17 wild populations of red clover from Serbia, and grouping of the accessions of red clover in the three clusters was due to the traits with the largest influence: green mass yield, dry matter yield, plant height, length and width of a leaf. The crude protein content, fibre content, and fat content were also the most contributing factor for PCA grouping of red clover populations, with the crude protein content and fibre content being negatively associated like in our study. The significant associations between red clover green mass yield, dry matter yield and plant height were determined in many research (Vasiljević *et al*., 2006; Tucak *et al*., 2013; Hoekstra *et al.*, 2017), inferring that breeding for a longer stem could cause an increase of the mass yield. Tucak *et al.* (2013) and Reiné *et al.* (2020) obtained negative associations between crude protein content and neutral detergent fiber content, crude protein content and acid detergent fiber content, and also positive association between neutral detergent fiber content and acid detergent fiber content what is compatible with the outcomes of our research. Reiné et al. (2020) examined nutritional quality of plant species in Pyrenian hay meadows of high diversity and determined negative associations between crude protein content and acid detergent fiber content, crude protein content and neutral detergent fiber content, and positive associations between neutral detergent fiber content and acid detergent fiber content for red clover.

Conclusion

In regard to very strong associations of red clover plant height, green mass yield and dry matter yield, and to moderately strong associations of plant height and internode number *per* stem, it could be anticipated that the indirect selection for higher red clover plants with higher numbers of internodes *per* stem can lead to higher green mass and dry matter yield. Low amounts of non-digestible cellulose fibers (NDF and ADF) lead to the enhancement of the feeding value of red clover for livestock by improving the forage fiber digestibility. The breeding for reduced concentration of NDF and ADF and elevated crude protein content of red clover might be successful in regard to obtained associations in this study, but this approach can reduce herbage yield (due to very weak positive and negative associations with green mass yield and dry matter yield, respectively). PCA biplot evaluation of the 46 red clover cultivars and populations from this study, on the basis of agronomic and forage quality traits, revealed grouping of the accessions to the six clusters which can facilitate choice of parents for selection and breeding for different agronomic and quality objectives.

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DIRECT ORGANOGENESIS OF *CATUNAREGAM SPINOSA* THROUGH NODAL AND INTERNODAL EXPLANTS

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Abstract

Catunaregam spinosa is a medicinal plant used for various therapeutic diseases. It is an exotic plant in Sri Lanka. Micropropagation is a productive plant generation method compared to conventional methods. Micropropagation of *C. spinosa* was studied using explants taken from nodes and internodes. Best surface sterilization protocol was standardized using different concentrations of Clorox® (7.5 % NaOCl) and Carbendazim® exposing to different time intervals. Organogenesis was tested with different concentrations (1.0 mg L^{-1} – 4.0 mg L^{-1}) of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA). Completely randomized design was used with 10 replicates per treatment. Leaf number, shoot length and percentage explants producing shoots were evaluated after four months of incubation. Data were statistically analyzed using ANOVA at significant level of $p = 0.05$. Best surface sterilization protocol found to be exposure to 0.2 % (w/v) Carbendazim for 10 mins, 10 % Clorox for 8 min. following two successive washings in sterile distilled water. Type of explant significantly affected the regeneration frequency and shoot growth. No plantlets were produced by both explants in media added with BAP and NAA in ratio of 1:1 and 2:2. Nodes recorded 100% regeneration except in media including 2.0 mgL^{-1} BAP with NAA (2 or 3 mgL^{-1}) and BAP alone. Nodes recorded highest mean number of leaves (11.2± 1.03) and shoot length (4.66 ±0.25 cm) in MS medium supplemented with 3.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA. Internodes required higher concentration of BAP (4.0 mg L^{-1}) in producing highest mean number of leaves (7.6± 0.84) and shoot length (3.57± 0.18 cm). Internodes produced calli rather plantlets in ratios of (1:1), (1:2), (1:4), (3:4), (4:4) and BAP or NAA alone. Results of the present study revealed that nodal explants found to be better in direct organogenesis of *C. spinosa*.

Key words: *Catunaregam spinosa, micropropagation, sterilization, nodes, internodes.*

Introduction

Catunaregam spinosa (Family Rubiaceae) is a traditional medicinal plant with multiple importance. It grows in many countries like India, Sri Lanka, Indonesia, Thailand, Vietnam, China, United State (California, Texas and Arizona) and even in Tanzania. *C. spinosa* grows up to 5-6 m as a well- armed thorny shrub or tree. Each part of *C. spinosa* is (leaves, fruits, flowers, seeds, root, stem and root bark) are employed in different disciplines for various purposes. The species is rich with diverse phytochemical profile including saponins, alkaloids, glycosides, flavonoids, triterpenoids and phenolic/tannins. The rich phytochemistry of *C. spinosa* leads to numerous ethnobotanical and pharmacological activities vtz. cytotoxicity, piscicidal, antihelminthic, insecticidal, anti- microbial and anti-inflammatory properties. Current world demands more ethnobotanical applications due to rising issues attributed to the western

medicine. Thus rapid generation of important flora is essential to compensate the raising demand.
Micropropagation is one of the productive methods of plant generation in plant biotechnology. It overcomes the constraints incurred in conventional propagation namely microbial and pest attacks, damages due to seasonal changes, seed viability and long term dormancy etc. Further vegetative propagation is insufficient to address the raising demand due to lack of planting materials and destruction of mother plants for harvesting (Chandran, *et al*., 2020). Micropropagation approves bulk propagation using low quantity of plant materials in controlled environment. Seed germination, grafting and cuttings are reported for conventional propagation of *C. spinosa* and have low success. Direct organogenesis through seeds, nodes, internodes and indirect organogenesis through leaf discs are possible methods in producing plantlets via *in-vitro* propagation. There is lack of evidences regarding *in vitro* seed germination yet Chauhan (1999) mentioned *in vitro* seed germination as a reliable method in producing plantlets of *C. spinosa*. Begum *et al*., (2003) observed maximum shoot multiplication (12.7 shoots per shoot tip) from seedlings of *C. spinosa* in MS medium supplemented with BAP and NAA each at 1.0 mg L^{-1} concentration. The present study intended to investigate the micropropagation of *C. spinosa* by evaluating the best surface sterilization protocol, best medium and best explant in producing *C. spinosa* plantlets using nodes and internodes as explants.

Materials and methods

One year old plants of *C. spinosa* obtained from seed germination were used as mother stock of explants. They were maintained in the open environment. Young stem cuttings with internodes and nodes (1.0 cm length) were used as explants. Explants were washed with running tap water for 1 h treated with 4 % Teepol (v/v; liquid detergent) and 2 % Dettol (v/v; disinfectant). Under aseptic conditions further sterilization was carried out using different concentrations of Clorox® contained with 7.5 % NaOCl (10, 15 %) and Carbendazim® (0.2, 0.4 %) exposing to different time intervals (Clorox; 8, 12 mins, Carbendazim; 5, 10 min.). Then each explant was sterilized with 70 % ethanol for 30 sec and thoroughly washed with two washings in sterile distilled water. Half MS (Murashige and Skoog, 1962) medium was prepared supplementing different concentrations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) (1.0 – 4.0 mg L^{-1}). The pH of the medium was adjusted to 5.6. Culture media were sterilized at 121 ºC and 1.5 kg/cm^2 pressure for 20 mins. Explants were aseptically cultured on glass bottles containing 20.0 mL of semi-solidified medium. Cultures were incubated under 116 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) white fluorescent light, 16 h photoperiod at 25 ºC. Completely randomized design was used with 10 replicates per treatment. Plant growth regulators free medium was used as control. Leaf number, shoot length and percentage explants producing shoots were evaluated after four months of incubation. Frequency of shoot production was calculated.

$$\text{Frequency of shoot production} = \frac{\text{Number of explants produced shoots}}{\text{Total number of explants}} \times 100$$

Data were statistically analyzed using ANOVA at significant level of p = 0.05. Results are presented as mean ± standard deviation.

Results and discussion

Optimizing surface sterilization protocol for explants

Best surface sterilization protocol found to be washing in 0.2 % (w/v) Carbendazim for 10 mins, 10 % Clorox for 8 min. followed by two successive washings in sterile distilled water (C1). It recorded highest percentage survival (85.5 %) and lowest contamination percentage (14.5 %). Some protocols exhibited high survival percentages and low contamination percentages at high concentration of Carbendazim and exposure time (C3, C12). However the protocols were still avoided due to possibility of occurring mutations. Time of exposure seems critical highly where the contamination percentage increased to 89.88 % after decreasing exposure time by half. At high concentrations of Clorox and long exposure time (C8) contamination percentage decreased (16.33 %) and concurrently survival rate also decreased due to death of explants after tissues getting bleached (Table 1). Yildiz, *et al*., (2012) discussed about effect of high concentration and long exposure time of sodium hypochlorite solutions on cell viability and *in vitro* cell regeneration capacity.

Table 1: Percentage survival and percentage contaminations of different treatments used for surface sterilization

Treatment code	Clorox		Carbendazim		% survival	% contamination
	Concentration (v/v)	Exposure time (mins)	Concentration (w/v)	Exposure time (mins)		
C1	**10**	**8**	**0.2**	**10**	**85.5**	**14.5**
C2	10	8	0.2	5	10.12	89.88
C3	10	8	0.4	10	91.75	8.25
C4	10	8	0.4	5	75.34	24.66
C5	10	12	0.2	5	60.36	39.64
C6	10	12	0.2	10	69.77	30.23
C7	15	12	0.2	5	15.89	80.43
C8	15	12	0.2	10	65.67	16.33
C9	15	8	0.4	5	70.44	29.56
C10	15	8	0.4	10	79.44	20.56
C11	15	12	0.4	5	81.68	18.32
C12	15	12	0.4	10	94.1	5.9

In vitro shoot generation

Growth conditions of the medium, healthiness of mother stock, genotype and type of explant affect the growth of plantlets through micropropagation. Optimization of required level of plant growth regulators is a prerequisite for an optimum growth of *in vitro* cultures. *In vitro* plant induction of *C. spinosa* in different concentration combinations of BAP and NAA is summarized in Table 2.

Table 2: Observational parameters after 4 months in MS media supplemented with different concentrations of BAP and NAA from nodal and intermodal explants

Treatment code	Concentration (mg L^{-1})		Mean no: of leaves± SD		Mean shoot length (cm) ± SD		Shoot producing frequency (%)	
	BAP	NAA	Node	Internode	Node	Internode	Node	Internode
T1	0	0	-	-	-	-	-	-
T2	0	1	-	-	-	-	-	-
T3	0	2	-	-	-	-	-	-
T4	0	3	-	-	-	-	-	-
T5	0	4	-	-	-	-	-	-
T6	1	0	3.3±1.15	2	1.9 ±0.3	1.5	30	10
T7	1	1	-	-	-	-	-	-
T8	1	2	-	-	-	-	-	-
T9	1	3	4.0±1.33	4.4 ±0.84	2.26±0.8	2.08±0.0	100	100
T10	1	4	8.2±1.13	-	3.28±0.2	-	100	-
T11	2	0	4.8±1.09	4	2.88±0.1	2.6	50	10
T12	2	1	6.2±1.13	5.4 ±0.96	3.47±0.9	2.3 ±0.26	100	100
T13	2	2	5.5±1	4.66±0.4	3.2±0.21	2.3±0.26	40	30
T14	2	3	2	5.33±1.5	2.15±0.8	2.4±0.34	30	30
T15	2	4	3.2±1.03	6.6 ±0.96	2.04±0.1	2.74±0.7	100	100
T16	3	0	7.25±1.3	5±1.41	3.78±0.2	2.55±0.9	80	20
T17	3	1	11.2±1.3	6.8± 1.03	4.66±0.5	2.89±0.4	100	100
T18	3	2	9.2±1.03	6.4± 1.26	4.01±0.4	2.68±0.1	100	100
T19	3	3	7.4±0.96	3.5± 0.92	3.66±0.0	2.2± 0.20	100	80
T20	3	4	7.2±1.03	-	3.91±0.9	-	100	-
T21	4	0	-	-	-	-	10	-
T22	4	1	9.4±0.96	7.6± 0.84	4.0±0.28	3.57±0.8	100	100
T23	4	2	8.4±0.84	5.0 ±1.05	3.82±0.8	2.93±0.3	100	100
T24	4	3	6.8±1.03	4.8 ±1.03	3.57±0.1	2.4±0.34	100	100
T25	4	4	5.2±1.03	-	3.05±0.9	-	100	-

Type of explant significantly affected the regeneration frequency and different responses in shoot growth of *C. spinosa*. No shoot formation was observed in growth regulators free MS medium. Nodal cultures of MS media alone with BAP (T6, T11 and T16) produced shoots in low frequency of 30, 50 and 80 % respectively. Nodes produced plantlets with highest mean number of leaves (11.2± 1.03), shoot length (4.66 ±0.25 cm) and 100 % frequency in MS medium supplemented with 3.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA after 4 months of incubation (Table 2; Plate 1a). Internodes recorded highest mean number of leaves (7.6± 0.84), shoot length (3.57± 0.18 cm) and 100 % frequency in MS medium supplemented with 4.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (Table 2; Plate 1b).

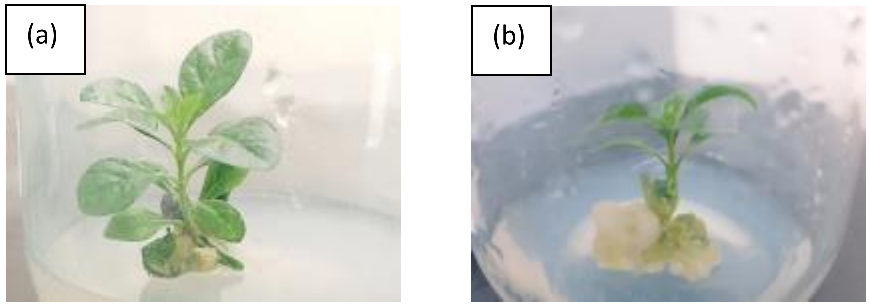

Plate 1: *C. spinosa* plantlets obtained from (a) nodal segment cultured on MS medium supplemented with 3.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (b) intermodal segment cultured on MS medium supplemented with 4.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA after 4 months of incubation

Frequency of internodes producing shoots was significantly low in MS medium alone with BAP compared to frequency of nodes producing shoots. No plantlets regenerated in media only added NAA (T2, T3, T4 and T5). However, addition of NAA remarkably enhanced the frequency of shoot formation and growth of shoots in both nodal and intermodal explants ($p < 0.05$). This reflects the synergistic effect of BAP and NAA combination in shoot development. Synergistic effect of cytokinin- auxin has discussed on different plant species; *Brassica* rapa var. turnip (Abbasi, et al., 2011) and *Solanum tuberosum* (Dhital, *et al*., 2010), *Salvia nemorosa* (Skala and Wysokinska 2004) which proves the finding of this research.
Cytokinin plays a crucial role in shoot formation (Makunga *et al*. 2005). At low concentration of BAP either low growth rate or tendency to produce calli was observed [T7 (1.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA), T8 (1.0 mg L^{-1} BAP and 2.0 mg L^{-1} NAA), T9 (1.0 mg L^{-1} BAP and 3.0 mg L^{-1} NAA) and T10 (1.0 mg L^{-1} BAP and 4.0 mg L^{-1} NAA)]. Increasing concentrations of BAP had no effect on shoot height and mean number of leaves level above 3.0 mg L^{-1} of BAP in nodal cultures independent of concentration of NAA ($p > 0.05$; 0.0953 and 0.0580 respectively). Thus threshold value of BAP for nodal shoot induction of *C. spinosa* could be suggested as 3.0 mg L^{-1}. Similarly, Chand and Singh, (2004) reported inhibitory effect of high concentration of cytokinin in *in vitro* shoot regeneration of *Pterocarpus marsupium* Roxb. using nodal explants.
High cytokinin to auxin ratio triggered *in vitro* shoot generation (Burdyn, *et al*., 2006). MS media supplemented with 3.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (T17) and 4.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (T22) recorded high mean number of leaves and mean shoot length in nodal and intermodal cultures respectively. Internodes produced highest mean number of leaves (7.6± 0.84) and shoot length (3.57± 0.18 cm) at 4.0 mg L^{-1}BAP and 1.0 mg L^{-1} NAA. Matured and developed vascular tissues in internodes facilitated transporting of sugar produced from hydrolysis of starch. It triggers the cell elongation causing shoot development (Dhital *et al*., 2010). Results indicated that BAP concentration required for intermodal explants are higher compared to that of nodes in producing plantlets yet have slow growth rate. In general, calli are produced in media supplemented with high auxin to cytokinin ratio and their equal concentrations (Ahmad and Spoor, 1999). In our study this scenario was observed in (1:1), (1:2), (1:4), (3:4), (4:4) ratios of BAP and NAA.

Conclusion

C. spinosa possesses potential of developing through micropropagation. Best surface sterilization protocol included 0.2 % (w/v) Carbendazim for 10 mins, 10 % Clorox for 8 min. following two successive washings in sterile distilled water. Both nodes and internodes are capable of develop into plantlets. However, nodes recorded higher frequencies, high mean number of leaves and shoot lengths at most of the BAP and NAA combinations compared to internodes. Best medium for nodal cultures produced plantlets in MS medium supplemented with 3.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA with highest mean number of leaves (11.2± 1.03), shoot length (4.66 ±0.25 cm) and 100 % frequency after 4 months of incubation. Internodes recorded highest mean number of leaves (7.6± 0.84), shoot length (3.57± 0.18 cm) and 100 % frequency in MS medium supplemented with 4.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA. The results conclude that tissue culture as a rapid and productive method of producing *C. spinosa* plantlets via best sterilization protocol and best MS medium composition.

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DETERMINATION OF THE DIFFERENCES IN BIOACTIVE COMPOUNDS OF PUMPKIN FROM THE SEED TO THE PEEL

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Abstract

This study was performed to determine the total phenolic content, total flavonoid content, total tannin content, antioxidant activity by DPPH and phenolic compounds by HPLC of pumpkin seed, pulp and peel. The peel of pumpkin contained the highest amounts of total phenolic (97.22 mg/100 g) and total flavonoid (114.76 mg/100 g). The pumpkin peel (1.93 mmol/kg) exhibited better antioxidant activity. The lowest antioxidant activity (1.05 mmol/kg) was observed in the seed, while the pulp of sample had the minimum total flavonoid content (29.05 mg/100 g). To total phenolic content and total tannin amount of the pulp and the seed, the values were closed to each other, and there was no significant difference. There was a decrease in the total phenolic content and antioxidant activity of pumpkin when progressed from the peel to the seed. The main phenolic compounds of pumpkin parts were gallic acid (12.05-28.67 mg/100 g), 3,4-dihydroxybenzoic acid (11.78-22.91 mg/100 g), catechin (19.16-29.67 mg/100 g) and rutin (4.03-10.16 mg/100 g). The highest amounts of these phenolic compounds were detected in the peel of pumpkin. The lowest phenolic acid contents (such as gallic acid, 3,4-dihydroxybenzoic acid, caffeic acid and syringic acid) were observed in the seed of pumpkin. The pulp of pumpkin had the minimum flavonoid amounts (such as catechin and rutin). In addition, pumpkin contained *p*-coumaric acid, ferulic acid, quercetin, cinnamic acid and kaempferol in small amounts in all parts. With respect to phenolic compounds, there were no significant changes between the peel, pulp and seed of pumpkin ($p>0.05$).

Keywords: *Bioactive properties, phenolic compounds, pumpkin, HPLC.*

Introduction

Pumpkin is a member of the Cucurbitaceae family which consists of 130 genera and more than 800 varieties (Rolnik and Olas, 2020). It is cultivated in a wide area such as Europe, Asia and Western America (Sathiya Mala and Kurian, 2016). Widely grown cultivars around the world are *Cucurbita maxima*, *Cucurbita pepo*, and *Cucurbita moschata (*Perez Gutierrez, 2016). In addition to being a vegetable prepared in various ways, pumpkin is an ingredient added to many products such as bread, soup, and pies because of nutritional value (Ceclu *et al*., 2020; Men *et al*., 2021). It was reported that the composition of pumpkin flour was 10-12% peel, 3-4% pulp, 79-82% flesh and 4-6% seed (Norfezah *et al*., 2011).
Recently, phenolic compounds extracted from natural sources have attracted attention because of biological activity Chiou *et al*., 2007). This activity of phenolic compounds is due to their antioxidant properties, because phenolics can also scavenge free radicals and chelate metals to inhibit lipoxygenase activity (Men *et al*., 2021). Pumpkin, which has low cost, easy cultivation and availability, is a good source of bioactive compounds (Cvetković *et al*., 2021). In several studies, the consumption of these compounds in diet contributes to the decrease of

cardiovascular diseases and cancer risks (Aune *et al*., 2018). Moreover, Ghahremanloo *et al*. (2017) informed that the extracts of pumpkin are prefered to overcome obesity for a long-term weight management. To be rich in vitamins and minerals, pumpkins reduce the potential cause of COVID 19 pandemic (Perez-Alvarez *et al*., 2021). Health-important components are found in sufficient quantities not only in the pulp part of the pumpkin, but also in the peel and seed parts (Hussain *et al*., 2022). The aim of this study was to assess the differences in bioactive properties of pumpkin peel, pulp and seed.

Material and Methods

Material

Spark pumpkin (Cucurbita pepo) was purchased from a local market in Konya, Turkey. Peel, pulp and seed parts of pumpkin were separated from each other, and ground before analyses.

Methods

Moisture content

Moisture contents of parts of pumpkin samples were detected at 105°C using an oven (Nüve FN055 Ankara. Turkey) until a certain weight was determined.

Extraction procedure

The ground parts of pumpkin (1 g) were mixed with 5 ml of methanol:water solution (80:20, v/v), and vortexed for 1 min, and then kept in ultrasonic bath for 10 min. After samples were centrifuged at 6000 rpm for 5 min, the supernatants were removed, and these steps were repeated twice (Toh *et al*., 2013).

Total phenolic content

Total phenolic contents of extracts were performed using Folin Ciocalteu (FC) reagent (Yoo *et al*., 2004). The extracts (0.5 ml) were mixed with 2.5 ml of FC reagent and 1.5 ml of sodium carbonate solution. The absorbance values of the samples, which were stored for 2 hours at room temperature in the dark, were measured at 725 nm in a spectrofotometer. Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent (GAE)/100 g.

Total flavonoid content

Total flavonoid contents of extracts were determined according to the method described by Hogan *et al*. (2009). The extracts (1 ml) were mixed with 0.3 ml of $NaNO_2$, 0.3 ml of $AlCl_3$ and 2 ml of NaOH, respectively. The absorbance of mixture was recorded at 510 nm with a spectrophotometer. The results were given as mg quercetin equivalent (QE)/100 g.

Total tannin content

Total tannin contents of extracts were determined by Folin Ciocalteu method described by Haile and Kang (2019). The extract (100 µl) was mixed with distilled water (7.5 ml), FC reagent (0.5 ml), and Na_2CO_3 (1 ml, 35%), respectively. The mixture was diluted to 10 ml with distilled water, and vortexed, and then kept at room temperature for 30 min. The absorbance was measured at 700 nm using a spectrophotometer. The results were given as mg of tannic acid equivalent (TAE)/100 g.

Antioxidant activity

Antioxidant activity values of extracts were determined using 2,2-diphenyl-1-picrazil (DPPH) as proposed by Lee *et al*. (1998). After the extracts (0.1 ml) were mixed with 2 ml of DPPH solution, the absorbance values of the samples, which were kept in the dark for 30 minutes at room temperature, were recorded at 517 nm in a spectrofotometer. The results were given as mmol trolox equivalent (TE)/kg.

Determination of phenolic compounds

The phenolic compounds of extracts were quantified at 280 nm by HPLC (Shimadzu LC 10A vp, Kyoto, Japan) equipped with Inertsil ODS3 analytical column (5 µm, 25 cm x 4.6 mm, GL Sciences, Japan) and a Diode Array Detector (Shimadzu SPD-M20). Phenolic compounds were separated by gradient elution method using mobile phases of A (0.5% acetic acid aqueous solution), and B (acetonitrile). The gradient program was as follows: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The flow rate of the mobile phases was 0.85 ml/min, and the injection volume was 20 µl. The column temperature was maintained at 40°C during the run. Reference standards for phenolic compounds were obtained from Sigma-Aldrich Co. LLC.

Statistical analyses

Statistical analyses of the results were carried out using SPSS-Statistics-22 statistical program. Data were analysed using one-way ANOVA for pumpkin parts. The means of significant variation sources were compared using Duncan Multiple Comparison Test with the help of MSTAT program. The significance level was given as $p<0.05$ unless stated otherwise.

Results and Discussion

Moisture contents and bioactive properties of samples are presented in Table 1. The moisture contents of pulp, peel and seed were equal to 95.10%, 88.30% and 6.72%, respectively. The highest total phenolic content (97.22 mg/100 g) was dedected in peel of pumpkin, followed by pulp (43.73 mg/100 g) and seed (39.70 mg/100 g). Similar to phenolic content, both total flavonoid (114.76 mg/100 g) and total tannin (120.44 mg/100 g) contents of pumpkin peel were higher than pulp and seed parts. Regarding to the total tannin amounts of pulp and seed, no significant differences were found. However, seed of pumpkin contained more flavonoids than pulp, with values of 87.26 mg/100 g and 29.05 mg/100 g, respectively. Similarly, previous studies reported by Nyam *et al*. (2013) and Saavedra *et al*. (2015) were revealed that peel of pumpkin contained higher polyphenols than seed. Priori *et al*. (2017) presented lower total phenolic content (26.31 and 79.86 mg/100 g) than current study. Similar total phenolic amount in peel of pumpkin (93.40 mg GAE/100 g) was dedected by Hussain *et al*. (2021). On the other hand, the highest total phenolic and flavonoid contents were obtained in seed of pumpkin (224.61 mg GAE/100 g and 139.37 mg CE/100 g, respectively) in study of Hussain *et al*. (2021). Sathiya Mala and Kurian (2016) reported that there was no significant difference in total phenolic amounts of pumpkin peel and pulp. In the study of Enneb et al. (2020), the condensed tannin amount was dedected as 85.35 mg CE/g in seeds and 25.65 mg CE/g in pulp extracts. Jing et al. (2011) recorded that the condensed tannin content of pumpkin seed was equal to 28.06 mg CE/g.

Table 1. Moisture contents and bioactive properties of pumpkin parts

Part of sample	Moisture content (%)	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg QE/100g)	Total tannin content (mg TAE/100 g)	Antioxidant activity (mmol TE/kg)
Pulp	95.18 ± 0.36^{*A}	43.73 ± 1.47^{B}	29.05 ± 1.35^{C}	54.52 ± 3.78^{B}	1.32 ± 0.07^{B}
Peel	88.30 ± 0.24^{B**}	97.22 ± 1.75^{A}	114.76 ± 11.02^{A}	120.44 ± 3.14^{A}	1.93 ± 0.04^{A}
Seed	6.72 ± 0.18^{C}	39.70 ± 3.87^{B}	87.26 ± 2.05^{B}	46.59 ± 0.26^{B}	1.05 ± 0.02^{C}

Mean (three replicates) ± standard deviation of each parameter.
Different superscript letters in the same column indicate significant differences *(**$p<0.01$)*

Peel of pumpkin (1.93 mmol/kg) exhibited higher level of antioxidant activity as compared to pulp (1.32 mmol/kg) and seed (1.05 mmol/kg) of sample. In a study of Kulczynski *et al*. (2020), antioxidant activities of *Cucurbita pepo* and *Cucurbita moschata* by DPPH were obtained as 120.07 mg TE/100 g and 122.83 mg TE/100 g, respectively. In another study, the inhibition against DPPH was found between 18.92 and 70.96% (Saavedra *et al*., 2015).
Table 2 shows the phenolic compounds of peel, pulp and seed of pumpkin. The concentrations of the major phenolic compounds of pumpkin ranged from 19.16 to 29.67 mg/100 g for catechin, from 12.0 to 28.67 mg/100 g for gallic acid, from 11.78 to 22.91 mg/ 100 g for 3,4-dihydroxybenzoic acid and from 4.03 to 10.16 mg/100 g for rutin. Phenolic compounds did not statistically vary their concentration based on parts of pumpkin ($p>0.05$). Furthermore, the highest concentrations of main phenolics were observed in peel of pumpkin. According to previous study (Enneb *et al*., 2020), quercetin, rutin and catechin amounts of pulp were determined as 0.350 mg/100 g, 0.130 mg/100 g, 0.267 mg/100 g, while these flavonoid concentrations of seed were found as 0.103 mg/100 g, 0.047 mg/100 g and nd (not dedected), respectively.

Table 2. Phenolic compounds of pumpkin parts

Phenolic compounds (mg/100g)	Pulp	Peel	Seed
Gallic acid	25.39 ± 1.89*	28.67 ± 0.73	12.05 ± 0.10
3,4-Dihydroxybenzoic acid	14.46 ± 1.81	22.91 ± 0.22	11.78 ± 0.09
Catechin	19.16 ± 2.31	29.67 ± 1.90	22.72 ± 0.95
Caffeic acid	2.84 ± 0.50	3.15 ± 0.62	0.19 ± 0.04
Syringic acid	2.18 ± 0.41	4.44 ± 0.83	0.52 ± 0.07
Rutin	4.03 ± 0.45	10.16 ± 2.15	5.97 ± 0.11
p-Coumaric acid	0.22 ± 0.01	0.72 ± 0.11	0.11 ± 0.01
Ferulic acid	0.30 ± 0.02	1.40 ± 0.23	0.09 ± 0.01
Quercetin	1.01 ± 0.09	0.66 ± 0.06	0.20 ± 0.03
Cinnamic acid	0.31 ± 0.04	0.09 ± 0.00	0.08 ± 0.05
Kaempferol	0.53 ± 0.05	0.66 ± 0.05	0.35 ± 0.12

*Different superscript letters in the same column are not significant (*p> 0.05).*

Kulczynski and Gramza-Michałowska (2019) informed that the highest amounts of rutin and quercetin were recorded as 46.93 mg/100 g and 4.51 mg/100 g, respectively. It was stated that the differences in results were caused by several reasons such as climate, variety, cultivation condition solvent and method used for extraction (Hussain *et al*., 2021).

Conclusions

Each part of the pumpkin, such as peel, pulp and seed, is rich in phytochemical components and has the potential to be used as a natural source of bioactive compounds. In particular, the total phenolic, total flavonoid, total tannin contents, antioxidant activity of the peel were found to be higher compared to the pulp and seed. In addition, gallic acid, 3,4-dihydroxybenzoic acid, caffeic acid, syringic acid concentrations were at maximum level in the peel. Catechin and rutin contents, which were among the main flavonoids, were determined the highest in the peel, followed the seed part. As a result, it is recommended that increasing the use of pumpkin peel, which is a by-product, as an ingredient in food products,can provide a product that is rich in bioactive properties.

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THE INFLUENCE OF THE ROOTSTOCK ON THE PROPERTIES OF LEAF OF PLUM (*PRUNUS DOMESTICA* L.) CULTIVARS

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Abstract

Properties of leaf of three plum cultivars (‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Cacanska Najbolja’) grafted on four rootstocks (Myrobalan, ‘Pixy’, ‘Fereley’ and ‘St. Julien A’) were studied in the region of Belgrade (Serbia). The average values for leaf surface, length and width, leaf petiole length and leaf shape index in cultivar/rootstocks combinations ranged from 40.08 cm^2 to 50.60 cm^2, from 9.12 cm to 10.43 cm, from 6.13 cm to 6.94 cm, from 1.74 cm to 2.71 cm, and from 1.37 to 1.58, respectively. The rootstocks had a statistically significant influence on the leaf surface, leaf width and leaf petiole length. Leaves of cultivar ‘Cacanska Najbolja’ had the highest average leaf surface, leaf length and width, while the cultivar ‘Cacanska Rana’ had the longest leaf petiole length. The meteorological conditions in the second year of the research influenced the highest values of the physical properties of leaf. Significant differences in chlorophyll *b* content and carotenoids were found among cultivars, rootstocks and cultivar/rootstock combinations, whereas differences in chlorophyll *a* content were not significant. The chlorophyll *b* content was the highest in leaves of ‘Cacanska Najbolja’ (46.77 μg/ml) and ‘Cacanska Lepotica’ (45.79 μg/ml), while the highest carotenoids contents were found in leaves of ‘Cacanska Najbolja’ (4.87 μg/ml) and ‘Cacanska Rana’ (4.79 μg/ml) cultivars. Differences among years in contents of chlorophyll *a*, chlorophyll *b* and carotenoids were found.

Keywords: *Prunus domestica L., rootstock, cultivar, physical properties of leaf, content of leaf pigments.*

Introduction

The leaf is one of the basic vegetative organs of a plant, which has limited growth, and in which the most important physiological processes such as photosynthesis, respiration and transpiration take place. In plum, the leaf is simple and it consists of a leaf plate and a petiole. It can be of different shapes: ovate, elliptical or obovate (UPOV, 2002).

The amount of nutrients produced in the leaf depends on its surface, number, and health condition, which results in the growth and yield of the fruit tree. For this reason, it is necessary to form a sufficient amount of healthy leaf mass with the application of appropriate cultural practices. In this way, it is possible to produce a larger amount of carbohydrates during the photosynthesis process, and thus more intense differentiation of flower buds, higher yield and better quality of fruits.

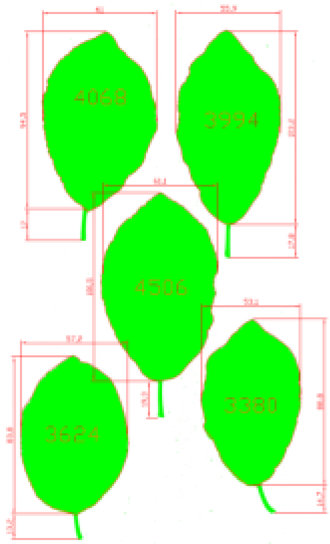
According to *Mišić* (1996), when leaves of plum are large, healthy and well lightened, this is a basic condition for abundant formation and normal development of flower buds. *Ayala* and *Lang* (2004) state that healthy leaf mass, which constantly produces photo assimilates during the vegetation, is the basis for the production of nutrients in the current vegetation and for the

formation of reserves for the next vegetation. *Roper* and *Loescher* (1987) found that there is a positive correlation between fruit mass and leaf surface, while *Seehuber et al.* (2011) state the optimal value of the ratio of the number of leaves and fruits in plum 5–15:1.
As photosynthesis is the basic process during which light energy is absorbed and converted into organic matter, the importance of the plant pigment chlorophyll (*a* and *b* forms) as an intermediary in transformation of the absorbed solar energy and its activity in the process of photosynthesis and synthesis of organic substances in plants are crucial (Pavlovic et al., 2014). The whole process is based on chlorophyll and therefore by monitoring its content in plants we can determine the productivity of photosynthesis. Photosynthetic pigments, chlorophyll *a* and chlorophyll *b*, are necessary for the absorption of light (*Richardson et al.*, 2002). On the other hand, the amount of chlorophyll *a* provides basic information about the efficiency of the photochemical reaction. *Bojović* and *Stojanović* (2005) state that the content of chlorophyll in the leaves of different plant material varies widely, depending on the degree of growth and development, species and cultivar, environmental factors, and that the ratio between chlorophyll *a* and chlorophyll *b* is usually 3:1.
The content of chlorophyll in the leaf is positively correlated with the physiological and health condition of the plant, considering that lower values result in a limited process of photosynthesis, which leads to a reduced production of organic assimilates. Chlorophyll content can be used as an indicator of the nutritional status of the tree.
Photosynthetic pigments also include carotenoids. They absorb light that chlorophyll does not receive, change its wavelength and then direct it to chlorophyll. Their basic function is to absorb light energy and quickly transfer it to chlorophyll. In addition to the role of auxiliary pigments for light absorption, carotenoids play an important role in protecting the photosynthetic apparatus. During the action of intense light, they bind oxygen and thus protect chlorophylls from photooxidative destruction (*Prasad et al.*, 2005).
The content of chlorophyll *a*, chlorophyll *b* and carotenoids depend on nutrition, e.g. mineral substances introduced into the soil through mineral fertilizers. The influence of nutrition is manifested on the photosynthetic activity through the surface of leaf. *Pilarski et al.* (2007) state that all factors that have a direct influence on the amount and composition of photosynthetic pigments result in photosynthetic activity. The concentration of chlorophyll in the leaf is correlated with the photosynthetic capacity, which also indicates the physiological state of the fruit tree (*Gamon* and *Surfus*, 1999).

Material and methods

The leaves of plum were taken from the plum orchard at the Experimental Station ‘Radmilovac’ of the Faculty of Agriculture in Belgrade (44°45′ N, 20°35′ E). Four rootstocks were included: one seedling rootstock (Myrobalan) and three clonal rootstocks (‘Pixy’, ‘Fereley’ and ‘St. Julien A’). Three table plums cultivars (‘Cacanska Rana’, ‘Cacanska Lepotica’ and ‘Cacanska Najbolja’) were grafted on each rootstock. Every combination of cultivar/rootstock was represented by six trees (two replications with three trees). The orchard was planted in spring 2010. The planting distance was 4 m between the rows, and within the row different distances were applied depending on the rootstock vigor: 2.3 m for Myrobalan seedling, 2.0 m for ‘Fereley’ and ‘St. Julien A’, and 1.7 m for ‘Pixy’. The training system was the Spindle. Standard cultural practices were applied, including drip irrigation. From every combination of cultivar/rootstock, 30 leaves were randomly picked in July, from the middle part of moderately vigorous one-year-old shoots.

The length, width, surface of leaf and the length of the petiole were measured using the program *AutoCAD software version 2011* (Picture 1).



Picture a. Morphometric analysis of the leaf (*AutoCAD software version 2011*)

The content of pigments in the leaf was determined by the method of extraction in dimethylformamide (*Moran* and *Porath*, 1980). In laboratory conditions, from every combination of cultivar/rootstock, fragments of the leaf blade were sampled (in three repetitions) up to a total weight of 0.1 g and transferred to test tubes with 3 ml of dimethylformamide each.

The extraction was performed in the dark place at a temperature of 4 °C for 24 hours. After this period, the absorbance of the extract was read on a spectrophotometer (*T60 Visible Spectrophotometer-PG Instruments Ltd, Leicester, UK)* at three wavelengths: 480 nm for carotenoids, 664 nm for chlorophyll *a* and 647 nm for chlorophyll *b*. To calculate the concentration of pigments (μg/ml) the formula according to *Wellburn* (1994) was used:

Chlorophyll *a*: ca = 11,65A664–2,69A647
Chlorophyll *b*: cb = 20,81A647–4,45A664
Carotenoids: ck = (1000A480–0,89ca-52,02cb)/245

Data of all measurements presented in the tables are the mean of three replicates ± standard deviation. *Duncan's* test was used to detect the significance of differences ($P \leq 0.05$) between mean values.

Results and discussion

The physical properties of the leaf (surface, length, width, length of petiole and leaf shape index) are shown in Table 1. Leaf surface ranged from 40.08 cm^2 (‘Cacanska Lepotica’/‘Pixy’) to 50.60 cm^2 (‘Cacanska Najbolja’/‘St. Julien A’). Significant differences for leaf surface were found between cultivar/rootstock combination. The highest average surface was found on leaf from trees grafted on ‘St. Julien A’ rootstock (46.87 cm^2), while the lowest values were found on leaf from trees grafted on ‘Pixy’ rootstock (44.02 cm^2).

Table 1. Properties of leaf of three plum cultivars grafted on four rootstocks (average 2013–2015)

Cultivar/rootstock combination		**Leaf surface (cm^2)**	**Length of leaf (cm)**	**Width of leaf (cm)**	**Petiole length (cm)**	**Leaf shape index (L/W)**
C. Rana/St. Julien A		44.86 ± 1.48 ^{c†}	9.55 ± 0.15 ^{ab}	6.64 ± 0.13 ^{cd}	2.54 ± 0.09 ^{b}	1.44 ± 0.01
C. Rana/Pixy		44.31 ± 1.41 ^{c}	9.53 ± 0.17 ^{ab}	6.55 ± 0.11 ^{cd}	2.71 ± 0.13 ^{a}	1.46 ± 0.01
C. Rana/Fereley		44.33 ± 1.95 ^{c}	9.29 ± 0.24 ^{ab}	6.70 ± 0.16 ^{bc}	2.71 ± 0.12 ^{a}	1.39 ± 0.02
C. Rana/Myrobalan		44.63 ± 2.01 ^{c}	9.21 ± 0.24 ^{b}	6.73 ± 0.16 ^{bc}	2.41 ± 0.08 ^{c}	1.37 ± 0.01
C. Lepotica/St. Julien A		45.16 ± 1.41 ^{c}	9.48 ± 0.09 ^{ab}	6.55 ± 0.18 ^{cd}	2.15 ± 0.05 ^{d}	1.46 ± 0.04
C. Lepotica/Pixy		40.08 ± 2.01 ^{e}	9.12 ± 0.15 ^{b}	6.13 ± 0.19 ^{f}	2.18 ± 0.06 ^{d}	1.50 ± 0.03
C. Lepotica/Fereley		42.99 ± 1.61 ^{cd}	10.26 ± 1.22 ^{ab}	6.46 ± 0.15 ^{de}	2.13 ± 0.10 ^{d}	1.58 ± 0.17
C. Lepotica/Myrobalan		41.56 ± 0.83 ^{de}	9.18 ± 0.11 ^{b}	6.28 ± 0.10 ^{ef}	1.95 ± 0.08 ^{e}	1.47 ± 0.03
C. Najbolja/St. Julien A		50.60 ± 2.28 ^{a}	10.43 ± 0.22 ^{a}	6.89 ± 0.18 ^{ab}	1.85 ± 0.05 ^{ef}	1.52 ± 0.02
C. Najbolja/Pixy		47.67 ± 2.18 ^{b}	9.97 ± 0.26 ^{ab}	6.69 ± 0.14 ^{bc}	1.95 ± 0.09 ^{e}	1.49 ± 0.01
C. Najbolja/Fereley		49.56 ± 2.91 ^{ab}	9.87 ± 0.33 ^{ab}	6.94 ± 0.21 ^{a}	1.91 ± 0.05 ^{e}	1.42 ± 0.01
C. Najbolja/Myrobalan		45.08 ± 1.60 ^{c}	9.64 ± 0.17 ^{ab}	6.55 ± 0.13 ^{cd}	1.74 ± 0.05 ^{f}	1.47 ± 0.02
Rootstock	St. Julien A	46.87 ± 1.07 ^{a}	9.82 ± 0.11	6.70 ± 0.09 ^{a}	2.18 ± 0.06 ^{b}	1.47 ± 0.02
	Pixy	44.02 ± 1.16 ^{c}	9.54 ± 0.12	6.46 ± 0.09 ^{b}	2.28 ± 0.07 ^{a}	1.48 ± 0.01
	Fereley	45.63 ± 1.33 ^{b}	9.81 ± 0.42	6.70 ± 0.10 ^{a}	2.25 ± 0.07 ^{ab}	1.46 ± 0.06
	Myrobalan	43.76 ± 0.91 ^{c}	9.35 ± 0.11	6.52 ± 0.08 ^{b}	2.03 ± 0.06 ^{c}	1.44 ± 0.01
Cultivar	C. Rana	44.53 ± 0.84 ^{b}	9.40 ± 0.10 ^{b}	6.66 ± 0.07 ^{b}	2.59 ± 0.05 ^{a}	1.41 ± 0.01 ^{b}
	C. Lepotica	42.45 ± 0.78 ^{c}	9.51 ± 0.31 ^{ab}	6.35 ± 0.08 ^{c}	2.10 ± 0.04 ^{b}	1.50 ± 0.04 ^{a}
	C. Najbolja	48.23 ± 1.15 ^{a}	9.98 ± 0.13 ^{a}	6.77 ± 0.09 ^{a}	1.86 ± 0.03 ^{c}	1.47 ± 0.01 ^{ab}
Year	2013	36.83 ± 0.41 ^{c}	8.73 ± 0.08 ^{c}	5.91 ± 0.04 ^{c}	1.88 ± 0.04 ^{c}	1.48 ± 0.01
	2014	51.34 ± 0.74 ^{a}	10.41 ± 0.30 ^{a}	7.12 ± 0.05 ^{a}	2.48 ± 0.05 ^{a}	1.46 ± 0.04
	2015	47.04 ± 0.57 ^{b}	9.74 ± 0.07 ^{b}	6.75 ± 0.05 ^{b}	2.20 ± 0.06 ^{b}	1.45 ± 0.01

Mean values followed by the same letter in a column for each cultivar/rootstock combination, rootstock, cultivar and year respectively are not significantly different according to Duncan’s multiple range test ($P \leq 0.05$)

The trend of the average leaf surface, among the cultivars, was as follows: ‘Cacanska Najbolja’ (48.23 cm^2) > ‘Cacanska Rana’ (44.53 mm^2) > ‘Cacanska Lepotica’ (42.45 cm^2). Significant differences among cultivars were found (Table 1). The significantly highest average leaf surface was in 2014 year (51.34 cm^2), while the lowest value was in 2013 year (36.83 cm^2). Differences among year of research were statistically significant. Our results for leaf surface were higher than the results obtained by *Razouk et al.* (2013; 2016), and lower than the results by *Mišić* (1996) and *Seehuber et al.* (2011). According to *Razouk et al.* (2013) average leaf surface values was from 19.3 cm^2 to 19.4 cm^2 in cultivar ‘Stanley’. *Razouk et al.* (2016) established the interval of the leaf surface 18.6–24.7 cm^2, depending on the irrigation method.

During the three-year period, there were slightly variations in leaf length between cultivar/rootstock combinations. Influence of rootstock was not significant on this parameter. On the other hand, cultivar ‘Cacanska Najbolja’ has the leaf with average length of 9.98 cm, which

was a significantly higher value compared to cultivar ‘Cacanska Rana’ (9.40 cm). The leaf length was the highest in 2014 (10.41 cm), and the lowest in 2013 (8.73 cm).
Significant differences of average width of leaf were found between cultivar/rootstock combinations (Table 1). The combination ‘Cacanska Najbolja’/‘Fereley’ had the highest width of leaf (6.94 cm), while the combination ‘Cacanska Lepotica’/‘Pixy’ had the lowest value (6.13 cm). The clonal rootstocks ‘St. Julien A’ and ‘Fereley’ influenced a significantly higher width of the leaf (6.70 cm) compared to the seedling rootstock (Myrobalan) (6.52 cm) and clonal rootstock ‘Pixy’ (6.46 cm). Cultivar ‘Cacanska Najbolja’ had the leaf with the highest value of width (6.77 cm), while the lowest value was in cultivar ‘Cacanska Lepotica’ (6.35 cm). Significant differences in leaf width among cultivars and years were found. The highest average width of the leaf was 7.12 cm in 2014 year and lowest was 5.91 cm in 2013 year. The results of our research of length and width of leaf were not in agreement with results that reported by *Surányi* (2005). According to *Milatovic* (2019) leaves of different cultivars of *Prunus domestica* L. have the average length of 5 cm to 9 cm and the width of 4 cm to 7 cm.
Average length of leaf petiole ranged from 1.74 cm to 2.71 cm. Significant differences for this parameter were found between cultivar/rootstock combination. The leaves from trees grafted on seedling rootstock (Myrobalan) had significantly the shortest petiole in relation to other three clonal rootstocks (‘Pixy’, ‘Fereley’ and ‘St. Julien A’). The cultivar ‘Cacanska Rana’ had the longest leaf petiole (2.59 cm), while the cultivar ‘Cacanska Najbolja’ had the shortest leaf petiole (1.86 cm). Differences in length of leaf petiole among cultivars were statistically significant. Also, meteorological conditions in the years of research had the significant influence on this parameter. The longest petiole of leaf was in 2014 year (2.48 cm), while the shortest petiole was in first year of investigation (1.88 cm). The analyzed cultivar/rootstock combination had an average length of leaf petiole of 2.2 cm which confirmed the statements *Milatović* (2019). On the other hand, these results are not in agreement with the findings of *Surányi* (2005).
The leaf shape index ranged from 1.37 (‘Cacanska Rana’/Myrobalan) to 1.58 (‘Cacanska Lepotica’/‘Fereley’). Approximate values of the leaf shape index were determined on all examined rootstocks (table 1). On the other hand, significant differences were found between cultivar ‘Cacanska Lepotica’ (1.50) and ‘Cacanska Rana’ (1.41). According to *Milatović* (2019) leaf shape index in 50 of different cultivars *Prunus domestica* L. had the value from 1.15 (Kisinjevska rana) to 1.90 (Zlatka). *Surányi* (2005), states the leaf shape index interval from 1.23 to 2.44 in cultivars of different species of the genus *Prunus*.
Average chlorophyll *a* content ranged from 19.17 µg/ml (‘Cacanska Najbolja’/Myrobalan) to 19.49 µg/ml (‘Cacanska Rana’/‘Pixy’). Among cultivars and rootstocks were not found significant variation in the average content of this pigment (table 2). The lowest average chlorophyll *a* content was in 2013 year (19.12 µg/ml).
The lowest chlorophyll *b* content was found in the leaf of the cultivar ‘Cacanska Rana’ grafted on the clonal rootstock ‘Fereley’ (40.82 µg/ml), while the highest content was in leaf of the combination ‘Cacanska Najbolja’/Myrobalan (47.78 µg/ml). However, significant differences for chlorophyll *b* content were found between cultivar/rootstock combination.
The lowest average chlorophyll *b* content was found in leaves from trees grafted on ‘Pixy’ rootstock (43.49 µg/ml) and the highest were found in leaves from trees grafted on ‘St. Julien A’ rootstock (46.98 µg/ml). The highest values of chlorophyll *b* content (for all four rootstocks) were found in leaves of cultivar ‘Cacanska Najbolja’ (46.77 µg/ml), then in ‘Cacanska Lepotica’ (45.79 µg/ml), while they were lowest in ‘Cacanska Rana’ cultivar (43.05 µg/ml). Years

manifested significant differences in chlorophyll *b* content. The significantly lowest value was in 2014 year compared to two other years (43.24 μg/ml).

Table 2. Content of pigments in the leaf of three plum cultivars grafted on four rootstocks (average 2013–2015)

Cultivar/rootstock combination		Chlorophyll *a* (μg/ml)	Chlorophyll *b* (μg/ml)	Carotenoids (μg/ml)
Cacanska Rana/St. Julien A		19.32 ± 0.12 †	45.83 ± 1.61 a-c	4.72 ± 0.15 a-d
Cacanska Rana/Pixy		19.49 ± 0.13	42.14 ± 1.27 cd	4.98 ± 0.09 a-c
Cacanska Rana/Fereley		19.32 ± 0.09	40.82 ± 1.53 d	5.07 ± 0.25 ab
Cacanska Rana/Myrobalan		19.45 ± 0.22	43.40 ± 2.25 b-d	4.39 ± 0.18 cd
Cacanska Lepotica/St. Julien A		19.26 ± 0.12	47.73 ± 1.04 a	4.20 ± 0.17 d
Cacanska Lepotica/Pixy		19.44 ± 0.15	43.47 ± 1.21 b-d	4.63 ± 0.19 a-d
Cacanska Lepotica/Fereley		19.26 ± 0.09	46.49 ± 1.13 ab	4.54 ± 0.31 b-d
Cacanska Lepotica/Myrobalan		19.43 ± 0.11	45.51 ± 0.99 a-c	4.42 ± 0.19 cd
Cacanska Najbolja/St. Julien A		19.22 ± 0.09	47.39 ± 0.84 a	4.94 ± 0.29 a-c
Cacanska Najbolja/Pixy		19.28 ± 0.11	44.85 ± 1.55 a-c	5.17 ± 0.20 a
Cacanska Najbolja/Fereley		19.39 ± 0.07	47.04 ± 1.09 ab	4.82 ± 0.24 a-d
Cacanska Najbolja/Myrobalan		19.17 ± 0.06	47.78 ± 0.97 a	4.54 ± 0.15 b-d
Rootstock	St. Julien A	19.26 ± 0.06	46.98 ± 0.69 a	4.62 ± 0.13 ab
	Pixy	19.40 ± 0.07	43.49 ± 0.78 c	4.92 ± 0.10 a
	Fereley	19.32 ± 0.05	44.78 ± 0.89 bc	4.81 ± 0.15 a
	Myrobalan	19.35 ± 0.08	45.56 ± 0.91 ab	4.45 ± 0.09 b
Cultivar	Cacanska Rana	19.39 ± 0.07	43.05 ± 0.87 b	4.79 ± 0.09 a
	Cacanska Lepotica	19.34 ± 0.06	45.79 ± 0.59 a	4.45 ± 0.11 b
	Cacanska Najbolja	19.26 ± 0.04	46.77 ± 0.58 a	4.87 ± 0.11 a
Year	2013	19.12 ± 0.03 b	46.55 ± 0.49 a	4.74 ± 0.09 ab
	2014	19.44 ± 0.07 a	43.24 ± 0.71 b	4.48 ± 0.08 b
	2015	19.44 ± 0.05 a	45.82 ± 0.86 a	4.89 ± 0.14 a

Mean values followed by the same letter in a column for each cultivar/rootstock combination, rootstock, cultivar and year respectively are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Significant differences of carotenoid contents among cultivar/rootstock combinations were found. The leaves from combination 'Cacanska Lepotica'/'St. Julien A' had the lowest carotenoid content (4.20 μg/ml), while the leaves from combination 'Cacanska Najbolja'/'Pixy' had the highest value (5.17 μg/ml). The seedling rootstock (Myrobalan) influenced the lowest carotenoid contents in leaves (4.45 μg/ml), while the rootstocks 'Fereley' and 'Pixy' influenced significantly higher carotenoid contents (4.84 μg/ml, and 4.92 μg/ml respectively). Cultivar 'Cacanska Najbolja' showed the highest amount of carotenoids content (4.87 μg/ml), followed by 'Cacanska Rana' (4.79 μg/ml), while the cultivar 'Cacanska Lepotica' had the significantly lower carotenoids content (4.45 μg/ml). Differences in carotenoids contents among years of research were significant.

The clonal rootstock 'St. Julien A' influenced the lowest chlorophyll *a* content in leaves and these results confirmed the statements of *Mestre et al.* (2017). The chlorophyll *b* content was higher compared to chlorophyll *a* content, which is in accordance with the previous findings of (*Sytykiewicz et al.,* 2013). According to *Pareek et al.* (2018) the ratio of chlorophyll *a* to

chlorophyll *b* is three, approximately (depending on the species) and depends on conditions of growing and exposure of leaves to sunlight.
The reason for the lower chlorophyll *a* content in the leaves can be explained by the fact that this pigment is less thermostable, and it is sensitive at higher air temperatures (*Erge et al.,* 2008). According to *Reig et al.* (2018) the influence of rootstocks (Adara, Ademir, Miral 3278 AD, Myrobalan B and Myrobalan 713 AD) was statistically significant on the chlorophyll content in the leaves of the cultivar R.C.Bavay. On the other hand, *Bolat et al.* (2006) state that different mineral nutrition results in different values of chlorophyll content in the leaf of the rootstocks Marianna GF 8-1, Myrobalan B and Pixy. Also, *Mestre et al.* (2017) have confirmed the influence of the rootstock on the chlorophyll content during two years of research. Studying two European plum cultivars, grafted on different rootstocks, Reig et al. (2018) found that in one cultivar, the rootstock had a statistically significant effect on the chlorophyll content in the leaves, while in the other cultivar this difference was not significant. *Mestre et al.* (2017) found that there are statistically significant differences in the chlorophyll content between the rootstocks. *Jakab-Ilyefalvi* and *Pamfil* (2011) established the interval of chlorophyll a content from 7.02 mg/l to 12.93 mg/l and chlorophyll b content from 0.25 mg/l to 1.11 mg/l, in four plum cultivars (Ivan, Iulia, Geta, Jubileu 50). According to the previous group of authors the chlorophyll *a* content and chlorophyll *b* content is significantly higher in healthy leaf than in leaf with signs of the disease. In the study of *Botu et al.* (2017) the chlorophyll content (SPAD readings), in July, was in the range 39.6–52.6, while the value of this parameter was significantly lower in September (1.4–12.1).
The values of photosynthetic pigments depend on the time of leaf sampling (*Botu et al.*, 2017), part of the leaf used for analysis (leaf blade or petiole) (*Pilarski et al.*, 2007), irrigation (*Razouk et al.*, 2016), health condition (*Jakab-Ilyefalvi* and *Pamfil*, 2011), but also the application of different mineral fertilizers (*Staneva et al*., 2019).

Conclusion

Based on the results of three-year tests of the influence of the rootstock on the properties of the leaves of plum (*Prunus domestica* L.) cultivars in the ecological conditions of the Belgrade, we can conclude that rootstocks had a significant influence on all examined leaf morphometric properties, except for leaf length. Also, the rootstock influenced the chlorophyll *b* content and carotenoids content in the leaf of grafted plum cultivars, while the influence on chlorophyll *a* content was not statistically significant.

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GROWTH OF MEDITERRANEAN SAGE SPECIES AND INTERSPECIFIC HYBRIDS UNDER LIMITED IRRIGATION IN A GREEN ROOF

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Abstract

Mediterranean sages (*Salvia* spp.) could be ideal for use in xeriscaping, as bee-friendly plants or in green roofs. *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, along with five new interspecific hybrids of them, were evaluated for their growth under regular (every 2–3 days when substrate moisture 17–23% v/v) and limited irrigation (every 4–5 days when substrate moisture 7–13% v/v), in a Mediterranean extensive green roof. A substrate (grape-marc compost: perlite: pumice, 3:3:4, v/v), with 10 cm depth, was used. *S. pomifera* ssp. *pomifera* × *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera* survived at the highest percentage. Limited irrigation resulted in the reduction of aboveground and root biomass of all sage types, but to varying degrees depending on sage type. *S. officinalis*, *S. officinalis* × *S. ringens* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest aboveground biomass reduction, in contrast to *S. fruticosa* that showed the highest, while *S. officinalis*, *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest reduction in root biomass. All studied sage types, with a reservation for *S. fruticosa*, grew satisfactorily under limited irrigation, being suitable for sustainable exploitation in xeriscaping, including extensive green roofs in arid regions. Especially the hybrids of *S. officinalis* or *S. ringens* or *S. pomifera* ssp. *pomifera* were found to be even more resistant to limited irrigation than their parental species.

Keywords: *Salvia fruticosa, Salvia officinalis, Salvia pomifera ssp. pomifera, Salvia ringens, Salvia tomentosa.*

Introduction

Green roofs (GRs) are one type of green infrastructure that can be applied to city buildings and provide significant environmental, social and economical advantages to the urban environment that mitigate the adverse effects of urbanization and make cities more safe, sustainable and resilient to the climate crisis (Caneva *et al.*, 2015; Shafique *et al.*, 2018). One of the most critical steps in green roof installation in these areas is the selection of drought- and heat-tolerant plant species that can thrive under extreme microclimate conditions (Savi *et al.*, 2016). The adaptation of many native Mediterranean plants to drought stress and their floristic diversity leads them to be ideal for use in extensive GRs in the Mediterranean and other areas with similar climatic conditions (Papafotiou *et al.*, 2013; Caneva *et al.*, 2015; Ondoño *et al.*, 2015; Tassoula *et al.*, 2021).
Mediterranean sages (*Salvia* spp.), as members of the macchia vegetation, are drought-resistant plants and could be ideal for use in xeriscaping, as bee-friendly plants or in green roofs. In order

to introduce new drought-resistant species with interesting floricultural characteristics in the floriculture industry, interspecific crossbreeding was undertaken between five native to Greece sage species and five hybrids, i.e., *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. pomifera* ssp. *pomifera* × *S. ringens*, were chosen for their ornamental traits (Papafotiou *et al.*, 2021). *S. officinalis* and *S. fruticosa* have been tested previously for growth on extensive GRs (Raimondo *et al.*, 2015; Papafotiou *et al.*, 2015; Kemp *et al.*, 2019), while the drought tolerance of *S. officinalis* has thoroughly been investigated (Raimondo *et al.*, 2015; Kemp *et al.*, 2019; Abate *et al.*, 2021). Besides, four of the studied hybrids used were found to respond better to water stress in greenhouse conditions compared to *S. fruticosa* (Papafotiou *et al.*, 2021).
In the present study, the species *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. ringens* and *S. tomentosa*, along with five new interspecific hybrids of them, were evaluated for their growth under regular and limited irrigation, in a Mediterranean extensive green roof.

Materials and methods

Rooted cuttings of five sage species native to Greece, i.e., *Salvia fruticosa*, *S. officinalis*, *S. pomifera* ssp. *pomifera*, *S. tomentosa* and *S. ringens*, along with five interspecific hybrids of them, i.e., *S. fruticosa* × *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera*, *S. officinalis* × *S. ringens*, *S. officinalis* × *S. tomentosa* and *S. pomifera* ssp. *pomifera* × *S. ringens*, about 8 weeks old, were planted on early April 2021 in plastic containers that were 40 cm (width) × 60 cm (length) × 22 cm (depth) in size. Each container had a green roof infrastructure fitted, i.e., moisture retention and protection of the insulation mat FLW-500, a drainage layer Diadrain-25H and a filter sheet VLF-150 (Landco Ltd., Diadem Green Roof Systems, Athens, Greece). Two plants of the same type per container and six containers per treatment were used. The containers were placed on a second-floor flat roof at the Agricultural University of Athens (37°59′ N, 23° 42′ E). The substrate used was grape marc compost: perlite: pumice (3: 3: 4, *v*/*v*) and had a 10 cm depth. Climatic conditions during this experiment are shown in Figure 1.

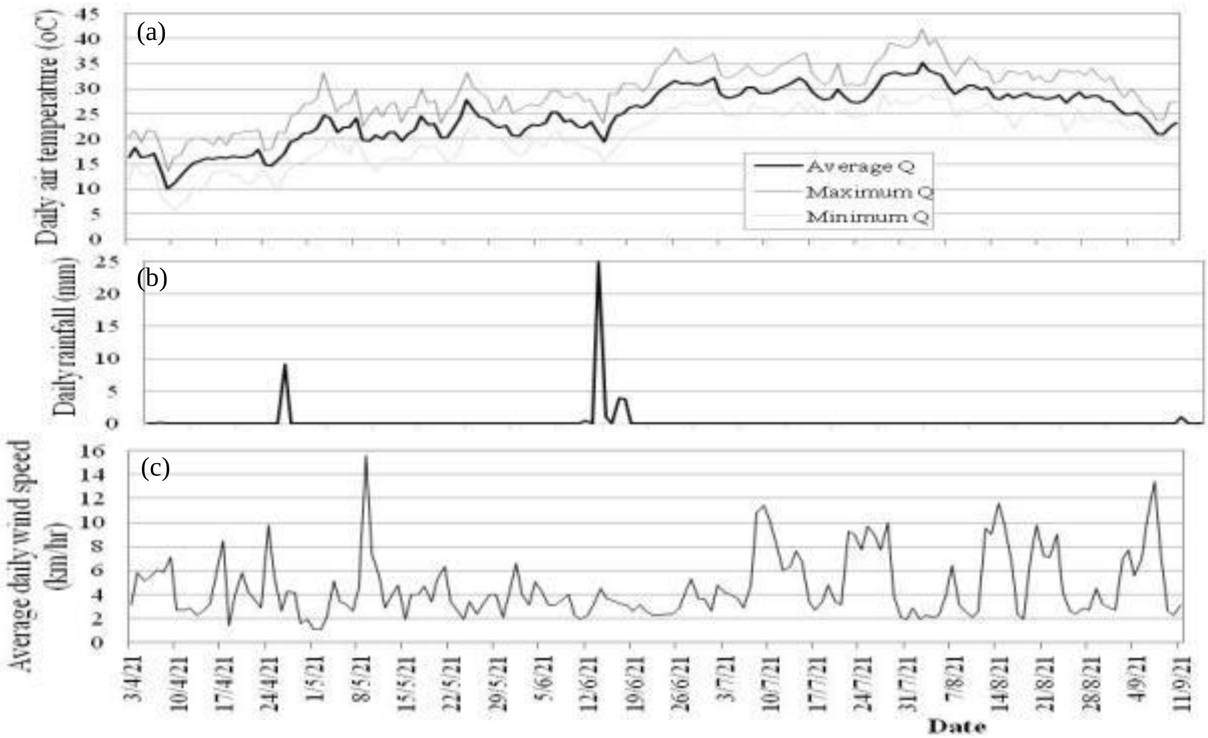
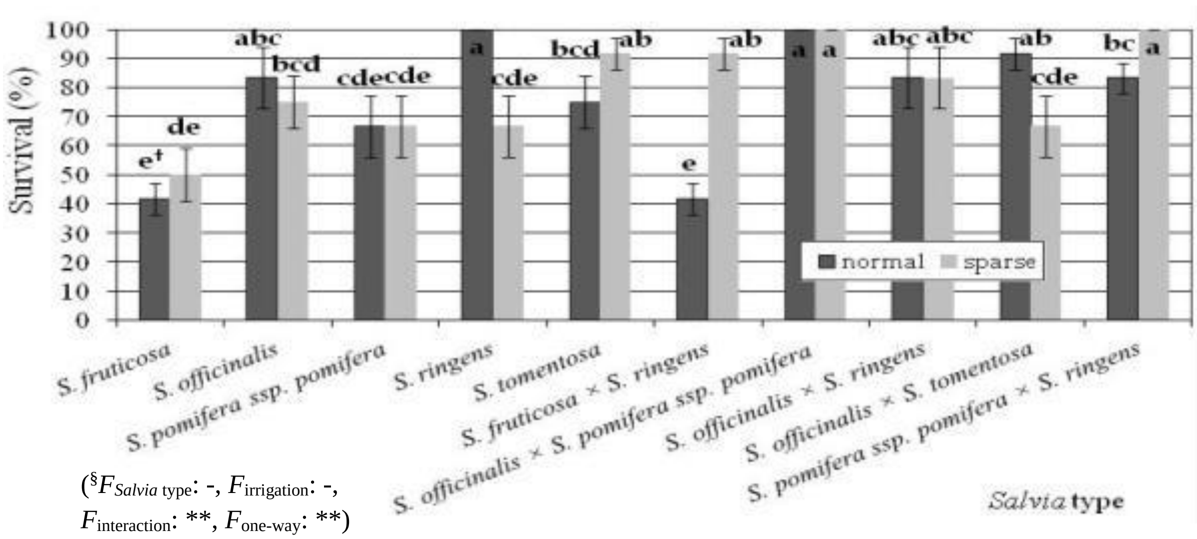


Figure 1. Climatic conditions, i.e., temperature (a), daily rainfall (b) and average daily wind speed (c) in the green roof, during the 5-month experimental period (from April to September 2021).

Two irrigation frequencies were applied, a regular (normal, when substrate moisture 17–23% v/v) and a limited one (sparse, when substrate moisture 7–13% v/v). In the first month of cultivation, the plants were irrigated normally every 3 days and sparsely every 5 days, while in the following months every 2 and 4 days, respectively. Automatic drip irrigation on the substrate surface was applied before sunrise by two drippers placed at equal distances from the center of the container and the plants (dripper supply 4 L h^{-1}, irrigation period: 60 min).
The experiment lasted for 5 months, until September 2021, and plant survival and growth were evaluated. The completely randomized design was used. The significance of the results was tested by either one- or two- way analysis of variance (ANOVA) and the means of the treatments were compared by Student’s *t* test at $p< 0.05$ (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

Five months after planting in an extensive green roof, *S. fruticosa* presented the lowest survival rate, regardless of irrigation frequency (Figure 2). Plant losses occurred sporadically during the June–September period, with most losses in July and August (monthly data not shown), which were the hottest, driest and most windy months (Figure 1a–c). *S. ringens* was the species with the highest survival rate under normal irrigation, but under sparse irrigation, its survival rate was significantly reduced. However, all three *S. ringens* hybrids showed a high survival rate under sparse irrigation. The hybrid *S. officinalis* × *S. pomifera* ssp. *pomifera* was the only *Salvia* type that showed 100% survival at both irrigation frequencies (Figure 2).



(§$F_{Salvia\ \mathrm{type}}$: -, $F_{\mathrm{irrigation}}$: -, $F_{\mathrm{interaction}}$: **, $F_{\mathrm{one\text{-}way}}$: **)

Figure 2. Survival (%) of *Salvia* species and interspecific hybrids five months after establishment in an urban Mediterranean green roof under normal and sparse irrigation during the hot and dry season (n = 6).

† Mean comparison with Student’s *t*-test at $p \leq 0.05$; means followed by the same letter were not significantly different at $p \leq 0.05$. § NS or **, non-significant at $p \leq 0.05$ or significant at $p \leq 0.05$, respectively.

The experimental factors (*Salvia* type and irrigation frequency) significantly affected the aboveground and the root dry weight (Figure 3). Sparse irrigation resulted in lower aboveground and root biomasses compared to normal irrigation. Concerning *Salvia* type, the highest aboveground biomass was recorded for the *S. officinalis* × *S. ringens* hybrid with no statistical difference from *S. tomentosa.* The same hybrid also showed one of the highest values of root biomass, along with *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* (Figures 3 and 4). Under normal irrigation, the species *S. fruticosa*, *S. tomentosa* and *S. pomifera* ssp. *pomifera* and the hybrids *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* × *S. ringens* developed the highest aboveground biomass compared to all other species and hybrids, followed by *S. ringens*, *S. fruticosa* × *S. ringens* and *S. officinalis* × *S. tomentosa* (Figures 3 and 4). As for the root biomass, this was highest in *S. fruticosa*, *S. tomentosa* and *S. officinalis* and the hybrids *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. officinalis* × *S. ringens* (Figures 3 and 4).

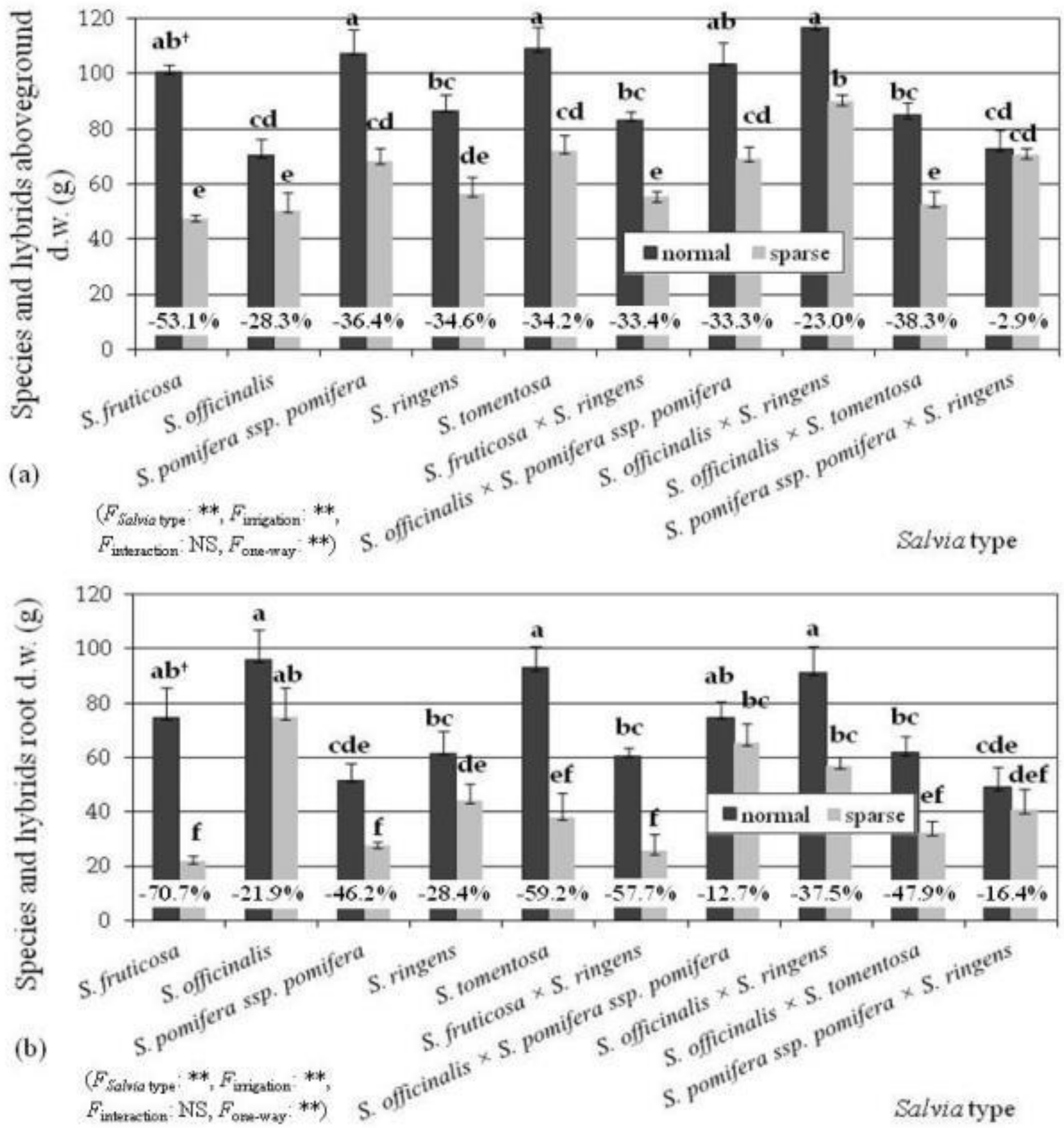


Figure 3. Aboveground (a) and root (b) dry weight (d.w.) of Greek sage species and interspecific hybrids after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation, as well as reduction percentage (%) of aboveground or root d.w. under sparse irrigation is presented.

Limited irrigation resulted in the reduction of aboveground and root biomass of all sage types, but in a different degree depending on sage type. *S. officinalis*, *S. officinalis* × *S. ringens* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest aboveground biomass reduction, in contrast to *S. fruticosa* that showed the highest, while *S. officinalis*, *S. ringens*, *S. officinalis* × *S. pomifera* ssp. *pomifera* and *S. pomifera* ssp. *pomifera* × *S. ringens* showed the lowest reduction in root biomass (Figure 3).

The survival and growth of all five sage species and five hybrids in an extensive green roof during the hot and dry season of the Eastern Mediterranean were affected by both the plant genotype and the irrigation frequency (Figure 2, 3). Regarding hybrid parents, *S. officinalis* is considered suitable for use in green roofs (Raimondo *et al.*, 2015; Papafotiou *et al.*, 2017), as supported by the present work as well. In general, the hybrids acclimatized more efficiently to the green roof conditions compared to their parents and the hybrids of *S. pomifera* ssp. *pomifera* showed the highest survival of all species and hybrids at both irrigation frequencies. All species and hybrids, except for *S. pomifera* ssp. *pomifera* × *S. ringens*, showed a reduction in aboveground plant biomass under water stress, which is an avoidance mechanism caused by a dehydration process (Alarcón *et al.*, 2006), leading to water loss reduction. In several plant species, under drought conditions, the root biomass is reduced less than the aboveground biomass, resulting in a higher root/aboveground ratio (Zhou *et al*., 2018), which optimizes water uptake (Chaves *et al.*, 2003). In the present work, all species and hybrids, except *S. officinalis*, *S. ringens* and *S. officinalis* × *S. pomifera* ssp. *pomifera*, showed a greater reduction in root biomass than aboveground biomass under sparse irrigation (Figure 3).

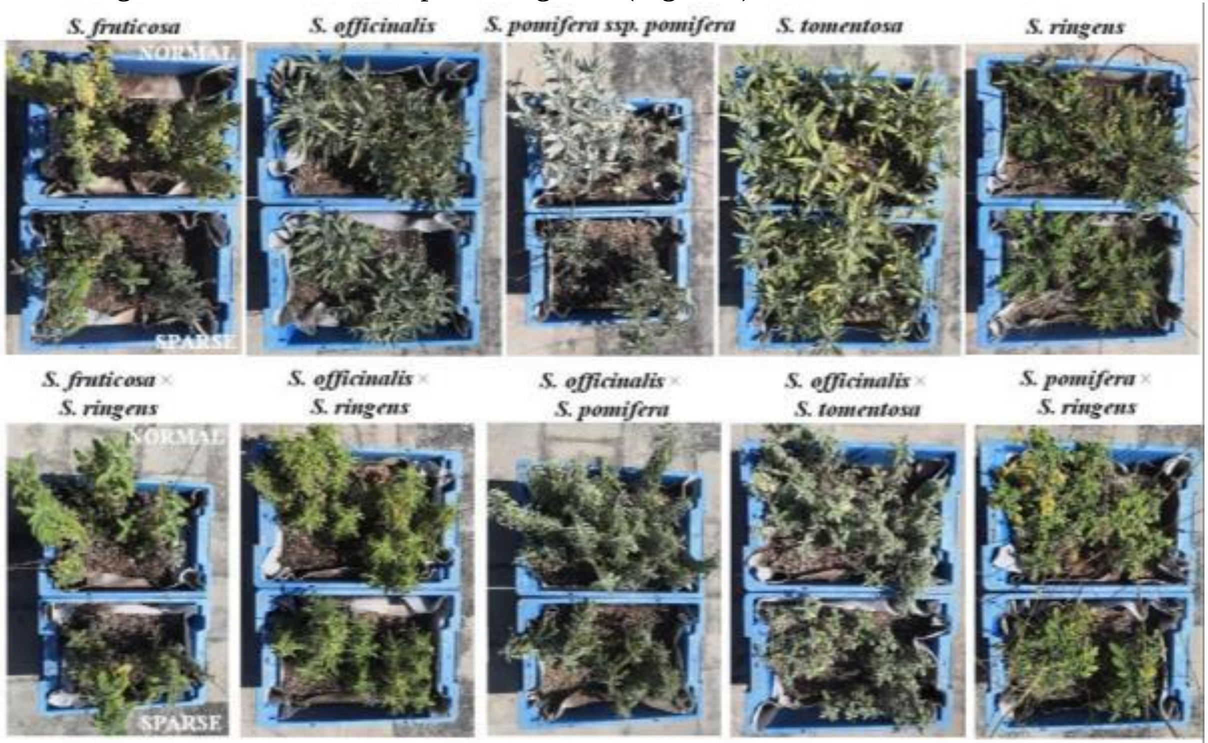


Figure 4. Typical aboveground growth of Salvia species and interspecific hybrids after five months of growth (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation frequencies.

Conclusions

All studied sage types, with a reservation for *S. fruticosa*, grew satisfactorily under limited irrigation, being suitable for sustainable exploitation in xeriscaping, including extensive green roofs in arid regions. Especially the hybrids of *S. officinalis* or *S. ringens* or *S. pomifera* ssp. *pomifera* were found even more resistant in limited irrigation than their parental species.

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MORPHOLOGICAL CHARACTERS OF NEW INTERSPECIFIC HYBRIDS OF SAGE ORIGINATED FROM *SALVIA OFFICINALIS, S. POMIFERA* SSP. *POMIFERA* AND *S. TOMENTOSA*

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Abstract

Native plant species or clones are an important source of new products for the floricultural industry. Greek flora is a bank of genetic material due to its biodiversity and includes *Salvia* spp. of high potential value, which have not been exploited like other commercial species. The present study aims to reveal the morphology of two new interspecific hybrids, between *S. officinalis* and *S. pomifera* ssp. *pomifera* or *S. tomentosa*, i.e *S. officinalis* × *S. pomifera* ssp. *pomifera* (OP) and *S. officinalis* × *S. tomentosa* (OT). A total of eight quantitative and 14 qualitative characters were selected and used based on descriptors for other plant species. The first group of characters involved leaf and flower morphometrics and the second characters of vegetation, flowers and fragrance. One way ANOVA was used for determination of the differences between the mean values of leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. OP and OT hybrids found to be closer to *S. officinalis*. Both *S. officinalis* and *S. pomifera* ssp. *pomifera* are strongly aromatic plants and their hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of *S. pomifera*. OT is a compact plant with numerous lateral shoots and a lighter aroma than that of *S. officinalis*. The present study revealed morphological characteristics to differentiate the new hybrids.

Keywords: *Dendrogram, descriptors, flower morphometrics, Mediterranean sage, leaf morphometrics, qualitative and quantitative characters.*

Introduction

Salvia is one the largest genera of flowering plants and includes 2.100 scientific plant names, 1042 being accepted species names (WFO, 2022). There are three distinct regions of its diversity, i.e., Central and South America, Eastern Asia and Central Asia and the Mediterranean (Walker *et al.*, 2004). Greek flora is a bank of genetic material and Salvia species native in Greece have a high potential value for floriculture industry; 30 taxa (species and subspecies) of the genus Salvia can be found in Greece, (Karousou *et al*., 2000). *S. officinalis* is one of the most widely used species in traditional medicine (Llurba-Montesino and Schmidt, 2018) being with *S. fruticosa* one of the most researched European species (Karalija *et al*, 2022). *S. officinalis* is a perennial subshrub, native to the coastal regions of the southern Europe with a habitat reaching south into northwest Greece (di Pietro, 2011). *S. pomifera* spp. *pomifera* is endemic in dry, rocky places in Peloponnese and Crete being unexploited (Strid, 2016). *S. tomentosa* resembles to *S. officinalis* and can be found in areas of macchia vegetation and on limestone slopes in the North-

Eastern and Central Greece and the North-Eastern and Eastern Aegean Islands (Dimopoulos *et al.,* 2013).

Considering that the floriculture industry is seeking the introduction of new native plant species, hybrids or clones for use either as pot or landscape plants, the introduction of artificial hybrids between Salvia species found in Greece is a challenge. It must be emphasized that few instances of natural hybridization have been documanted between native Salvia species (Celep *et al.*, 2020).

Morphological analysis is an effective tool for both characterizing and distinguishing hybrids and studying their relationships (Santos et al., 2011). Morphological traits are widely used for studies in hybrids and assessing variability in plant species (Khurshid *et al.,* 2004; Arabaci *et al.*, 2021); leaf size and flower characters have been used as descriptors of a number of *Salvia* species (Celep *et al.,* 2011; Leontaritou *et al*., 2020; Bertsouklis *et al.*, 2021). In the present study, we aim to test quantitative and qualitative morphological traits that could be the base of the development of suitable descriptors for exploring the phylogenetic relations between new artificial Salvia hybrids and their parental species aiming to facilitate their use by the floricultural industry.

Materials and Methods

Two Salvia species *S. officinalis* (O) and *S. tomentosa* (T), one subspecies, *S. pomifera* ssp. *pomifera* (P) and two new hybrids *S. officinalis* × *S. tomentosa* (OP) and *S. officinalis* × *S. pomifera* ssp *pomifera* (PT) were sampled for identification in 2022, at Agricultural University of Athens (37°58΄58.051΄΄N and 23°42΄17.499΄΄E). The hybrids were products of the SALVIA-BREED-GR research project and both had *S. officinalis* as seed parent. A total of eight quantitative (leaf and flower/inflorescence morphometrics) and 14 qualitative characters (characters of vegetation, flowers and fragrance) were selected and used based on descriptors for other plant species (Table 1, Figure 1). Some of these descriptors had been used in a previous study of morphometrics of *S. fruticosa* in Greece (Bertsouklis *et al*., 2021). One-way ANOVA was used for determination of the differences between the mean values of leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA) and the dendrograms were generated based on Euclidean distance-squared of morphological character analysis (Greenacre and Underhill, 1982). Principal coordinate analysis (PCA) was used in order to verify cluster analysis and to assist in visualizing the data, and statistical analysis of morphological markers was conducted by the software NTSYS-pc version 2.11f (Rohlf, 1992).

Results and Discussion

Regarding the quantitative characteristics, analysis revealed that were differences in all morphological traits (Table 2). T had the longest and widest leaves, as well as the longest inflorescences (Table 2). The quantitative characteristics of the hybrids, revealed that OP had intermediate leaf length, width, length/width, while the inflorescence and flower length had no difference to that one of O and internode length had no difference to that one of P. OT had the smallest flower and calyx length compared to its parents with the smallest internode length of all species and hybrids analyzed being a compact plant with numerous lateral shoots. Cluster analysis separated the species and their hybrids according to their morphological characteristics in three main branches (Figure 2). Both hybrids, OP and OT, were found to be closer to O (Figure 2). T and P were found to be distinct from the hybrids. O and P are strongly aromatic plants and their hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of P, while OT has a lighter aroma than that of O.

Table 1. Morphological traits measured in two Salvia species (O, T), one subspecies (P) and two interspecific hybrids (OP, OT) were used as descriptors. There is a scoring code for each one depending on its status among different individuals tested.

Code	Descriptor	Score code - descriptor state					
1	Leaf pubescent	1: Low	2: Medium	3: High			
2	Leaf texture	1: Leathery-elastic	2: Membranaceous-smooth	3: Membranaceous-tough			
3	Leaf shape	1: Elliptical to lanceolate	2: Elliptical				
4	Leaf colour of upper side	1: Light green	2: Green				
5	Leaf simple	1: Yes	2: No				
6	Leaf lobes	1: Yes	2: No				
7	Existence of dark/linear zone on stems	1: Yes	2: No				
8	Colour of petals	1: Pink	2: Light pink	3: Light pink-purple	3: Light purple	5: Purple	6: Dark purple
9	Inflorescence density	1: Low	2: Medium	3: High			
10	Existence of dark/linear zone on calyx	1: Yes	2: No				
11	Intensity of dark/linear zone on calyx	1: Low	2: Medium	3: High			
12	Calyx pubescent	1: Yes	2: Medium	3: High			
13	Colour of calyx	1: Light green	2: Green	3: Dark green			
14	Strength of leaf and flower fragrance	1: Low	2: Medium	3: High			

Cluster analysis was confirmed by PCA analysis and five components have been arranged in decline order according to their importance, explaining the 95.2% of the total variability among the different individuals. The suggested descriptors grouped in the same principal component have strong correlation and each component is strongly correlated with a group of the used descriptors so it could be estimated their contribution to variability (Table 3, Figure 3). Morphological traits have been used to study the variability of *S. fruticosa* in a previous work (Bertsouklis *et al.*, 2021). The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for new Salvia hybrids serving the aim to distinguishing plants with special ornamental characteristics, which could be the initial plant material for breeding programs and clonal propagation of plants to be introduced to the floricultural industry.

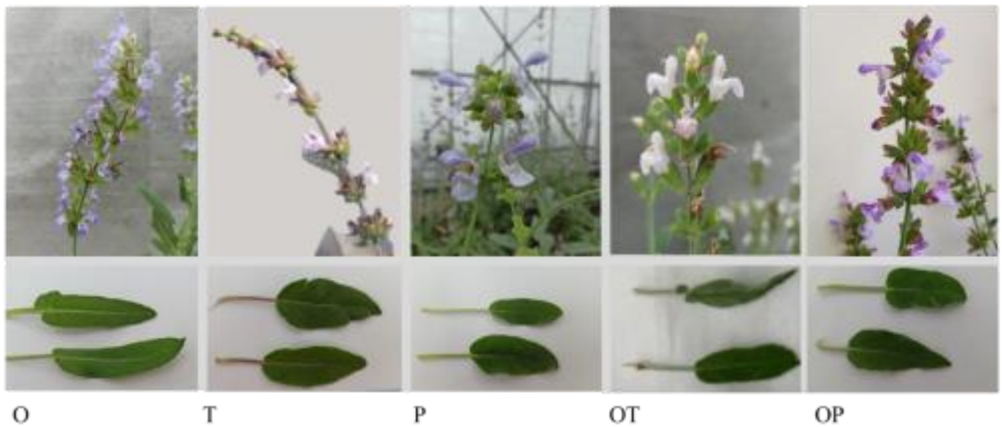

Figure 1. Leaves and inflorescences of *Salvia* spp and interspecific hybrids used in analysis

Table 2. Leaf, stem and inflorescence traits of two *Salvia* spp. (O, T), one subspecies (P) and two interspecific hybrids (OP, OT)

	Leaf				**Inflorescence**			**Stem**
Code	**Length (cm)**	**Width (cm)**	**Length/ Width**	**Thickness (mm)**	**Length (cm)**	**Flower length (cm)**	**Calyx Length (cm)**	**Internode length (cm)**
O	4.4 bc	1.4 d	3.2 a	0.7 c	32.3 b	2.3 c	1.2 c	1.0 b
P	4.4 bc	2.4 b	1.8 c	0.7 c	24.8 c	3.7 a	1.6 a	1.2 a
T	7.1 a	2.9 a	2.5 b	0.7 c	37.4 a	3.0 b	1.4 b	1.0 b
OP	4.9 b	2.0 c	2.6 b	0.9 b	30.4 b	2.3 c	1.0 d	1.2 a
OT	3.9 c	1.4 d	2.8 b	1.8 a	13.8 d	2.1 d	1.0 d	1.0 b
F	***	***	***	***	***	***	***	***

Mean separation in columns by Student's t test at $P \leq 0.05$, ***significant at $P \leq 0.001$, Values followed by different lowercase letter within each trait are significantly different

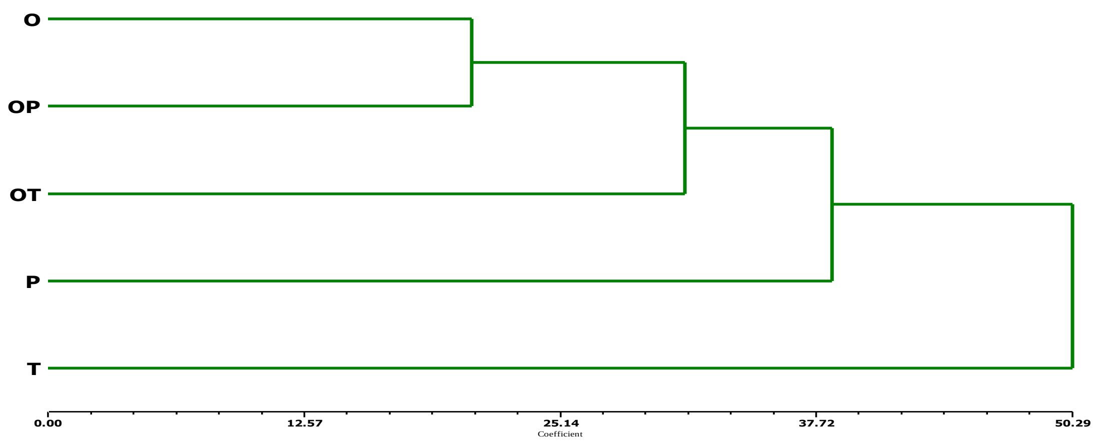

Figure 2. UPGMA dendrogram of two new interspecific hybrids (OP, OT) originated from two *Salvia* spp (O, T) and one subspecies (P) based on Euclidean distance-squared of morphological character analysis

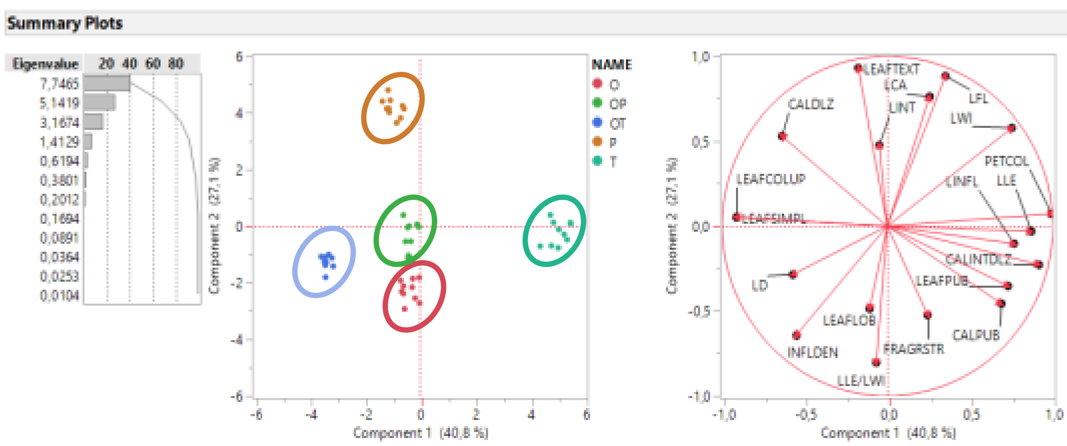

Figure 3. Evaluation of the descriptors and their contribution to the variability of the species and hybrids studied

Table 3. Results of principal components calculation

Principal Components				
1	2	3	4	5
% Contribution of variability				
40.77	27.06	16.67	7.43	3.26
Related descriptors				
CALINTDLZ LEAFPUB LWI LINFL	LFL LEAFTEXT	CALDLZ FRAGRSTR LEAFLOB	CALPUB LD LCA INFLDEN	LLE LLE/LWI LINT

LEAFC0LUP
PETCOL
LEAFSIMPL

Leaf pubescent (LEAFPUB), Leaf texture (LEAFTEXT), Leaf shape (LEAFSHAPE), Leaf colour of upper side (LEAFCOLUP), Colour of petals (PETCOL), Inflorescence density (INFLDEN), Existence of dark/linear zone on calyx (CALINTTDLZ), Intensity of dark/linear zone on calyx (CALDLZ), Calyx pubescent (CALPUB) Colour of calyx (CALCOL), Strength of leaf/flower fragrance (FRAGRSTR), Leaf Length (LLE), Leaf Width (LWI), Leaf Width/Leaf Length (LLE/LWI), Leaf Thickness (LD), Inflorescence Length (LINFL), Flower length (LFL), Calyx Length (LCA), Internode length (LINT), Leaf simple (LEAFSIMPLE), Leaf lobes (LEAFLOB)

Conclusions

The present experimental procedure leads to the develpoment of suitable descriptors in order to explore the phylogenetic relations between new artificial sage hybrids and their parental species. The use of suitable descriptors may facilitate the needs for increased demand that floriculture or ornamental horticulture face and in producing new hybrids for commercial purposes.

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A FIRST STUDY ON FLOWER MORPHOLOGY OF SIX ARBUTUS SPECIES, ONE HYBRID AND ONE CULTIVAR OF THE MEDITERRANEAN BASIN AND NORTH AMERICA

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Abstract

Three Mediterranean Arbutus species and their natural hybrid, as well as three North American species and one Californian cultivar of mysterious origin were studied microscopically; a comparison of the internal anatomy of the flowers of these species is reported for the first time. Morphological features were found to be similar in general but unique to the *Arbutus*. However, the length of the anther appendages, the spurs, seems to provide a heritage trait linking the Californian cultivar ‘Marina’ to the *A. canariensis* and support other morphological findings between the East Mediterranean hybrid, *A.* × *andrachnoides,* and its parents *A. andrachne* and *A. unedo*.

Keywords: *Anther appendages, blossom, spur, strawberry tree.*

Introduction

Fossil findings of plants from the upper Cretaceous period in a clay pit in New Jersey have shown the presence of spurs in the anthers of flowers having some characteristics similar to modern Ericales and even Ericaceae (Crepet, 2009). Present day Ericaceae have been studied including the development of the stamens and their appendages (Hermann and Palser, 2000). Many of these were found to have “awns” and “spurs”. The prevalence of appendages in Ericaceae have suggested a different mechanism involving the pollination process. *Arbutus* genus, (f. Ericaceae), includes many popular shrubs or tree species in both hemispheres. *A. unedo* L. is prominent in the Mediterranean region (“Strawberry Tree”). It is being utilized in the northwest coast of the western hemisphere and in the Mediterranean region in landscaping as an attractive and simply maintained bush. Its bell-shaped blossoms in the fall are generally white with a rare variety originated in Ireland with a pink to ruby color called *A. unedo* var *rubra*. *A. andrachne* L. of the central and eastern Mediterranean commonly (Greek or Eastern Strawberry tree), has also the potential in landscape use, reforestation, and pharmaceutical industry (Bertsouklis and Papafotiou, 2013; Bertsouklis and Papafotiou, 2016). *A.* × *andrachnoides* Link is believed to be a natural hybrid between *A. unedo* and *A. andrachne* and it is native to Greece and the eastern Mediterranean region (Arabatzis, 2001; Torres *et al.*, 2002). Gökbayrak and Engin (2021), studied morphological changes during flower growth of *A. andrachne,* but it is challenging, to differentiate *A.* × *andrachnoides* from *A. andrachne* (Bačić et al., 1992; Cullen et.al. 1997). A significant difference has been found in the length/width ratio of the leaves (Bertsouklis and Papafotiou, 2016) and in a pattern difference of the stomata and the surrounding tissue when studied with electron scanned microscopy (Bačić *et al.,* 1992). *A. canariensis*

Duhamel is an indigenous tree of the Canary Islands portraying many of the same traits of the Mediterranean *Arbutus* but offering pink to ruby blossoms instead of white. In the natural habitat, *A. canariensis* like *A. unedo* is a fall bloomer whereas *A. andrachne* is a spring bloomer. In the western hemisphere, *Arbutus menziesii* Pursh is prevalent for much of the Pacific Northwest extending from central Vancouver Island south to mid California. *A. xalapensis* Kunth is found in Texas and northeastern Mexico. *A. peninsularis* is indigenous to the *Sierra de la Laguna* mountains in the southern region of Baja California in Mexico. *A. menziesii* is found along the seashore, often in rocky regions, bending and twisting out to open space to obtain the sunshine (Martin, 2022). It also can grow to immense size; a circumference of 10 m has been reported (Coe, 1983). *A. xalapensis* behaves similarly but turns frequently beyond bone color to a white appearance, this occurrence depends on the brightness and harshness of its sun exposure during the year. A mysterious and popular cultivar *Arbutus* ‘Marina’ has been propagated in California particularly in the regions with a Mediterranean climate. The tree is known to have originated in California, at the San Francisco 1915 Panama American Exposition. It is thought to have been brought to the exposition from somewhere in the Mediterranean region. Details of its history after the exposition are described (Baldwin, 2020). However, of pertinence here is that in the early 1990’ Briggs Nursery Inc. near Olympia WA, USA, contracted to produce plantlets by micro propagation techniques. This action fueled the starting of wide distribution of it in California and apparently elsewhere. Demoly (2004) names and describes two hybrids: *Arbutus* × *thuretiana* Demoly and *Arbutus* × *reyorum* Demoly. The first is a cross between *A. canariensis* and the *A. andrachne* and the latter a second cross between the *A.* × *andrachnoides* and the *A. canariensis*. Further, he suggests that latter hybrid was displayed in the 1915 Panama American Exposition but doesn’t indicate how it could have arrived there (Demoly, 2004). World War I was erupting at that time, which added to confusion of normal record keeping. However, Demoly (2004) also writes that a plant called *Arbutus* ‘Marina’ was introduced in France in 1993 from the Madrona Nursery located in England. Liam Mackenzie the 1986 founder of that nursery revealed he used to travel in the early 1990’s to the Courson Flower Show, held south of Paris, to sell plants and probably sold *A.* ‘Marina’ there. However, he had himself acquired *A.* ‘Marina’ plants from Peter Catt of Liss Forest Nursery in Hampshire, UK (Mackenzie, 2018). Mr. Catt, formerly of the Liss Forest Nursery, though unsure of dates, said he had bought many plants from Briggs Nursery in that early time frame (Catt, 2018). Demoly compared the morphology of the *A.* ‘Marina’ to the *A.* × *reyorum* and feels justifying maintaining the name of the cultivar as *Arbutus* × *reyorum* ‘Marina’ (Demoly, 2018). Nevertheless, there is no other direct evidence that they are the same. This investigation was prompted finding the link between the spurs of the *A.* ‘Marina’ and the *A. canariensis* compared to other ` trees.

Materials and Methods

Flowers of the *Arbutus* species were obtained from various regions for examination. *A. unedo*, *A. andrachne* and *A.* × *andrachnoides* were obtained in the region of Tatoi Former Royal Palace (Varympompi, Athens, Greece, N 38°08’14.0”, E 23° 47’59.0”. *A. menziesii* and *A. unedo* were acquired near Anacortes, WA, USA (N 48° 30’54.011 W 122° 37’10.169); *A. peninsularis* blossoms were obtained from the Sierra de la Laguna mountains (Baja California, Mexico, N 23° 32’44.489” W 110°00’37.68”); *A. xalapensis* flowers were picked and sent from Dripping Springs TX, USA (N 30°11′31″ W 98° 05′07″) near Austin; *A. canariensis* and A. ‘Marina’ were obtained, with permission, from the Golden Gate Botanical Garden in San Francisco. The significance of the results was tested by one-way analysis of variance (ANOVA). The spur length means were compared by Student’s t test at P≤0.05 (JMP 11.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The blossoms of all *Arbutus* species studied had a similar form. Ten anthers with each having two pollen sacs and two spurs were found in all species (Figure 1). The filament of the stamens broadens at increasing distance from the anther until it suddenly reduces in diameter just before it attaches to the corolla. White dense hairs, almost luminous in color, encompass the lower one third of each stamen. Its purpose is not known. The spurs taper from their attachment point to the anther to their tip. The internal parts of the blossoms of *A.* ‘Marina’ and *A. canariensis* have similar appearances (Figure 1, 2). The spurs seem to be shorter and thicker where they attach to the anther, and with less complicated curvature compared to *A. unedo* (Figure 3) and the other *Arbutus* species (Figure 4, 5). *A. unedo* has the longest spur length (Table 1, Figure 3). *A.* × *andrachnoides* has a value closer to *A. andrachne*, along with other traits found in a previous study (Bertsouklis and Papafotiou, 2016). The length of all three of these species were found to be different and longer that both *A. canariensis* and ‘Marina’. According to Pascual *et al.* (1993) *A. canariensis* in the Canary Islands had thick and short spurs like what we found in the blossoms from San Francisco.

Table 1. Spur length measure of the spurs of *A. unedo*, *A.* × *andrachnoides*, *A. andrachne* *A.* ‘Marina’ and *A. canariensis.*

Species	Mean Length (mm)
A. unedo	1.27 ± 0.18 a
A. × *andrachnoides*	1.19 ± 0.18 b
A. andrachne	1.10 ± 0.13 b
A. ‘Marina’	0.69 ± 0.10 c
A. canariensis	0.69 ± 0.14 c

Mean (± SE) separation in columns by Student’s *t*-test, *P*≤0.001. Means followed by the same letter are not significantly different, *n*=15-66.

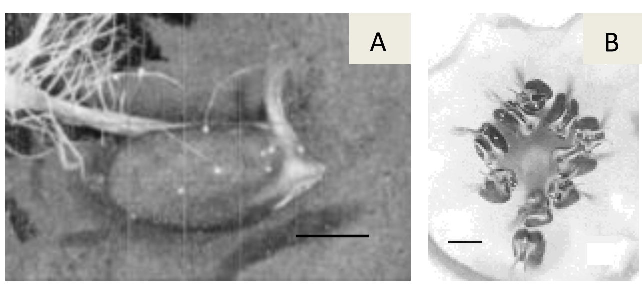

Figure 1. *A*. ‘Marina’, A. Single anther B. View into the blossom with the opening end of the corolla removed exposing the anthers (10) and their spurs. Bar represents length of 1 mm.

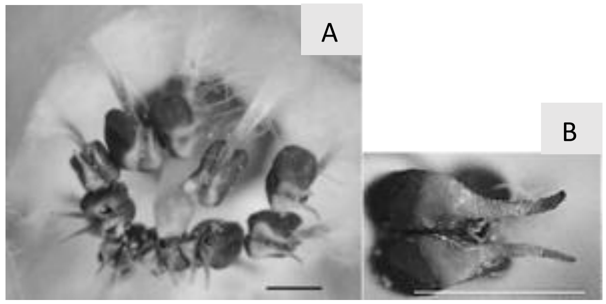

Figure 2. *A. canariensis* A. Stamens B. Single anther with its dual spurs like the view of Figure 1A for the *A*. ‘Marina’. Bar represents length of 1 mm.

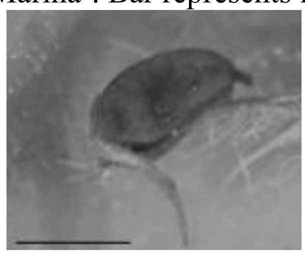

Figure 3. *A. unedo,* single anther with dual pollen sacs and the dual spurs. Bar represents length of 1 mm.

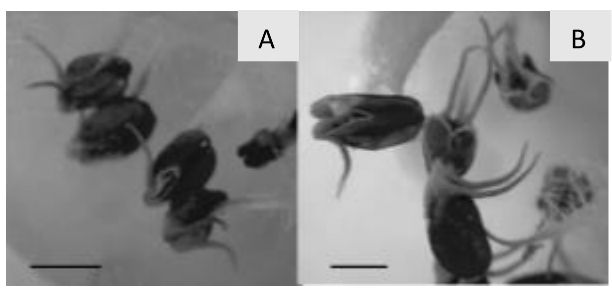

Figure 4. Anthers and spurs of *A. andrachne* and *A.* × *andrachnoides.* Bar represents length of 1 mm.

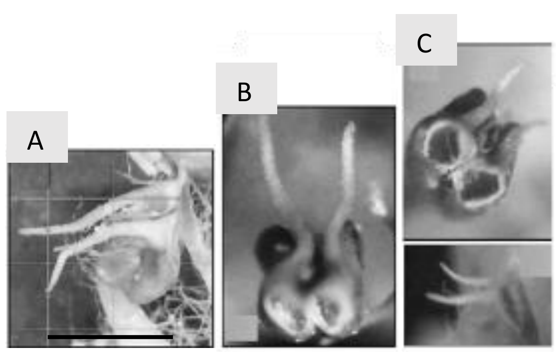

Figure 5. Anthers and spurs of three western hemisphere Arbutus trees. A. *A. menziesii*, an oblique view of two spurs. B. *A. pensinsularis*, apical view of single anther. C. *A. xalapensis*, a lateral view of an anther showing the curved spurs. Bar represents length of 1 mm.

Conclusions

The dominate morphology of the dual spurs in the *Arbutus* species studied and their definitive structure found implies that the cultivar *A.* 'Marina' have the *A. canariensis* in their heritage line. The color of the blossoms and the peeling bark further supports this indication. Additionally, this finding defends the premise of Demoly (2004) that it is the hybrid *Arbutus* x *reyorum* Demoly However, by itself it does not rule out that it could be *Arbutus* x *thuretiana* Demoly that he also has identified as a hybrid of the *A. canariensis*. The spur length of the Greek hybrid, *A. × andrachnoides,* agrees with other reported morphology findings that place *A. × andrachnoides* closer to *A. andrachne.*

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MEDITERRANEAN SAGE HYBRID *Salvia officinalis* × *S. ringens* AS GROWN UNDER REDUCED IRRIGATION IN A GREENHOUSE AND AN EXTENSIVE GREEN ROOF

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Abstract

Tolerance in xerothermic conditions of the new interspecific sage hybrid *S. officinalis* × *S. ringens* was examined aiming to its exploitation in extensive green roofs and xeriscaping. Two irrigation frequencies, a regular (every 2–3 days, when substrate moisture 17–23% v/v) and a reduced (every 3–5 days, when substrate moisture 7–13% v/v) were applied in a greenhouse (cultivation in 14 cm pots on peat: perlite, 2:1, v/v) and a green roof experiment (cultivation in 10 cm deep substrate mixture of grape-marc compost: perlite: pumice 3:3:4, v/v, alongside with the parental species, *S. officinalis* and *S. ringens*). Both experiments started in April 2021 and lasted 3 and 5 months, respectively. In the greenhouse, most growth parameters of the hybrid, i.e., plant height, lateral shoot number and length and aboveground dry weight were not affected significantly by reduced irrigation, excepting root dry weight which was reduced. In the green roof, *S. officinalis* × *S. ringens* had higher plant height and aboveground dry weight than the parental species. Under reduced irrigation, all *Salvia* types had lower plant diameter, as well as aboveground and root dry weight compared to regular irrigation. *S. officinalis* × *S. ringens* hybrid, as well as its parental species *S. officinalis* and *S. ringens* grew satisfactorily under reduced irrigation, resulting to their recommendation for sustainable use in xeriscaping, including extensive green roofs.

Keywords: *drought resistance, Mediterranean sage, interspecific hybrid,* Salvia officinalis, Salvia ringens.

Introduction

Mediterranean sages (*Salvia* spp. family Lamiaceae) are drought-resistant plants, part of the macchia shrubland, which could be ideal for use in xeriscaping, suitable for use in extensive type urban green roofs and valuable as bee-friendly plants. Aiming to introduce new drought-resistant species with interesting floricultural characteristics in the floriculture industry, interspecific crossbreeding was undertaken between sage species native to Greece (Papafotiou *et al.*, 2021). One of the crosses was between *S. officinalis*, used as the seed parent, and *S. ringens*, used as the pollen parent, resulting to the creation of a new interspecific hybrid *S. officinalis* × *S. ringens*.
S. officinalis L. is a strongly aromatic shrub, up to 60 cm tall, with greenish above and white felted beneath, oblong to elliptical leaves and violet-blue, pink or white flowers in May–July, that prefers garrigue, stony pastures, scrub, rocky places. It is widespread on the Apennines and eastern Adriatic coast, found in Northern Greece and the Ionian islands as well. It is one of the most important species of the genus *Salvia* worldwide, as it is cultivated in many varieties as medicinal, culinary and ornamental (Blamey and Grey-Wilson, 1993; Tutin *et al.*, 1972).

S. ringens Sibth. & Sm. is a hardy low, up to 30 cm tall, herbaceous plant, with dark green, pinnatisect or pinnate with 3–6 pairs of small lateral segments, appressed-hairy leaves and tall, branching flowering stems with large dark violet-blue flowers during late spring through summer. It is found in areas with garrigue vegetation, forest glades and streams between 490 m and 1300 m, in South and Eastern parts of Balkan Peninsula, including North and Central Greece (Tutin *et al.*, 1972). It is resistant to low temperatures.
S. officinalis × *S. ringens* has inherited from *S. ringens* the segmented leaves and the long flowering stems. It is taller than *S. ringens* and produces many lateral shoots, while its segmented leaves are unique and particularly decorative, but they have very light aroma, as *S. ringens* does. Flowering stems are about 40 cm long with more flowers than those of *S. ringens*. Flower color is light violet-blue, resembling the flower color of *S. officinalis* (Papafotiou *et al.*, 2021).
The drought tolerance of *S. officinalis* has been thoroughly investigated (Raimondo *et al.*, 2015; Kemp *et al.*, 2019; Abreu and Munné-Bosch, 2008; Bettaieb *et al.*, 2011; Abate *et al.*, 2021), while it has been tested for growth on extensive green roofs as well (Raimondo *et al.*, 2015; Papafotiou *et al.*, 2022; Kemp *et al.*, 2019; Kokkinou *et al.*, 2016). *S. ringens* and *S. officinalis* × *S. ringens* hybrid were also tested as for their adaptation in a green roof with promising results (Papafotiou *et al.*, 2022).
In the present study, *S. officinalis* × *S. ringens* hybrid was grown under two irrigation frequencies, one considered adequate and one deficient, during the hot and dry Eastern Mediterranean summer, in a greenhouse and, alongside with its parental species (*S. officinalis* and *S. ringens*), in an extensive green roof. The aim was to evaluate its growth and tolerance in xerothermic conditions, in order to facilitate its introduction as a suitable species for extensive green roofs and xeriscaping.

Materials and Methods

Aiming to test growth and drought tolerance of the new interspecific sage hybrid *S. officinalis* × *S. ringens* in a greenhouse, in early April 2021, four-week-old rooted cuttings were transplanted singly in plastic pots, 14 cm in diameter, which contained 1 L of peat-perlite mixture 2:1 (v/v). Climatic conditions during the experiment are shown in Figure 1 a-b. Plants were fertilized monthly with 2 g/L water-soluble fertilizer (20-20-20 plus, HUMOFERT, Metamorfosi, Greece). In each pot, 100 ml of fertilizer was applied. Two irrigation frequencies were applied, i.e., (i) when the moisture content of the substrate was 20–23% v/v (normal irrigation) and (ii) when the moisture content of the substrate was 8–13% v/v (sparse irrigation). In the first month of cultivation, the plants under normal irrigation were irrigated every 3–4 days and under sparse irrigation every 5 days, while in the following months every 2 and 3–4 days, respectively.
Aiming to evaluate growth and drought tolerance of the hybrid *S. officinalis* × *S. ringens*, alongside with its parental species *S. officinalis* and *S. ringens*, in an extensive green roof, in early April 2021, rooted cuttings about 8 weeks old were planted in plastic containers, 40 cm (width) × 60 cm (length) × 22 cm (depth) in size. Each container had a green roof infrastructure fitted, i.e., moisture retention and protection of the insulation mat FLW-500, a drainage layer Diadrain-25H and a filter sheet VLF-150 (Landco Ltd., Diadem Green Roof Systems, Athens, Greece). Two plants of the same type per container with six containers per treatment were used. The containers were arranged following a completely randomized design on a second-floor flat roof (12 m approximate height) at the Agricultural University of Athens (37°59′ N, 23° 42′ E). The substrate used was grape marc compost: perlite: pumice (3: 3: 4, v/v) and had a depth of 10

cm. Climatic conditions during this experiment are shown in Figure 1 c-e. Irrigation frequencies, like the greenhouse experiment, were applied i.e., a regular (when substrate moisture 17–23% v/v) and a reduced (when substrate moisture 7–13% v/v). In the first month of cultivation, the plants were irrigated normally every 3 days and sparsely every 5 days, while in the following months every 2 and 4 days, respectively. Automatic drip irrigation on the substrate surface was applied before sunrise by two drippers placed at equal distances from the center of the container and the plants (dripper supply 4 $L \cdot h^{-1}$, irrigation period: 60 min, adequate to allow water to drain off the container).
Results concerning various growth parameters were recorded after three and five months of cultivation in the greenhouse and the green roof experiment, respectively.
The completely randomized design was used. The significance of the results was tested by either one- or two- or three-way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at $P < 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

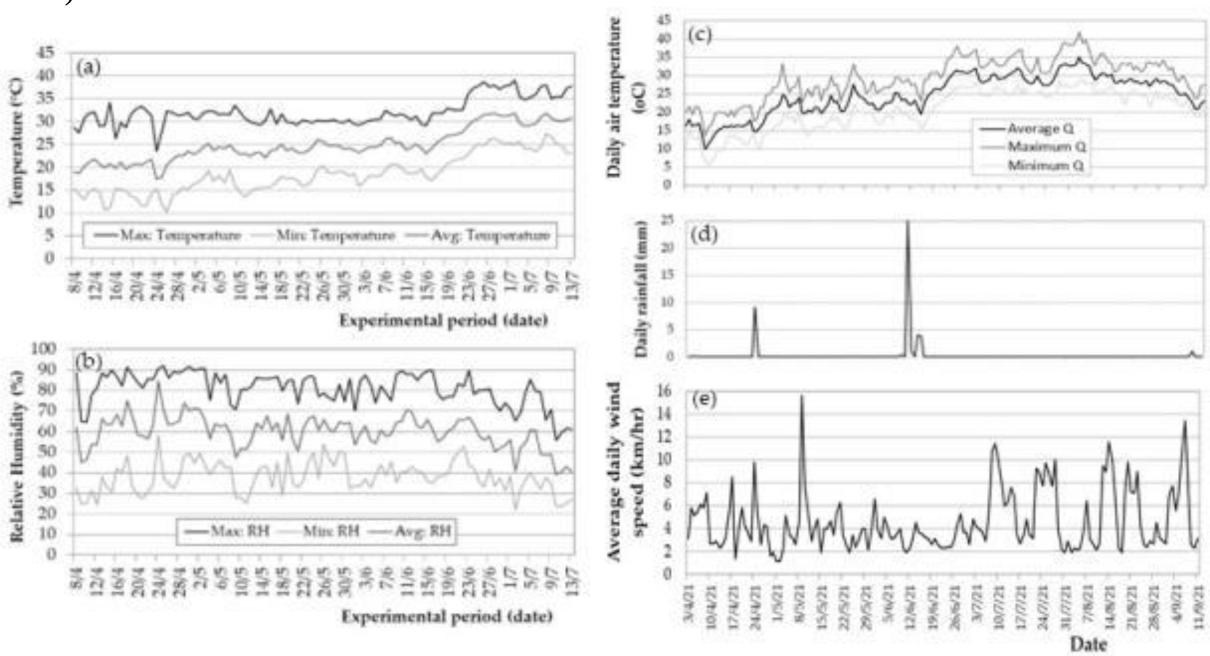

Figure 1. Climatic conditions, i.e., temperature (a) and relative humidity (b) inside the glass greenhouse where the experiment was conducted, during the 3-month experimental period (from April 2021 until July 2021), as well as temperature (c), daily rainfall (d) and average daily wind speed (e) at the green roof, during the 5-month experimental period (from April to September 2021).

Results and Discussion

In the greenhouse, all plants survived and most growth parameters of the hybrid were not affected significantly by sparse irrigation, excepting root dry weight, which was reduced (Table 1, Figure 2).
In the green roof, *Salvia* types did not differ in their survival rate, which was ranging between 67% - 100% (Table 2). *S. officinalis* × *S. ringens* had higher plant height and aboveground dry weight than the parental species (Table 2, Figure 3). Under sparse irrigation, all *Salvia* types had

lower plant diameter, as well as aboveground and root dry weight compared to normal irrigation (Table 2, Figure 3). Nevertheless, their growth was satisfactory (Figure 3).
Growth parameters of *S. officinalis* × *S. ringens* hybrid in the greenhouse were less affected by sparse irrigation than those in the green roof, as only root dry weight was reduced (Tables 1 & 2, Figures 2 & 3). Water deficiency has been reported to reduce plant height, as well as aboveground and leaf biomass in *S. fruticosa* (Chrysargyris *et al.*, 2016] and *S. officinalis* (Sabry *et al.*, 2016; Soltanbeigi *et al.*, 2021). Decreases in plant height are common under drought stress (Wang *et al.*, 2003), due to the lack of water which leads to clogging of vascular tissue and reduction of cell elongation (Abdalla and El-Khoshiban, 2007). The vigorous canopy growth of *S. officinalis* × *S. ringens*, which seemed to have been inherited from *S. ringens*, in combination with its rich root system, which seemed to have been inherited from *S. officinalis*, probably contributed to its better adaptation in the green roof under both irrigation frequencies (Table 2, Figure 3). This is reinforced by the fact that the root system is the main plant organ for adaptation to drought stress conditions (Abobatta, 2019; Zhou *et al.*, 2018).

Table 1. Growth of the new interspecific hybrid *S. officinalis* × *S. ringens* for three months (April–July 2021) in a greenhouse under normal and reduced irrigation (data per plant).

Irrigation frequency	Plant height (cm)	Lateral shoot number	Lateral shoot mean length (cm)	Lateral shoot total length (cm)	Aboveground d.w. (g)	Root d.w. (g)	Root d.w./ Aboveground d.w.
Normal	27.5 a[†]	9.0 a	10.1 a	87.4 a	12.9 a	3.4 a	0.3 a
Sparse	27.0 a	7.6 a	10.6 a	75.0 a	12.1 a	2.4 b	0.2 b
Significance [§] $F_{\text{one-way ANOVA}}$	NS	NS	NS	NS	NS	**	*

[†] Mean values (n = 10) in each column followed by the same lowercase letter (a-b) did not differ significantly at $P \leq 0.05$ using Student’s *t*-test.
[§] NS or * or **, non-significant at $P \leq 0.05$ or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

Figure 2. Typical aboveground and root system of the new interspecific hybrid *S. officinalis* × *S. ringens*, after growing for three months in greenhouse conditions, under normal (N) and sparse (S) irrigation frequency. Size bar = 10.0 cm.

Table 2. Comparative evaluation of growth of two Greek sage species (*S. officinalis* and *S. ringens*) and their interspecific hybrid (*S. officinalis* × *S. ringens*) after growing for five months (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation frequency.

Data		Per container	Per plant		Per container (2 plants)		
Salvia type	Irrigation Frequency	Survival (%)	Foliage height (cm)	Plant diameter (cm)	Above ground d.w. (g)	Root d.w. (g)	Root d.w./ Above ground d.w.
S. officinalis	Normal	83.3 a[†]	23.9 b	29.2 ab	70.3 c	95.9 a	1.4 a
	Sparse	75.0 a	20.8 c	25.4 c	50.4 d	74.9 ab	1.5 a
S. ringens	Normal	100.0 a	18.1 c	29.8 a	86.6 b	61.5 b	0.7 b
	Sparse	66.7 a	14.1 d	25.5 bc	56.6 cd	46.5 b	0.8 b
S. officin. × *S. ringens*	Normal	83.3 a	25.6 ab	27.0 abc	116.9 a	91.3 a	0.8 b
	Sparse	83.3 a	28.5 a	23.9 c	90.0 b	57.1 b	0.6 b
Significance [§]							
$F_{\textit{Salvia}\text{ type}}$		NS	-	NS	**	*	**
$F_{\text{irrigation frequency}}$		NS	-	**	**	**	NS
$F_{\text{interaction}}$		NS	**	NS	NS	NS	NS
$F_{\text{one-way ANOVA}}$		NS	**	**	**	**	**

[†] Mean values (n = 8-12) in each column using Student’s t test; means followed by the same letter are not significantly different at $P \leq 0.05$

[§] NS or * or **, non-significant at $P \leq 0.05$ or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

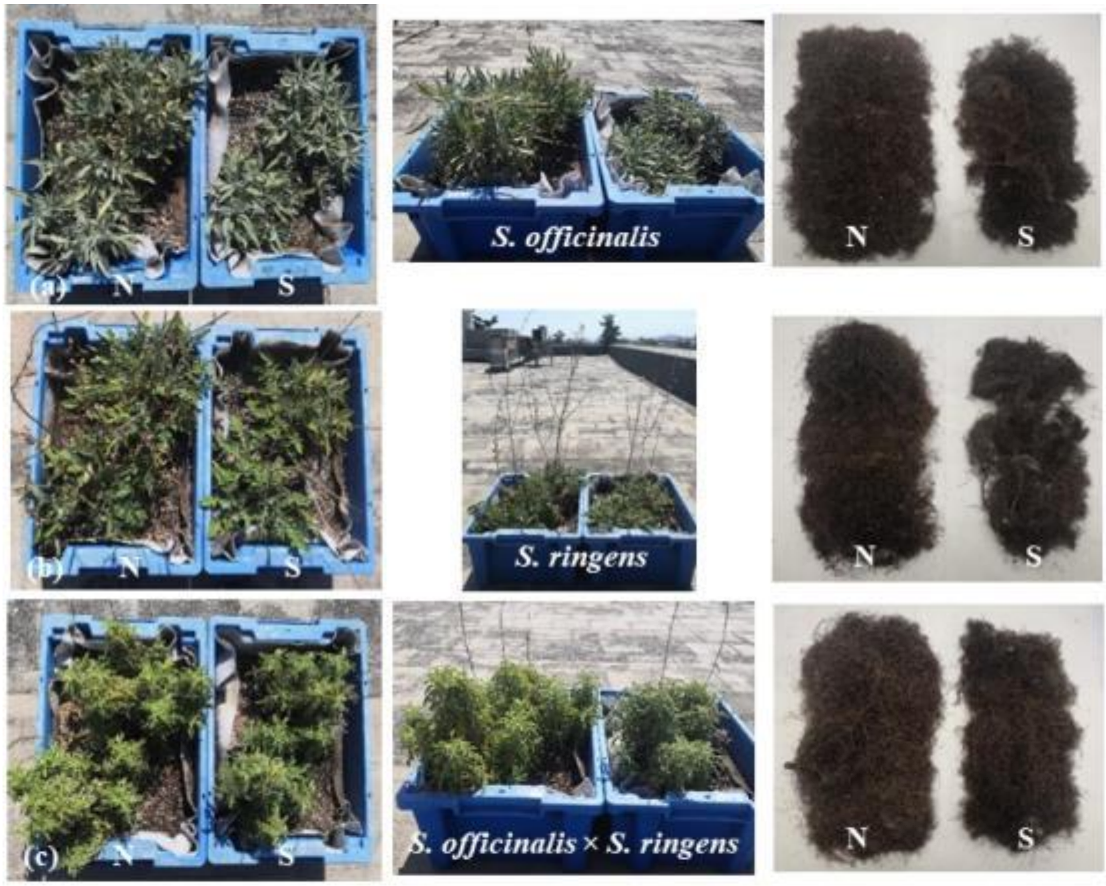

Figure 3. Typical growth of aboveground and root system of *S. officinalis* (a), *S. ringens* (b), and *S. officinalis* × *S. ringens* (c), after five months cultivation (April - September 2021) in an extensive green roof, under normal (N) and sparse (S) irrigation.

Conclusions

Under limited irrigation, growth of all three *Salvia* types was reduced compared to regular irrigation. However, *S. officinalis* × *S. ringens* hybrid, as well as its parental species *S. officinalis* and *S. ringens* grew satisfactorily under limited irrigation, resulting to their recommendation for sustainable use in xeriscaping, including extensive green roofs.

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GROWTH OF THE NEW *Salvia officinalis* × *S. tomentosa* HYBRID UNDER REDUCED IRRIGATION IN GREENHOUSE AND GREEN ROOF CONDITIONS

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Abstract

Aiming to the exploitation of a new interspecific Mediterranean sage hybrid *Salvia officinalis* × *S. tomentosa*, along with its parental species *S. officinalis* and *S. tomentosa*, in extensive green roofs and xeriscaping, their resistance to xerothermic conditions was examined in a Mediterranean green roof and in a greenhouse under regular and reduced irrigation. In the green roof the hybrid and the parental species were grown on 10 cm deep substrate mixture of grape-marc compost: perlite: pumice 3:3:4, v/v, while in the greenhouse in 14 cm plastic pots, on peat: perlite 2:1, v/v. Planting took place in April 2021 and the experiment lasted 3 and 5 months, in the greenhouse and green roof, respectively. Two irrigation frequencies, a regular (every 2–3 days when substrate moisture 17–23% v/v) and a reduced (every 3–5 days when substrate moisture 7–13% v/v) were applied. In the greenhouse, the hybrid growth was very satisfactory under reduced irrigation, since only plant height was reduced from all growth parameters recorded (lateral shoot number and length, aboveground and root dry weight). In the green roof, *S. tomentosa* had higher plant height and diameter and aboveground dry weight than *S. officinalis* and their hybrid, while root dry weight was higher in the parental species compared to the hybrid. Reduced irrigation resulted in significant reduction of all growth parameters of all *Salvia* types. However, their growth remained satisfactory. So, both the hybrid and its parents are recommended for sustainable use in xeriscaping, including extensive green roofs.

Keywords: *drought resistance, Mediterranean sage, interspecific hybrid,* Salvia *hybrid,* S. officinalis, Salvia tomentosa.

Introduction

Greek *Salvia* species being drought resistant could be ideal plants for use as ornamentals, in arid and semi-arid regions, particularly in xeriscaping, which has gained worldwide acceptance in recent years due to the limited irrigation water requirements. Native plants are ideal for use in xeriscaping, since they have low irrigation and cultivation needs, a great adaptability to different soil and climatic conditions, high ornamental value, as well as a contribution to the preservation of the character of the landscape (Bayramoğlu *et al.*, 2015; Martinetti *et al.*, 2018; Tassoula *et al.*, 2021). Following the trend of floriculture industry, which is constantly seeking to introduce new varieties to the market, a new interspecific sage hybrid *Salvia officinalis* × *S. tomentosa* was created through the crossbreeding between *S. officinalis*, as the seed parent, and *S. tomentosa*, as the pollen parent (Papafotiou *et al.*, 2021). *S. officinalis* is a strongly aromatic, rather grayish shrub up to 60 cm, with leaves oblong to elliptical, rough greenish above but white felted

beneath and flowers violet-blue or pink. It is cultivated worldwide with many varieties as pharmaceutical and ornamental (Tutin *et al.*, 1972; Blamey & Grey-Wilson, 1993). *S. tomentosa* is similar to *S. officinalis*, up to 80 cm, but has leaves with a rounded or heart-shaped base and flowers usually violet with reddish-brown calyces (Blamey & Grey-Wilson, 1993). Both *Salvia* species flower in late spring to early summer. Their interspecific hybrid *S. officinalis* × *S. tomentosa* is a compact plant that produces numerous lateral shoots with grey-green leaves smaller than those of *S. officinalis*. It also forms many flowering stems with light pink flowers, while it has a lighter aroma than that of *S. officinalis* (Papafotiou *et al.*, 2021).
Aiming to exploit the new interspecific Mediterranean sage hybrid *Salvia officinalis* × *S. tomentosa*, along with its parental species *S. officinalis* and *S. tomentosa*, in extensive green roofs and xeriscaping, their resistance to xerothermic conditions was examined in a Mediterranean green roof and in a greenhouse under regular and reduced irrigation.

Materials and Methods

Aiming to test growth and drought resistance of the new interspecific sage hybrid *S. officinalis* × *S. tomentosa*, two experiments were set in early April 2021.
In the first experiment, that took place in a greenhouse, four-week-old rooted cuttings of this hybid were transplanted singly in plastic pots, 14 cm in diameter, which contained 1 L of peat-perlite mixture 2:1 (v/v). Climatic conditions during the experiment are shown in Figure 1 a-b. Plants were fertilized monthly with 2 g/L (100 ml fertilizer per pot) water soluble fertilizer (20-20-20 plus, HUMOFERT, Metamorfosi, Greece). Two irrigation frequencies were applied: (i) when the moisture content of the substrate was 20–23% v/v (normal irrigation) and (ii) when the moisture content of the substrate was 8–13% v/v (sparse irrigation). In the first month of cultivation, the plants under normal irrigation were irrigated every 3–4 days and under sparse irrigation every 5 days, while in the following months every 2 and 3–4 days, respectively. This experiment lasted for 3 months, until July 2021.

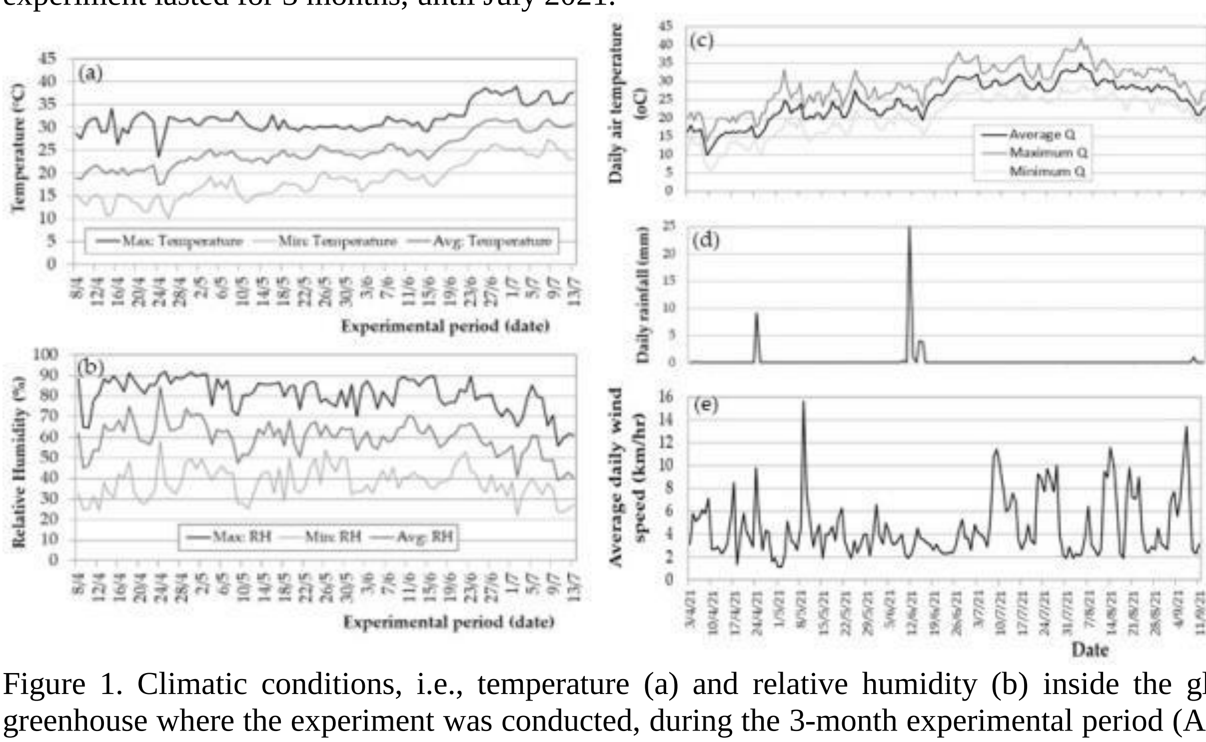

Figure 1. Climatic conditions, i.e., temperature (a) and relative humidity (b) inside the glass greenhouse where the experiment was conducted, during the 3-month experimental period (April

2021 - July 2021), as well as temperature (c), daily rainfall (d) and average daily wind speed (e) in the green roof, during the 5-month experimental period (April - September 2021).

In the second experiment, that was established in an extensive green roof, rooted cuttings, about 8 weeks old, of the hybrid *S. officinalis* × *S. tomentosa*, alongside with its parental species *S. officinalis* and *S. tomentosa*, were planted in plastic containers 40 cm × 60 cm × 22 cm in size. Each container had a green roof infrastructure fitted, i.e., moisture retention and protection of the insulation mat FLW-500, a drainage layer Diadrain-25H and a filter sheet VLF-150 (Landco Ltd., Diadem Green Roof Systems, Athens, Greece). Two plants of the same type per container and six containers per treatment were used. The containers were arranged following the completely randomized design on a second-floor flat roof at the Agricultural University of Athens (37°59′ N, 23° 42′ E). The substrate used was grape-marc compost: perlite: pumice (3: 3: 4, v/v) and had a 10 cm depth. Climatic conditions during this experiment are shown in Figure 1 c-e. Irrigation frequencies similar to those of the greenhouse experiment were applied, i.e., when substrate moisture 17–23% v/v (normal) and when substrate moisture 7–13% v/v (sparse). In the first month of cultivation, the plants were irrigated normally every 3 days and sparsely every 5 days, while in the following months every 2 and 4 days, respectively. Automatic drip irrigation on the substrate surface was applied before sunrise by two drippers placed at equal distances from the center of the container and the plants (dripper supply 4 L h^{-1}, irrigation period: 60 min). This experiment lasted for 5 months, until September 2021.
Plant growth was evaluated at the end of the experiments. The completely randomized design was used. The significance of the results was tested by either one- or two- way analysis of variance (ANOVA) and the means of the treatments were compared by Student’s *t* test at $P < 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

In the greenhouse experiment, all plants survived and the hybrid grew very satisfactorily under reduced irrigation, since only plant height was reduced from all growth parameters recorded (Table 1, Figure 2).
In the green roof experiment, survival rate was affected neither by *Salvia* type nor by irrigation frequency and was ranging between 66.7% and 91.7% (Table 2). *S. tomentosa* had higher plant height and diameter and aboveground dry weight than *S. officinalis* and their hybrid, while root dry weight was higher in the parental species compared to the hybrid. Reduced irrigation resulted in significant reduction of all growth parameters in all *Salvia* types. However, their growth remained satisfactory (Table 2, Figure 3).
In other *Salvia* species, such as *S. fruticosa* (Chrysargyris *et al.*, 2016) and *S. officinalis* (Sabry *et al.*, 2016; Soltanbeigi *et al.*, 2021), water deficiency was also reported to reduce plant height and plants yield components (aboveground and leaf fresh and dry weight). Regarding other Labiatae species, in *Lavandula latifolia*, *Mentha piperita* and *Thymus capitatus*, the aboveground fresh weight was reduced by drought stress, whereas in *Salvia sclarea*, *Salvia lavandutifolia* and *Thymus mastichina* it remained unaffected. As regards the aboveground dry weight, only in *L. latifolia* there was a significant reduction under water deficit conditions (García-Caparrós *et al.*, 2019).
The root system was found to play a key role in plant drought resistance in *S. officinalis* (Abate *et al*. 2021), a result that was also supported by our research since *S. officinalis* showed the

largest root/aboveground fresh and dry matter under both normal and sparse irrigation. In order to obtain drought tolerant genotypes, it is important to enlarge root-to-shoot ratios, since enhanced root growth in plants is fundamental to improve drought tolerance and yield under water stress in various crops (Idrissi *et al.*, 2015; Mathew *et al.*, 2018; Mwenye *et al.*, 2018).

Table 1. Growth of the new interspecific hybrid *S. officinalis* × *S. tomentosa* for three months (April–July 2021) in a greenhouse under normal and reduced irrigation (data per plant).

Irrigation frequency	Plant height (cm)	Lateral shoot number	Lateral shoot mean length (cm)	Lateral shoot total length (cm)	Aboveground d.w. (g)	Root d.w. (g)	Root d.w./ Aboveground d.w.
Normal	30.1 a[†]	10.3 a	10.2 a	105.1 a	9.4 a	2.6 a	0.3 a
Sparse	24.6 b	8.1 a	9.0 a	73.5 a	9.0 a	2.6 a	0.3 a
Significance [§]							
$F_{\text{one-way ANOVA}}$	*	NS	NS	NS	NS	NS	NS

[†] Mean values (n = 10) in each column followed by the same lowercase letter (a-b) did not differ significantly at $P \leq 0.05$ using Student’s *t*-test.
[§] NS or * or **, non-significant at $P \leq 0.05$ or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

Figure 2. Typical aboveground and root system of the new interspecific hybrid *S. officinalis* × *S. tomentosa*, after growing for three months in greenhouse conditions, under normal (N) and sparse (S) irrigation frequency. Size bar = 10.0 cm.

Table 2. Comparative evaluation of growth of two Greek sage species (*S. officinalis* and *S. tomentosa*) and their interspecific hybrid (*S. officinalis* × *S. tomentosa*) after five-month cultivation (April–September 2021) in an urban Mediterranean green roof under normal and sparse irrigation frequency.

Data		Per container	Per plant		Per container (2 plants)		
Salvia type	Irrigation Frequency	Survival (%)	Foliage height (cm)	Plant diameter (cm)	Aboveground d.w. (g)	Root d.w. (g)	Root d.w./ Aboveground d.w.
S. officinalis	Normal	83.3 a[†]	23.9 c	29.2 bc	70.3 b	95.9 a	1.4 a
	Sparse	75.0 a	20.8 c	25.4 c	50.4 c	74.9 ab	1.5 a
S. tomentosa	Normal	66.7 a	37.0 a	43.2 a	109.4 a	93.1 a	0.8 a
	Sparse	91.7 a	32.0 b	32.8 b	72.0 b	38.0 bc	0.5 b
S. officinalis × *S. tomentosa*	Normal	91.7 a	29.9 b	30.2 bc	85.1 b	61.8 abc	0.7 b
	Sparse	66.7 a	24.0 c	26.0 c	52.5 c	32.2 c	0.6 b
Significance [§]							
$F_{\mathit{Salvia}\ \mathrm{type}}$		NS	**	**	**	*	**
$F_{\mathrm{irrigation\ frequency}}$		NS	**	**	**	**	NS
$F_{\mathrm{interaction}}$		NS	NS	NS	NS	NS	NS
$F_{\mathrm{one\text{-}way\ ANOVA}}$		NS	**	**	**	**	**

[†] Mean values (n = 8-12) in each column using Student’s t test; means followed by the same letter are not significantly different at $P \leq 0.05$

[§] NS or * or **, non-significant at $P \leq 0.05$ or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively.

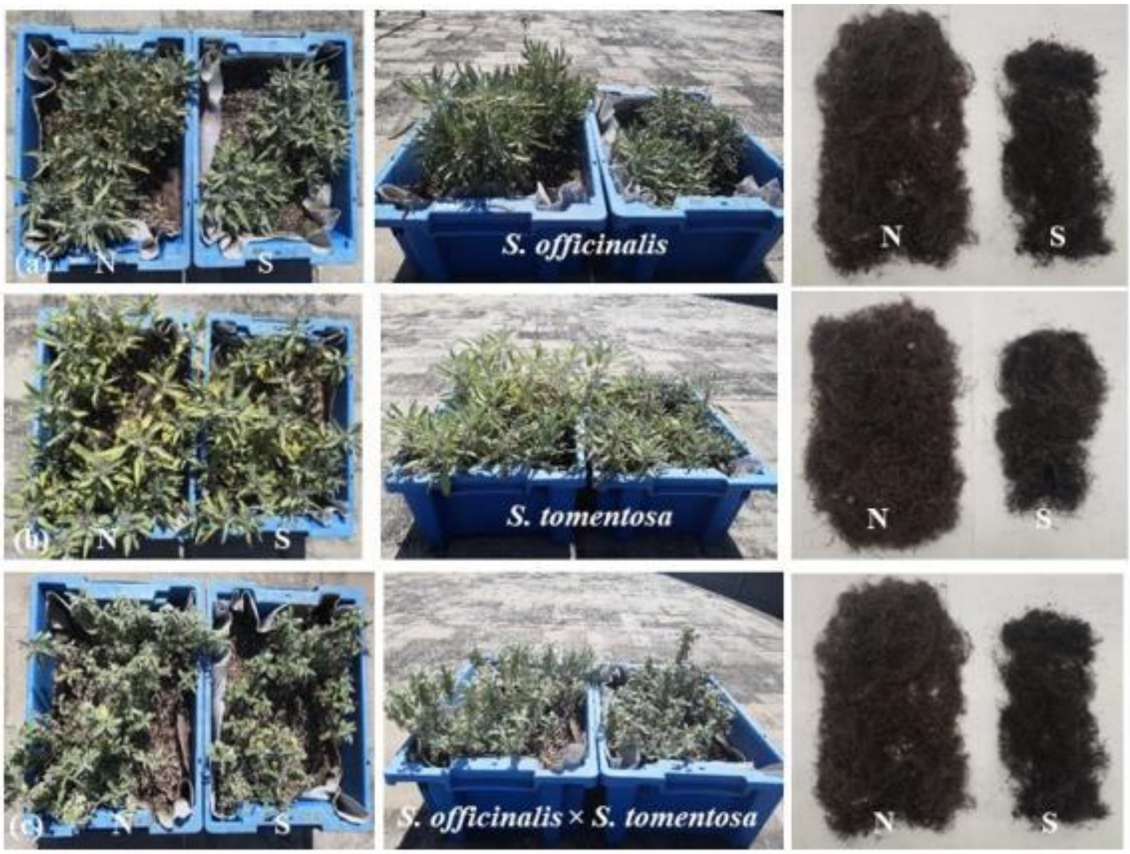

Figure 3. Typical growth of aboveground and root system of *S. officinalis* (a), *S. tomentosa* (b), and *S. officinalis* × *S. tomentosa* (c), after five months cultivation (April - September 2021) in an extensive green roof, under normal (N) and sparse (S) irrigation.

Conclusions

Despite the reduction of all growth parameters of all three *Salvia* types under reduced irrigation in the extensive green roof, their growth remained satisfactory. So, both the hybrid *S. officinalis* × *S. tomentosa* and its parents, *S. officinalis* and *S. tomentosa*, are recommended for sustainable use in xeriscaping, including extensive green roofs.

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THE EFFECT OF CYTOKININ TYPE AND CONCENTRATION ON *in vitro* MULTIPLICATION OF *Salvia fruticosa*

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Abstract

Salvia fruticosa Mill. (Lamiaceae), Greek sage, is a perennial sage species endemic of the Mediterranean region, part of the macchia vegetation, with a wider distribution from Sicily to Israel. Aiming to impove and promote the species for ornamental and medicinal use, in the present work the effect of cytokinin type and concentration on its *in vitro* propagation was studied. 6-benzyladenine (BA), zeatin (ZEAT) or meta-Topolin (mT) at 0.0, 0.4, 0.8, 1.6 or 3.2 mg L^{-1} were combined with 0.01 mg L^{-1} 1-naphthaleneacetic acid (NAA) in the culture medium (solid MS). Shoot tip or single-node explants from microshoots originated from explants excised from *in vitro* grown seedlings were used. The response for shoot production was very high in shoot tip explants (over 90%) in most media, while in nodal explants was slightly lower (75-85%) when the medium was supplemented with ZEAT or BA. The presence of cytokin compared to the hormone-free medium promoted slightly the number of shoots produced decreasing simultaneously their length. However, cytokinin in high concentrations, regardless of cytokinin type, resulted in the formation of hyperhydrated shoots, which reached up to 62% in the substrate with 3.2 mg L^{-1} ZEAT. There was an indication that most normal shoots (2.4-2.5 shoots explant) were produced on 0.8 or 1.6 mg L^{-1} BA medium from nodal explants, while the highest multiplication rate was observed on 0.8 mg L^{-1} mT medium. The increase in concentration of all three types of cytokins tested resulted in an increase in the number of shoots produced, but this increase was mainly reflected in hyperhydrated shoots.

Keywords: *6-benzyladenine, hyperhydricity, Mediterranean sage, meta-Topolin, zeatin.*

Introduction

S. fruticosa Mill. (Lamiaceae), Greek sage, is a strongly aromatic, perennial evergreen shrub, up to 1.20 m high, growing mainly in bushy rocky areas, often on coastal cliffs, at altitudes 1-700 m (Blamey and Grey-Wilson, 1993; Thanos and Doussi, 1995). It is endemic to the eastern Mediterranean, including southern Italy, North Africa and the Canary Islands, while in Greece, it is found in the Central country, the Peloponnese and the Aegean islands (Thanos and Doussi, 1995). In Greece, it has been traditionally used as a medicinal, culinary and melliferous plant since the antiquity (Clebsch and Barner, 2003). Nowadays, it is widely used for the preparation of an herbal tea (faskomilo) (Hanson, 2004).

The biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants, while plant tissue culture techniques offer an integrated approach for the production of active compounds and standardized quality phytopharmaceutical for herbal and pharmaceutical industries (Debnath *et al.*, 2006; Sidhu, 2011). There are a few reports about the use of tissue culture techniques in *S. fruticosa* aiming to its micropropagation and the

accumulation of essential oils or other compounds (Karam et al., 2003; Arikat et al., 2004). Meta-topolin (mT) is a natural aromatic cytokinin that is shown to be more effective in morphogenesis than other cytokinins, regarding regeneration capacity, reduced hyperhydricity and root inhibition (Krishna Vrundha *et al.*, 2021), which would worth being tested on *in vitro* shoot multiplication of *S. fruticosa*. It has been used on *in vitro* propagation of *Salvia sclarea* and was found superior to other cytokinins (Erişen *et al.*, 2020)
So, in the present study the effect of cytokinin type and concentration on *in vitro* propagation of *S. fruticosa* was studied, aiming to impove and promote the species for ornamental and medicinal use.

Materials and Methods

Shoot tip or single-node explants excised from microshoots of *in vitro* cultures of *S. fruticosa* initiated from *in vitro* grown seedlings were used. The explants were cultured on MS medium (Murashige and Skoog, 1962) with 30 g L^{-1} sucrose either without plant growth regulators (control) or supplemented with three different cytokinins, i.e. 6-benzyladenine (BA), zeatin (ZEAT) or meta-Topolin (mT), at four concentrations, i.e., 0.4, 0.8, 1.6, and 3.2 mg·$L^{–1}$ in combination with 0.01 mg L^{-1} 1-naphthaleneacetic acid (NAA).
All media were solidified with 8 g L^{-1} agar and their pH was adjusted to 5.7 before agar addition and autoclaving (121 ^oC for 20 min). The cultures were maintained at 25 ^oC with a 16 h photoperiod at 37.5 μmol m^{-2} s^{-1} fluorescent light, provided by cool-white fluorescent lamps. Data were collected after 30 days of culture.
The "multiplication index" of each culture was calculated by multiplying the percentage of explants that produced shoots by the mean number of shoots per responding explant, and by the mean node number per shoot.
The completely randomized design was used. The significance of the results was tested by either one- or two- or three-way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at $P < 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The 3-way ANOVA in most parameters measured revealed significant interaction of the three nain factors of the experiment. The type and concentration of cytokinin had a significant effect on the length of normal shoots, while the explant type had an effect on the number of normal shoots and their node number, as well as on the multiplication index. Nodal explants resulted in higher number of normal shoots produced per responded explant, while tip explants in higher node number per shoot and higher multiplication index.
The response for shoot production was very high in tip explants (over 90%) in most media, while in nodal explants was slightly lower (73-85%) when the medium was supplemented with ZEAT or BA (Table 1). Cytokinin in high concentrations, regardless of cytokinin type, resulted in the formation of hyperhydrated shoots, which reached up to 62% when nodal explants were cultured on a medium with 3.2 mg L^{-1} ZEAT or mT (Table 1).
The presence of cytokinin compared to the hormone free medium promoted slightly the number of shoots produced per explant decreasing simultaneously their length. There was an indication that the highest number of normal shoots (2.4-2.5 shoots per responded explant) was produced on 0.8 or 1.6 mg L^{-1} BA medium when nodal explants were used, while the longest shoots (2.2 cm) with a higher number of nodes (3.3 nodes /shoot) were produced when tip explants were

cultured on hormone-free medium, but with no significant difference from many other media (Table 1, Figure 1). The increase in concentration of all three types of cytokinins tested resulted in an increase in the number of produced shoots, but this increase was mainly reflected in hyperhydrated shoots. The highest number of hyperhydrated shoots was observed on the 3.2 mg L^{-1} ZEAT medium for both explant types (2.3-2.4 hyperhydrated shoots per responded explant) (Table 1).

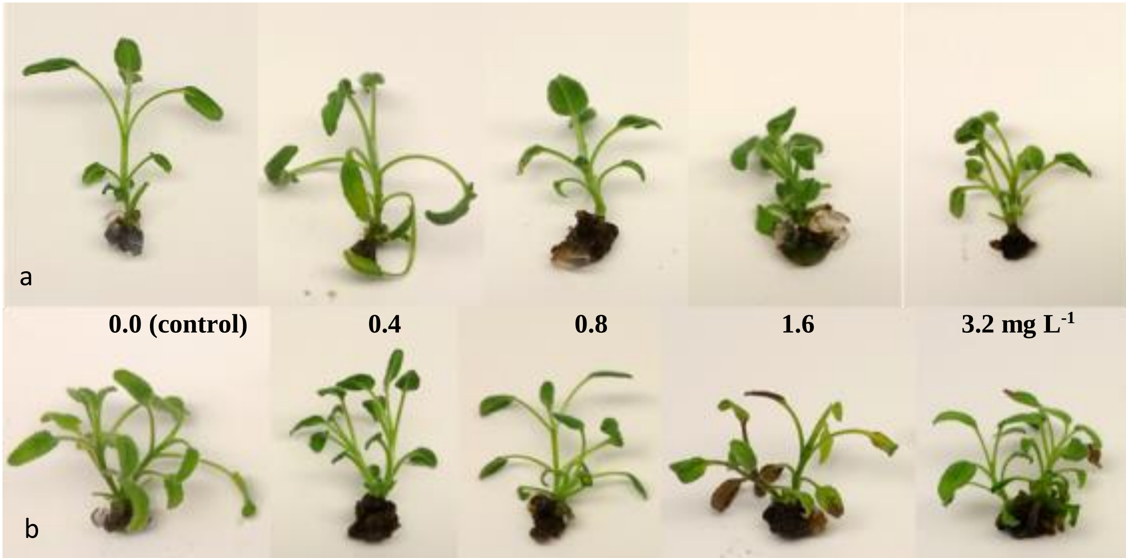
Taking into consideration the highest percentage of explants that responded to form normal shoots, hormone-free medium and media with 0.4 mg L^{-1} ZEAT or BA and 0.4 or 0.8 mg L^{-1} mT were distinguished (Table 1). In previous studies on micropropagation of *S. fruticosa* (Arikat *et al.*, 2004) and *S. officinalis* (Petrova *et al.*, 2015) was also shown that shoot proliferation was favored by low concentrations of BA (0.2-0.5 mg L^{-1}). In the present study, the medium with 0.8 mg L^{-1} mT was superior to others, since it resulted to the highest multiplication rate and good tissue quality (Figure 1), followed by that with 0.4 mg L^{-1} mT (Table 1). This result verifies other studies on mT, which have shown its superiority in shoot regeneration capacity, along with increased plant tissue quality (Kőszeghi *et al.*, 2014; Erişen *et al.*, 2020; Krishna Vrundha *et al.*, 2021).

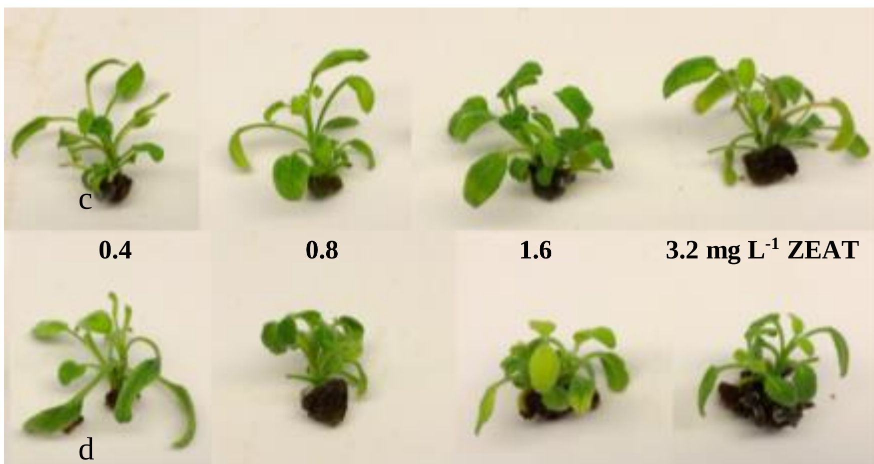
Table 1. Effect of explant type and cytokinin type and concentration on shoot multiplication of explants excised from *in vitro* seedlings of *S. fruticosa* and cultured in a medium with marked cytokinin type and concentration in combination with 0.01 mg L^{-1} NAA.

Cytokinin concn (mg L^{-1})	Shoot production[1] (%)	Shoot production[2] (%)	Mean NSh[Ŧ] number	Mean NSh length[Ŧ] (cm)	Mean NSh node number[Ŧ]	Mean HSh[ŦŦ] number	Multiplication index for NSh
Shoot tip explant							
0.0 (Hf[ŦŦŦ])	84 a[z]	8 m	1.0 f	2.2 a	3.3 a	0.1 l	2.8 bcd
0.4 ZEAT	83 a	13 l	1.1 f	1.6 cdef	2.5 bcd	0.2 k	2.3 def
0.8 ZEAT	67 def	21 j	1.4 def	1.3 cdef	2.5 bcd	0.3 jk	2.3 def
1.6 ZEAT	59 gh	29 h	1.3 ef	1.1 efg	2.3 cde	0.6 hi	1.8 fgh
3.2 ZEAT	21 lm	58 a	2.2 ab	0.7 hi	1.2 f	2.3 a	0.6 i
0.4 BA	80 ab	20 j	1.6 cde	0.8 ghi	1.2 f	0.3 jk	1.5 ghi
0.8 BA	67 def	27 h	1.6 cde	0.8 ghi	1.6 ef	0.5 i	1.7 fgh
1.6 BA	67 def	33 fg	1.9 abcd	0.7 hi	1.7 def	0.8 fg	2.2 def
3.2 BA	67 def	33 fg	2.0 abc	0.7 hi	1.6 ef	0.8 fg	2.1 efg
0.4 mT	73 c	21 j	1.7 bcd	2.0 ab	2.9 ab	0.6 hi	3.6 b
0.8 mT	77 b	23 i	2.2 a	1.8 abcd	2.7 bc	0.6 hi	4.6 a
1.6 mT	65 ef	35 f	1.8 bcd	1.6 cde	2.4 cde	0.9 def	2.8 cde
3.2 mT	62 fg	38 de	2.1 abc	1.4 cdef	1.7 def	1.0 de	2.2 def
Nodal explant							
0.0 (Hf[ŦŦŦ])	71 cd	17 k	1.7 bcd	1.4 cdef	2.3 cde	0.3 jk	2.8 cde
0.4 ZEAT	50 ij	29 h	2.0 abc	1.2 def	1.7 def	0.7 gh	1.7 fgh

0.8 ZEAT	46 jk	33 fg	1.9 abcd	1.1 efg	1.7 def	1.0 de	1.5 ghi
1.6 ZEAT	46 jk	37e	2.0 abc	1.0 fgh	1.7 def	1.0 de	1.6 gh
3.2 ZEAT	13 m	62 a	2.0 abc	0.8 ghi	1.3 f	2.4 a	0.3 i
0.4 BA	67 def	20 j	2.0 abc	0.7 hi	1.5 ef	0.6 hi	2.0 efg
0.8 BA	50 ij	27 h	2.5 a	0.6 i	1.2 f	1.0 de	1.5 ghi
1.6 BA	40 k	40 cd	2.4 a	0.6 i	1.2 f	1.0 de	1.2 hi
3.2 BA	23 l	50 b	2.2 ab	0.6 i	1.1 f	1.2 c	0.5 i
0.4 mT	56 hi	27 h	1.7 bcd	1.9 abc	2.7 bc	0.6 hi	2.6 def
0.8 mT	67 def	29 h	2.2 ab	1.4 cdef	2.4 cde	0.9 def	3.5 bc
1.6 mT	58 gh	42 c	1.7 bcd	1.2 def	1.6 ef	1.4 b	1.6 gh
3.2 mT	38 k	62 a	1.5 def	1.0 fgh	2.0 def	1.6 b	1.1 hi
$F_{cytokinin}$	-	-	-	***	-	-	-
$F_{concentration}$	-	-	-	*	-	-	-
$F_{explant}$	-	-	*	NS	**	-	***
$F_{cytok\ x\ concn}$	***	***	**	NS	*	***	**
$F_{cytok\ x\ explant}$	***	***	NS	NS	NS	NS	NS
$F_{concn\ x\ explant}$	**	***	NS	NS	NS	***	NS
$F_{cyt\ x\ exp\ x\ concn}$	***	***	NS	NS	NS	***	NS
$F_{one\text{-}way}$	***	***	***	***	***	***	***

[z]Mean separation in columns by Student’s t, $P \leq 0.05$.
NS: not significant or *, **, ***: significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively, n=30.
Multiplication Index = Shooting (%) x mean shoot number₸ x Mean node number₸
[1]The explants produced normal and hyperhydrated shoots
[2]The explants produced hyperhydrated shoots only
₸NSh = normal shoot
₸₸HSh = hyperhydrated shoot
₸₸₸Hf = hormone free


a
0.0 (control)
0.4
0.8
1.6
3.2 mg L^{-1}
b


c
0.4
0.8
1.6
3.2 mg L^{-1} ZEAT
d

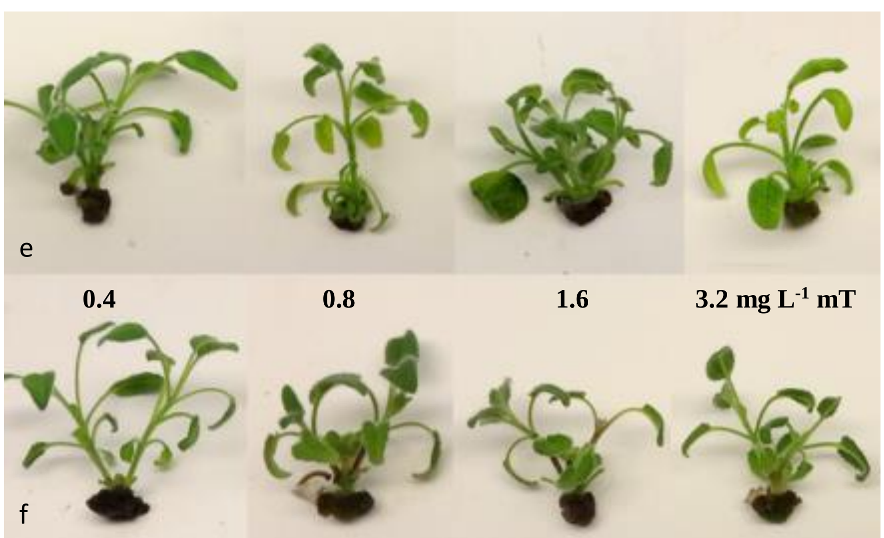

e
0.4
0.8
1.6
3.2 mg L^{-1} mT
f

Figure 1. Typical response of shoot tip (a, c and e) and nodal (b, d and f) explants of *S. fruticosa*, after 4-week culture *in vitro* on solid MS medium without plant growth regulators (control) or supplemented with marked concentration (mg L^{-1}) of BA or ZEAT or mT, respectively, in combination with 0.01 mg L^{-1} NAA.

Conclusions

The increase in concentration of all three types of cytokinins tested (ZEAT, BA, mT) resulted in an increase in the number of shoots produced, but this increase was mainly reflected in hyperhydrated shoots. Low concentration (0.4 or 0.8 mg L^{-1}) was preferable for all tested cytokinins, but meta-Topolin at 0.8 mg L^{-1} was superior to others, due to high shoot multiplication rate simultaneously with good plant tissue quality.

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EFFECT OF EXPLANT TYPE AND BENZYLADENINE CONCENTRATION ON *in vitro* MULTIPLICATION OF *Salvia tomentosa*

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Abstract

Salvia tomentosa Mill. (Lamiaceae) is a Mediterranean perennial sage species, part of the macchia vegetation. Aiming to impove and promote the species for ornamental and medicinal use, in the present work the effect of explant type and benzyladenine (BA) concentration on *in vitro* blastogenesis of the species was studied. Shoot tip or single-node explants from microshoots originated from explants excised from *in vitro* grown seedlings were used. The explants were cultured on MS medium either without plant growth regulators (control) or enriched with 0.4 or 0.8 or 1.6 or 3.2 mg L^{-1} BA in combination with 0.01 mg L^{-1} naphthalynacetic acid (NAA). Shoot tip explants responded at higher percentage (59-66%) to form shoots compared to nodal ones (27-48%), at BA concentration 0.4-1.6 mg L^{-1}. In the control, both explant types produced shoots at the same higher percentage (67-69%), whereas at 3.2 mg L^{-1} BA the lowest percentage of shoot production was observed (16-21%). The percentage of explants that produced hyperhydrated shoots was 13-33%, depending on the treatment. More shoots (1.6-1.7) were produced at 1.6 mg L^{-1} BA from shoot tip explants and at 0.0-0.8 mg L^{-1} BA from nodal explants, whereas the number of hyperhydrated shoots was highest (2.1-2.9) at 1.6 (only for nodal explants) or 3.2 mg L^{-1} BA. The highest multiplication rate and the longest shoots (5.1-5.8 cm) with the highest node number (4.8-5.0) were observed in the control for both explant types, followed by the response at 0.4 mg L^{-1} BA. In conclusion, the increase of BA concentration resulted in an increase in the number of produced shoots, but hyperhydricity was increased simultaneously.

Keywords: *benzyladenine (BA), hyperhydricity, Mediterranean sage, micropropagation, native plant.*

Introduction

Salvia tomentosa Miller (Lamiaceae), Balsamic sage, is a strongly aromatic, medicinal, perennial semi-woody herbaceous plant (Hedge, 1982), up to 80 cm, which grows in areas of maquis vegetation and on limestone slopes. Its geographical distribution extends from South Eastern Europe to Transcaucasia (Guner *et al.*, 2000). In Greece, it spreads in North-Eastern Greece and in the North-Eastern and Eastern Aegean Islands (Dimopoulos *et al.*, 2013). In traditional medicine, *S. tomentosa* is used to heal wounds (Aşkun *et al.*, 2010) and relieve stomach and abdominal pain (Ulubelen and Miski 1979), while it is consumed as an herbal tea in some Mediterranean countries (Dincer *et al.*, 2013). The aerial parts of the plant have antimicrobial and antioxidant properties, due to the significant quantities in secondary metabolites such as phenolics and terpenoids (Haznedaroglu *et al.*, 2001; Tepe *et al.*, 2005; Aşkun *et al.*, 2010).
Modern biotechnological methods like *in vitro* micropropagation technique have the benefits of the large scale multiplication of disease-free plants, faster cloning and the conservation of

desired genotypes, in a very short span of time, along with the potential for the production of high-quality plant-based medicine (Máthé *et al.*, 2015). Micropropagation is also used in the propagation of medicinal and aromatic plants (MAPs) as a tool to conserve rare, threatened, and valuable MAPs, and to massively produce high-value plant material for cultivation without seasonal constraints (Grigoriadou *et al.*, 2019). Micropropagation protocols are worked out for many plant species cultured *in vitro* to obtain high regeneration rates, aiming to facilitate commercially feasible micropropagation and enable their possible sustainable use (Máthé *et al.*, 2015; Grigoriadou *et al.*, 2019).

To the best of our knowledge, no studies have been performed on the *in vitro* propagation of *S. tomentosa*. Therefore, a first approach was made to its micropropagation with the aim of improving and promoting the species for ornamental and medical use. In the present work the effect of explant type and benzyladenine (BA) concentration on *in vitro* blastogenesis of the species was studied.

Materials and Methods

Shoot tip or single-node explants excised from microshoots of *in vitro* cultures of *S. tomentosa* initiated from *in vitro* grown seedlings were used. Explants were cultured on MS medium (Murashige and Skoog, 1962) with 30 g L^{-1} sucrose either without plant growth regulators (control) or supplemented with benzyladenine (BA), at four concentrations, i.e., 0.4, 0.8, 1.6, or 3.2 $mg \cdot L^{-1}$ in combination with 0.01 mg L^{-1} naphthalynacetic acid (NAA).

All media were solidified with 8 g L^{-1} agar and their pH was adjusted to 5.7 – 5.8 before agar addition and autoclaving (121 ºC for 20 min). The cultures were maintained at 25 ºC with a 16 h photoperiod at 37.5 μmol m^{-2} s^{-1} fluorescent light, provided by cool-white fluorescent lamps. Data were collected after 30 d of culture.

The "multiplication index" of each culture was calculated by multiplying the percentage of explants that produced shoots by the mean number of shoots per responding explant, and by the mean node number per shoot.

The completely randomized design was used. The significance of the results was tested by either one- or two-way analysis of variance (ANOVA) and the means of the treatments were compared by Student's *t* test at $P < 0.05$ (JMP 13.0 software, SAS Institute Inc., Cary, NC, 2013, USA).

Results and Discussion

The 2-way ANOVA in most of the parameters measured revealed significant interaction of the two nain factors of the experiment, i.e., BA concentration in the medium and explant type. Only the length and the node number of the shoots were affected by the BA concentration, as the inrease in the BA concentration resulted in a decrease in both of these parameters (Table 1).

Shoot tip explants responded at higher percentage (59-66%) to form shoots without hyperhydricity compared to nodal ones (27-48%), at BA concentration 0.4-1.6 mg L^{-1}, result that has also been reported for *S. officinalis* (Vlachou *et al.*, 2021). In the control, both explant types produced shoots at the same higher percentage (67-69%), whereas at 3.2 mg L^{-1} BA the lowest percentage of shoot production was observed (16-21%) (Table 1). The percentage of explants that produced hyperhydrated shoots was 13-33%, depending on the treatment (Table 1).

The presence of BA at high concentrations in the medium increased the number of shoots (normal and hyperhydrated) produced per explant. More shoots (1.6-1.7) were produced at 1.6

mg L^{-1} BA from shoot tip explants and at 0.0-0.8 mg L^{-1} BA from nodal explants, whereas the number of hyperhydrated shoots was highest (2.1-2.9) at 1.6 (only for nodal explants) or 3.2 mg L^{-1} BA (Table 1, Figure 1).

The highest multiplication rate and the longest shoots (5.1-5.8 cm) with the highest node number (4.8-5.0) were observed in the control for both explant types, followed by the response at 0.4 mg L^{-1} BA (Table 1, Figure 1). In previous studies on micropropagation of various *Salvia* species, such as *S. fruticosa* (Arikat *et al.*, 2004), *S. officinalis* (Petrova *et al.*, 2015), *S. sclarea* (Grigoriadou et al., 2020) or *S. wagneriana* (Ruffoni *et al.*, 2016), respectively low BA concentration (0.2-0.5 mg L^{-1}) favored shoot proliferation, in most cases combined with low auxin concentration as in the present study. Regarding the effect of the explant type on proliferation, in previous works on other *Salvia* spp, i.e., *S. fruticosa* (Arikat *et al.*, 2004), *S. sclarea* (Ghanbar *et al.*, 2016), *S. valentina* and *S. blancoana* subsp. *mariolensis* (Cuenca and Amo-Marco, 2000), maximum shoot proliferation has been reported from nodal explants compared to the shoot tips, whereas in the present study the explant type did not have such a significant effect on shoot proliferation of *S. tomentosa*. This is probably due to the fact that single node explants were used in this work, while in previous works nodal explants bear more nodes (Arikat *et al.*, 2004).

Table 1. Effect of BA concentration on shoot multiplication of shoot tip or nodal explants excised from S. tomentosa seedlings grown in vitro, in the presence of 0.01 mg L-1 NAA.

BA concn (mg L^{-1})	Shoot production [1] (%)	Shoot production [2] (%)	Mean NSh$^{₸}$ number	Mean NSh length$^{₸}$ (cm)	Mean node number$^{₸}$	Mean HSh$^{₸₸}$ number	Multiplication index
Shoot tip explant							
0.0 (Hf$^{₸₸₸}$)	69 a	28 cd	1.0 c	5.1 ab	4.8 a	0.3 f	3.3 b
0.4 BA	64 ab	28 cd	1.0 c	4.6 abc	3.8 b	0.4 f	2.4 cd
0.8 BA	66 a	27 d	1.3 bc	3.4 cde	3.4 bc	0.3 f	2.9 bc
1.6 BA	59 b	29 b	1.7 a	2.9 de	2.6 c	0.5 ef	2.6 bc
3.2 BA	21 ef	25 ef	1.3 bc	3.1 cde	3.1 bc	2.6 a	0.8 e
Nodal explant							
0.0 (Hf$^{₸₸₸}$)	67 a	25 ef	1.6 ab	5.8 a	5.0 a	0.6 de	5.4 a
0.4 BA	48 c	26 de	1.6 ab	4.2 bcd	3.4 bc	0.7 d	2.6 bc
0.8 BA	27 de	33 a	1.7 a	2.3 e	2.3 c	1.1 c	1.1 e
1.6 BA	29 d	17 f	1.3 bc	3.1 cde	3.4 bc	2.1 b	1.3 de
3.2 BA	16 f	13 g	1.3 bc	2.3 e	2.8 bc	2.9 a	0.6 e
$F_{\text{concn BA}}$	-	-	-	***	***	-	-
F_{explant}	-	-	-	NS	NS	-	-
$F_{\text{concn BA x expl}}$	***	***	**	NS	NS	***	***
$F_{\text{one-way}}$	***	***	***	***	***	***	***

[z]Mean separation in columns by Student's t, $P \leq 0.05$.

NS: not significant or *, **, ***: significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively, n=30.
Multiplication Index = Shooting (%) x mean shoot number[Ŧ] x Mean node number[Ŧ]
[1]The explants produced normal and hyperhydrated shoots
[2]The explants produced hyperhydrated shoots only
[Ŧ]NSh = normal shoot
[ŦŦ]HSh = hyperhydrated shoot
[ŦŦŦ]Hf = hormone free

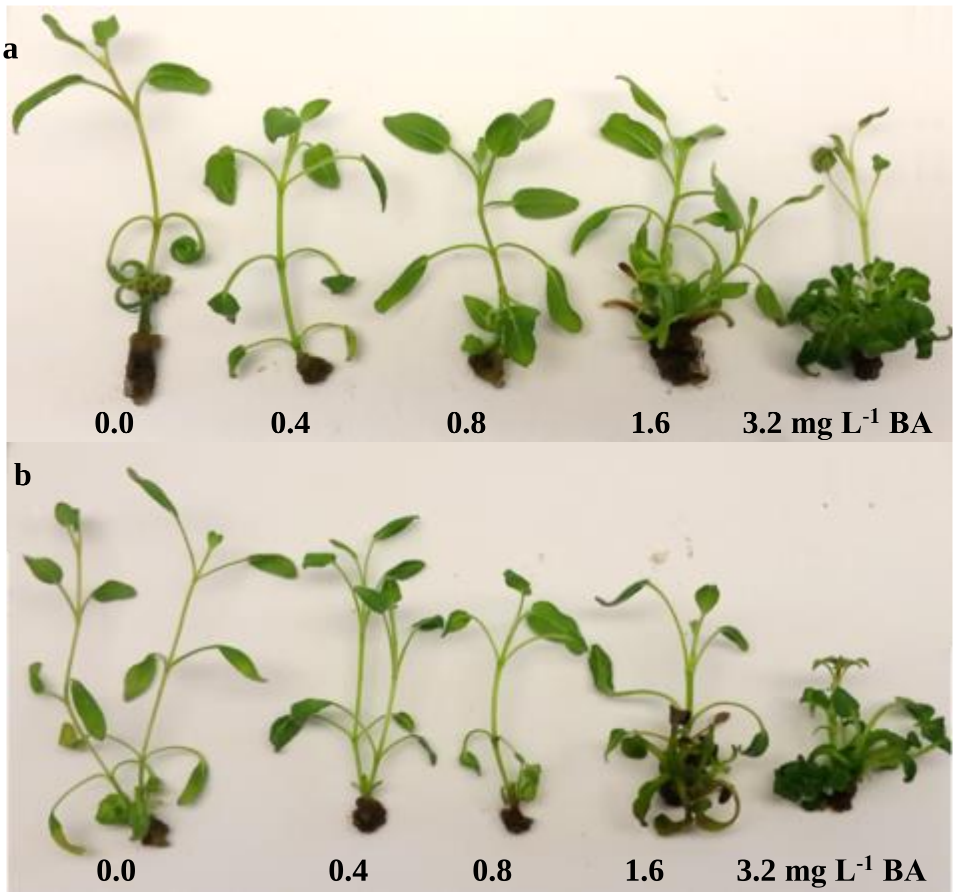

Figure 1. Typical response of shoot tip (a) and nodal (b) explant of *S. tomentosa* cultured *in vitro* on MS medium with marked BA concentration (mg L^{-1}) in the presence of 0.01 mg L^{-1} NAA.

Conclusions

The increase of BA concentration in the medium resulted in an increase in the number of produced shoots, but hyperhydricity was increased simultaneously.
Higher multiplication rates along with low hyperhydricity were achieved when either shoot tip or nodal explants were cultured on MS medium without plant growth regulators or enriched with the lowest BA concentration tested, 0.4 mg L^{-1}, in combination with 0.01 mg L^{-1} NAA.

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VARIABILITY OF STEM HEIGHT IN WHEAT AND TRITICALE UNDER INFLUENCE OF APPLIED INSECTICIDES

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Abstract

Stem height have significant impact in forming of yield. The aim of this study was estimation of variability of stem height in wheat and triticale grown in different environmental conditions. The three wheat and three triticale varieties were included in field experiment in three vegetative seasons. The experiment was set up as a randomized block design in three replications. Insecticides Desis (deltamethrin) and Bifentrin (bifenthrin) were applied in phase of booting and before of heading in aim of protection from attack of *Oulema melanopus*. The results showed that wheat Belija had significantly higher stem height in each year (83.75 cm, 83.85 cm, 83.18 cm) than wheat varieties Aurelia (66.53 cm, 68.26 cm, 68.37 cm) and Zemunska Rosa (72.44 cm, 79.83 cm. 79.18 cm). The stem height was the highest in triticale Admiral (97.27 cm) in first year, while in Zenit was in second (111,15 cm), third years (105.58 cm) and in average for all three years (104,52 cm). The least stem height in first and third year had triticale Agrounija while in second and in average for all three years had Admiral. The application of insecticides deltamethrin caused depression of stem height in wheat from 1.0% (Aurelia) to 2.99% (Belija), while in triticale from 0.3% (Admiral) to 0.7% (Zenit). Bifentrin caused greater depression of stem height in wheat from 1.21% (Zemunska Rosa) to 3.78% (Belija) as well in triticale from 0,73% in Zenit to 1.20% in Agrounija, while it is increased stem height for 0.1% in Admiral. The results indicate that stem height varied and depended on genetic and environmental factor and from their interactions (G/E).

Key words: *wheat, triticale, stem height, varieties.*

Introduction

Stem height is very important traits of plant architecture which is influence to efficient competition for acceptance of sunlight and photosynthesis, translocation water, mineral element, organic matter and affecting lodging resistance, density of wheat crops, grain harvest index and grain yield (Zečević et al., 2005; Berry and Berry, 2015; Madić et al., 2016). The developing of semi-dwarf varieties contribute significantly increasing yield of varieties which efficiently response to applied fertilizers, translocation mineral elements, water and organic matter from stem to spike and increasing of lodging (Medvedev et al., 2016; Grant et al., 2018). This period known as a period of `Green revolution`. For optimal grain yield of wheat is stem height ~ 0.7 m, and shortening the height is not reasonable for further improvement of lodging resistance was found in the studied wheat variety (Flintham et al., 1997). Variability of stem height depends from environment and genetic structure of varieties. The significant contribution in reduction of

stem height have *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) genes which response to gibberellin insensitivity, and *Rht8* gibberellin sensitive, that resulted in reducing plant height, number of grain and grain yield (Zanke et al., 2014; He et al., 2016; Würschum et al., 2017; Chai et al., 2022; Xiong et al., 2022). The *Rht-B1b* and *Rht-D1b* used in ~95% of modern wheat varieties around the world, while within 19 height mutant (*Rht3, Rht4, Rht12, Rht13, Rht14, Rht16, Rht18, Rht23, Rht24*,…) known in wheat, only *Rht8* used in some Europen varieties (Hedden, 2003; Würschum et al., 2015). The *Rht8* gene reduces the plant height (Ellis et al., 2004; Rebetzke et al., 2012) but it has negligible effect on coleoptile length (Liatukas and Ruzgas, 2011; Grover et al., 2018). The combinations of *Rht8* and *Rht4* can reduce plant height and improve yield components in the rainfed cultivation (Yingying et al., 2018; Dua et al., 2020). Also, temperature related to stem elongation, and end of stem elongation as well as final height, depends of variability genotypes for temperature response which have high heritablility (H^2=0.81) Kronenberg et al. (2021) what is importan for adaptability (Kaya et al., 2015). This confirm that environmental factor and its interaction with genotype have influence on stem height in wheat (Knežević et al., 1993; 2020). For improving competitiveness of crops against weeds, pathogens and pests, used pesticides for protection. However, the application of insecticides may also affect the plants along with the pests (Božinović et al., 1997; Dhungana et al., 2020). The application of insecticides can block photosynthesis, gases exchanges through stomates, reduce plant height due to chemical toxicity (Weinberger et al. 1978; Varshney et al. 2012). Pesticides can adversely affect plant growth and development which can affect decreasing of height plant in wheat which is connected with increasing of crop resistance to lodging (Abboud et al., 2014; Kuznetsov et al., 2020)
The aim of this study was establish (i) variability of plant height in wheat varieties grown under different environmental condition of three years of experiment (ii) effect of applied insecticides on stem height in wheat varieties grown in different environmental conditions.

Materials and Methods

This research encompasses three wheat and three triticale varieties. Selected wheat genotypes were sown in experimental field of Maize Institute Zemun Polje in Serbia. The experiment was set up as a randomized block design in three replications on plots of 1 m^2, in three growing seasons (2018/19 2019/20 and 2020/21). Sowing was done with a machine seeder, with the planned crop density based on 500 germinating seeds. The plants were harvested for analysis in the phenology phase of full maturity. The 30 plants at the full maturity stage (10 plants per replication) used for analysis of stem height in wheat and triticale varieties. The analysis of variance (ANOVA) was performed according to a random block system with one factor and thre factors by using the SPSS program. F-test was used to assess the significance of differences among average values of stem height and tested by LSD (0.01; 0.05).

Results and Discussion

The stem height of the wheat and triticale was different and in studied wheat varieties was lower than in triticale varieties in all three vegetation season (2018/19, 2019/20 and 2020/21). Among wheat, the smallest stem height was expressed in Aurelia 66.53 cm in first year, and the largest 83.85 cm in second year in variety Belija as well as in each vegetation season and in average for all three vegetation season (table 1). The stem height was significantly different between wheat varieties within each year ($p<0.01$). The stem height of wheat Zemunska Rosa and average value of stem height of all three varieties was significantly lower ($p < 0.01$) in first than in second and third vegetation season (table 1).

Table 1. Variability of stem height in wheat and triticale varieties

Variety		Wheat				**Variety**		Triticale			
		2018/19	2019/20	2020/21	Average			2018/19	2019/20	2020/21	Average
		$\bar{X}$ (cm)	$\bar{X}$ (cm)	$\bar{X}$ (cm)	**$\bar{X}$ (cm)**			**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**
Aurelia		66.53^{c}	68.26^{c}	68.37^{c}	67.72^{c}	Admiral		97.27^{a}	100.64^{c}	99.87^{b}	99.26^{b}
Belija		83.75^{a}	83.85^{a}	83.18^{a}	83.60^{a}	Agrounija		92.17^{b}	107.45^{b}	99.51^{b}	99.71^{b}
Zemunska Rosa		72.44^{b}	79.83^{b}	78.18^{b}	76.82^{b}	Zenit		96.85^{a}	111.15^{a}	105.58^{a}	104.52^{a}
	Average	**74.24**	**77.32**	**76.58**	**76.04**			**95.43**	**106.41**	**101.65**	**101.17**
Genotype	Lsd 0.05	2.362	2.398	2.635	1.423	Genotype	Lsd 0.05	3.188	2.989	3.566	1.877
	Lsd 0.01	3.108	3.156	3.467	1.870		Lsd 0.01	4.195	3.933	4.693	2.468
Year	Lsd 0.05	**1.423**	Genotype × Year	Lsd 0.05	2.464	Year	Lsd 0.05	1.877	Genotype × Year	Lsd 0.05	3.251
	Lsd 0.01	**1.870**		Lsd 0.01	3.240		Lsd 0.01	2.468		Lsd 0.01	4.275

Among triticale, the smallest stem height had variety Agrounija 92.17 cm in first year and the largest had Zenit 111.15 cm in second year. In average for three vegetation seasons, stem height was the smallest in variety Admiral (99.26 cm), and the highest in Zenit (104.52 cm) table 1.

The significant differences between triticale varieties for stem height in each growing seasons were established ($p<0.01$), and in average value in three-years. The stem height in average for all three varieties of triticale was s ignificantly lower in first vegetation season than in the second and third. The stem height of triticale Agrounija and Zenit and average value of all three varieties was significantly different ($p < 0.01$) between growing seasons (table 1).

Similar results for genotypes and environment influence on stem height in wheat varieties was found in other study (Zečević et al., 2004; Zečević et al., 2008; Branković et al., 2015; Knežević et al.2020).

By analysis of variance established significant differences among varieties, between year and in interaction genotype/year for stem height in both species wheat and triticale (table 2).

Table 2. Analysis of variance for stem height in wheat and triticale

Source of variance		Wheat					Triticale				
	df	*SS*	*MS*	*F*	*Lsd0,05*	*Lsd0,01*	*SS*	*MS*	*F*	*Lsd0,05*	*Lsd0,01*
Genotypes (G)	2	68545.856	34272.928	482.598	1.423	1.870	9197.205	4598.602	37.190	1.877	2.468
Year (Y)	2	2782.229	1391.114	19.588	1.423	1.870	32763.906	16381.953	132.486	1.877	2.468
Genotype/Year	4	3068.740	767.185	10.803	2.464	3.240	8093.521	2023.380	16.364	3.251	4.275
Error	1611	114409.236	71.018				199201.487	123.651			
Total	1620	9556883.496					16828945.17				

In experiment with application insecticides with active chemical substance: deltamethrin and bifentrin on wheat and triticale were established significant decrease of stem height in comparison to control variant, depends of variety, treatment, year and their interaction. In comparison with control variant, treatment by deltamethrin caused the highest decreasing of stem height for 2.99% in wheat Belija and 0.65% in triticala Agrounija, while treatment by bifentrin influenced the highest decreasing of stem height for 3.75% in wheat Belija and for 1.20% in triticala Agrounija. However, increasing of stem height for 0.1% was recorded in triticale Admiral treated by bifentrin (table 3).

Table 3. Variation of stem height in wheat and triticale varieties treated by insecticides

Wheat						Triticale					
Variety	Treatman	2019	2020	2021	Average	Variety	**Treatman**	2019	2020	2021	Average
		$\bar{X}$ (cm)	$\bar{X}$ (cm)	$\bar{X}$ (cm)	**$\bar{X}$ (cm)**			**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**	**$\bar{X}$ (cm)**
Aurelia	Control	65.67^{e}	72.55^{cd}	66.69^{d}	68.30^{d}	Admiral	Control	96.94^{ab}	102.58^{c}	98.48^{c}	99.33^{c}
	Decis	65.00^{e}	69.99^{d}	68.34^{d}	67.78^{d}		Decis	98.07^{a}	99.29^{c}	99.65b^{c}	99.00^{c}
	Bifentrin	68.91^{de}	62.24^{e}	70.08^{d}	67.08^{d}		Bifentrin	96.81^{ab}	100.04^{c}	101.49^{abc}	99.45^{c}
Belija	Control	87.38^{a}	86.04^{a}	83.14^{a}	85.52^{a}	Agrounija	Control	89.11^{c}	112.75^{a}	99.14^{c}	100.33^{bc}
	Decis	78.66^{b}	85.83^{a}	84.38^{a}	82.96^{ab}		Decis	95.99^{ab}	104.76^{bc}	98.29^{c}	99.68^{c}
	Bifentrin	85.22^{a}	79.70^{b}	82.02^{ab}	82.31^{b}		Bifentrin	91.41^{bc}	104.85^{bc}	101.11^{abc}	99.12^{c}
Zemunska Rosa	Control	70.89^{cd}	84.30^{a}	77.48^{bc}	77.56^{c}	Zenit	Control	97.75^{a}	113.91^{a}	103.42^{abc}	105.03^{a}
	Decis	72.34^{c}	76.30^{bc}	80.21^{ab}	76.28^{c}		Decis	96.52^{ab}	110.26^{ab}	106.09^{ab}	104.29^{ab}
	Bifentrin	74.11^{c}	78.91^{b}	76.85^{c}	76.62^{c}		Bifentrin	96.27^{ab}	109.27^{ab}	107.23^{a}	104.26^{ab}
Average	Control	74.64	80.96	75.77	77.13	Average	Control	**94.60**	**109.75**	**100.35**	**101.56**
Average	Desis	72.00	77.37	77.64	75.67	Average	Desis	**96.86**	**104.77**	**101.34**	**100.99**
Average	Bifentrin	76.08	73.62	76.32	75.34	Average	Bifentrin	**94.83**	**104.72**	**103.28**	**100.94**
Total average		**74.24**	**77.32**	**76.58**	**76.04**	Total average		**95.43**	**106.41**	**101.65**	**101.17**
Lsd $_{0.05}$	Genotype x Treatman	3.9139	3.7487	4.5415	2.3534	Lsd $_{0.05}$	Genotype x Treatman	5.4820	5.0356	6.1730	3.2185
Lsd $_{0.01}$		5.1506	4.9332	5.9764	3.0942	Lsd $_{0.01}$		7.2140	6.6266	8.1235	4.2317
Lsd $_{0.05}$	Year	1.3587	Genotype x Treatman x Year	Lsd $_{0.05}$	4.0762	Lsd $_{0.05}$	Year	1.8582	Genotype x Treatman x Year	Lsd $_{0.05}$	5.5747
Lsd $_{0.01}$		1.7864		Lsd $_{0.01}$	5.3593	Lsd $_{0.01}$		2.4432		Lsd $_{0.01}$	7.3296

On the base of analysis of variance were established that plant height varied significantly ($p < 0.01$) with the variety, year, and insecticide. Interaction year/insecticides were significant, and variety/insecticides were significant, and interaction variety/insecticides/year, was significant only in wheat Belija (table 4).

Table 4. Analysis of variance for stem height in wheat and triticale treated by insecticides

Source of variance		Wheat					Triticale				
	df	*SS*	*MS*	*F*	*Lsd$_{0,05}$*	*Lsd$_{0,01}$*	*SS*	*MS*	*F*	*Lsd$_{0,05}$*	*Lsd$_{0,01}$*
Genotype (G)	2	68545.856	34272.928	529.07	1.358	1.786	9197.205	4598.602	37.953	1.8582	2.4432
Treatman (T)	2	974.439	487.219	7.521	1.358	1.786	129.751	64.876	0.565	1.8582	2.4432
Year (Y)	2	2782.229	1391.114	21.475	1.358	1.786	32769.906	16381.953	135.205	1.8582	2.4432
Genotype/ Treatman (G/T)	4	351.207	87.802	1.355	2.353	3.094	90.399	22.600	0.187	3.2185	4.2317

Genotype/Year	4	3068.740	767.185	11.843	2.354	3.094	8093.521	2023.380	16.699	3.2185	4.2317
Treatman/Year	4	5758.160	1439.540	22.222	2.354	3.094	4226.049	1056.512	8.750	3.2185	4.2317
Genotype/ Treatman /Year	8	4131.571	516.446	7.972	4.076	5.359	1740.627	217.578	1.496	5.5747	7.3296
Error	1593	103193.858	64.780				193014.661	121.164			
Total	1620	9556883.496					16828945.17				

In similar studies found that application of pesticides have depresive effect on stem height in wheat (Božinović et al., 1997; Varshney et al. 2012**;** Korshunov et al., 2015; Kuznetsov et al., 2020) which might be due to differences in the genetic background of the breeding material and the test environments, phytotoxic effect of applied pesticides (Losert et al., 2016; Babaytseva and Gamberova, 2018; Spolidorio and Lollato, 2019).

Conclusions

Based on the obtained results established that stem height in the studied wheat and triticale varieties was significantly different among varieties in each year and between year. The stem height varied in all three vegetation season which depends on varieties and their response to environmental conditions. In three year study established that stem height in wheat varied from the lowest 67.72 cm in Aurelia and the highest 83.60 cm in Belija variety, while in triticale varied from the lowest 99.26 cm in Admiral and the highest 104.52 cm in Zenit variety. In three years of application insecticides was found that deltamethrin affect decreasing of stem height for 1.0% in Aurelia, 1.65% in Zemunska Rosa and 2.99% in Belija, while bifentrin had greater affect on stem height decreasing in Aurelia (1.78%), Belija (3.78%) and Zemunska Rosa (1.21%). Both insecticides had less effect on decreasing stem height in triticale than in wheat. Deltamethrin influenced decreasing of stem in average for 0.5% and bifentrin for 1.20% in Agrounija and 0.73 in Zenit, while caused increasing stem height for 0.1% in Admiral. Based on the results established that stem height varied significantly with variety, year conditions, insecticides and their interaction.

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PHENOTYPIC VARIABILITY AND SIMILARITY OF NUMBER OF PRODUCTIVE TILLERS IN WHEAT VARIETIES (*TRITICUM AESTIVUM* L.)

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Abstract

Number of productive stem tillers influence on crop density, number of fertile spikes which is directly related to grain yield. Aim of this study is estimation variability of productive tillering of wheat varieties grown under different environmental condition. The 50 wheat varieties are included for investigation, during two years (2015-2017) in experiment which was set up as a randomized block design in three replications on the field in Kraljevo, Serbia. Sparse sowing was performed in order to enable the examined plants to fully manifest their traits. Sixty plants at the full maturity stage (20 replication^{-1}) were used for analysis of number of tillers. The analysis of variance was performed by MSTAT C (5.0 version). Similarity among wheat was analyzed by hierarchical method of Euclidean distance. The results showed significant differences in number of tillers among varieties in both years, estimated by F-test. In average in the first year the smallest number of tillers 7.57 had Evropa 90 while the highest number of tillers (10.15) had Pobeda variety. In second year, the number of tillers varied from the lowest 8.42 in Evropa 90 to the highest 10.33 in Partizanka and Zastava. The similarity with Euclidean distance illustrated on dendogram contained five clusters in first year and six cluster of varieties in second year. The prominent cluster contains different number and composition of varieties with the highest degree of similarity. The differences in average number of productive tillers were determined by genetic and environmental factor as well as by interaction genotype/environment.

Key words: *wheat, variety, tillers, similarity, environment*

Introduction

Considering that the resources of arable land are limited, there is intensive work on the creation of genotypes with higher genetic potential for yield. For improving the genetic potential for yield, breeders need includes work on engineering the architecture of the plant's vegetative (root, stem, leaf) and generative (ear, seed) organs, which will have a greater capacity for increasing seed yield and better quality (Knežević et al., 2018; 2021). In order to achieve this, it is necessary to know the genetic control of traits, components of yield and quality, their mutual

connection in order to obtain the desired combination in the newly created variety ((Branković et al., 2015; Knežević et al., 2006; 2015). Tillering is directly influence to population structure in wheat crop, primarly crop density number of spike m^{-2}, number of grains $plant^{-1}$ (Madić et al., 2006; Xu et al., 2015), the number of fertile spikes, which is a critical component of grain yield (Wang et al., 2019). Among the three stages of tiller development: 1) axillary meristem initiation, 2) axillary bud development, and 3) axillary bud outgrowth. The first stage is determined mainly by genetic factors (Hyles et al., 2017), whereas the third is regulated mainly by environmental factors and management practices (Assuero and Tognetti, 2010; Assuero et al., 2012). Wheat which carry of reduced tillering (e.g., *tin*) genes have shown that inhibition of tillering stimulate the development of deeper roots, increases the tiller number, and increases the formation of large spikes under drought environments (Houshmandfar et al. 2019; 2020).
The number of productive tillers, as well as the total number of tillers, depends on environmental conditions, vegetation area, i.e. density of sowing or crops, mineral nutrition, moisture, light, temperature (Elhani et al., 2007; Kondić et al., 2016; Tilley et al., 2019). Abiotic stress factors at the time of shoot growth can inhibit their formation, and at a later stage their extinction can occur (Xie et al. 2016). Lack of nutrition can have a direct impact on the development and appearance of shoots, as well as the balance of auxin and cytokinin, which can also affect the termination of dormancy of the side buds (Valerio et al., 2009). In the conditions of a larger vegetation area, optimal mineral nutrition and soil moisture, the plants have greater general tillering (Paunovic et al., 2007). Under conditions of water deficit and drought, tillering is reduced, which can be used as a selection criterion, especially in a drought breeding program (Mitchell et al., 2006).
The aim of the work is to determine the potential of productive tillering of the stem plant in divergent genotypes of winter wheat varieties grown in different agro-ecological conditions.

Materials and Methods

In this study of productive tillering in wheat were included 50 varieties. Selected wheat genotypes were sown in experiment which was set up as a randomized block design in three replications, on plots size 1 m^2 on the field in Kraljevo, Serbia in two growing seasons (2015/16 and 2016/17). The seeds of varieties were sown at the distance of 0.10 m in rows of 1.0 m length among which was the distance of 0.2 m. For analysis of number of tillers, were used 60 plants in full maturity stage (20 plants per replication). Using the program MSTAT C 5.0 version the analysis of variance was performed according to a random block system with one factor and significant differences were estimated by F-test values and tested by test value of $LSD_{0.05}$ and $LSD_{0.01}$. Similarity among wheat analyzed by hierarchical method of Euclidean distance.

Weather conditions

The average temperature was 9.96 °C and total amount of precipitation was 651mm in first year was higher than in the second year 2016/17 in which average temperature was 8.74 °C and total amount of precipitation was 523 mm, and than in ten year periods recorded temperature 8.50 °C and precipitation 417.8 mm (Table 1). For plants growth in the second year was more favorable regime of temperature and precipitation. In the two months (October-November) the amount of precipitation and average temperature values were similar in both year of experiment favorable for seed germination and development of plants. During the February-April amount of precipitation in the first year (250.5 mm) was higher than in the second (174.0 mm), although the distribution of rainfall was more favorable in the second year experiment (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 ºC	2015/16	11.6	7.3	3.3	-0.1	8.8	7.8	14.1	15.5	21.3	**9.96**	
Temperature ºC	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**	
Precipitatin (mm)	2015/16	56.8	64.0	9.0	86.2	52.7	157.9	39.9	135.9	48.6	72.3	**651.0**
Precipitatin (mm)	2016/17	84.1	77.6	9.4	22.0	35.0	57.0	82.0	100.0	56.0	41.1	**523.1**
2000-2010		61.0	44.3	44.6	30.0	29.9	33.2	52.9	52.6	69.3	46.4	**417.8**

Results and Discussion

The number of productive tillers in the first year of experiment varied in range of 7.57 (Evropa 90) to 10.15 (Pobeda) with average value 8.91 for all 50 varieties, while in second year varied from 8.42 (Evropa 90) to 10.33 (Partizanka and Zastava) with average value 9.48 for 50 varieties (table 2).

Table 2. Variability of number of tillers in wheat varieties

	Cultivars	First year	Second year	Average			First year	Second year	Average
1	Evropa 90	7,57±0,10	8,42±0,18	7,99±0,14	26	Jarebica	9,10±0,17	9,45±0,18	9,28±0,18
2	Dejana	7,92±0,10	8,68±0,14	8,30±0,12	27	Fortuna	8,88±0,16	9,17±0,17	9,03±0,17
3	Sila	8,48±0,13	10,18±0,24	9,33±0,19	28	Sasanka	8,80±0,17	9,47±0,17	9,14±0,17
4	Omega	8,77±0,17	9,20±0,18	8,99±0,18	29	Danica	8,85±0,17	9,55±0,15	9,20±0,16
5	Lasta	8,05±0,12	9,15±0,14	8,60±0,13	30	Somborka	9,27±0,15	9,45±0,16	9,36±0,16
6	Milica	8,42±0,17	9,93±0,27	9,18±0,22	31	Kremna	8,82±0,15	9,00±0,17	8,91±0,16
7	Parizanka	8,37±0,17	10,33±0,25	9,35±0,21	32	KG-75	8,90±0,17	9,78±0,19	9,34±0,18
8	Pobeda	10,15±0,27	10,18±0,26	9,35±0,27	33	Šumadija	8,80±0,20	9,22±0,18	9,01±0,19
9	Dična	9,10±0,20	9,28±0,21	9,19±0,21	34	Levčanka	9,20±0,17	9,15±0,16	9,18±0,17
10	NS Rana 5	9,23±0,24	9,25±0,24	9,24±0,24	35	Oplenka	9,13±0,15	9,28±0,23	9,21±0,19
11	Alfa	10,10±0,27	9,28±0,21	9,70±0,24	36	Gruža	8,63±0,21	9,53±0,23	9,08±0,22
12	Rodna	8,12±0,24	9,30±0,30	8,71±0,27	37	Gružanka	8,70±0,18	9,77±0,23	9,24±0,21

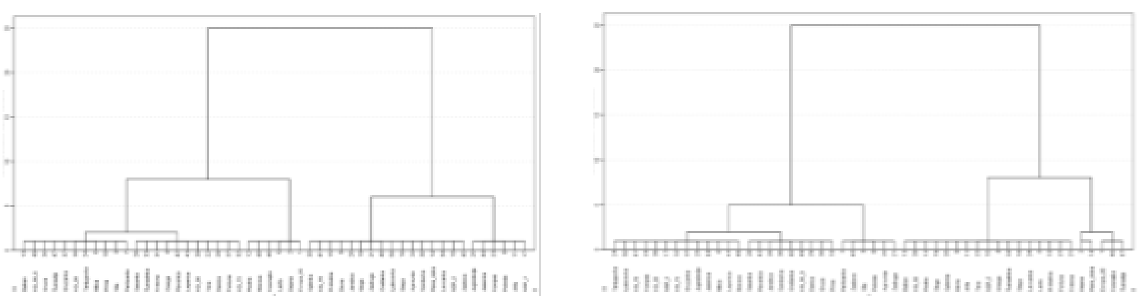
13	Balkan	8,60±0,20	9,32±0,21	8,96±0,21	38	KG-58	8,70±0,18	9,82±0,20	9,26±0,19
14	Rana Niska	9,20±0,27	8,83±0,23	9,02±0,25	39	KG-56	8,95±0,21	9,32±0,20	9,14±0,21
15	Proteinka	9,15±0,23	9,15±0,17	9,15±0,20	40	Orašanka	9,33±0,20	9,63±0,19	9,48±0,20
16	Stepa	9,37±0,23	9,15±0,25	9,26±0,24	41	KG-78	9,12±0,25	9,93±0,25	9,53±0,25
17	NSR-2	9,85±0,31	9,78±0,26	9,86±0,29	42	Ravanica	8,92±0,19	9,47±0,17	9,20±0,18
18	Prima	8,45±0,21	9,58±0,23	9,02±0,22	43	Lepenica	8,93±0,24	9,97±0,23	9,45±0,27
19	Sloga	9,08±0,20	9,28±0,24	9,18±0,23	44	Jasenica	9,62±0,26	9,72±0,27	9,67±0,27
20	Agrounija	9,28±0,27	10,17±0,23	9,73±0,25	45	Zastava	9,23±0,26	10,33±0,29	9,78±0,28
21	Zadruga	9,05±0,17	10,23±0,24	9,64±0,21	46	Kosmajka	8,15±0,14	8,28±0,14	8,22±0,14
22	Tera	8,85±0,17	9,27±0,23	9.06±0,20	47	Šumadija	8,62±0,20	8,05±0,15	8,34±0,18
23	Kompas	9,53±0,15	9,82±0,21	9,68±0,18	48	Morava	8,12±0,12	9,63±0,20	8,88±0,16
24	Tanjugovka	8,70±0,17	9,85±0,26	9,28±0,22	49	KG 56 S	8,60±0,19	9,63±0,20	9,12±0,20
25	Jugoslavija	9,60±0,18	9,70±0,15	9,65±0,17	50	Ljubičevka	9,35±0,28	9,85±0,28	9,60±0,28
	Average						8,91±0,12	9,48±0,21	
	V						17.1	17.1	17.1

The average value of productive tillering in the first year was lower than in the second year of the experiment and 8.91 shoots, while in the second year the average value for all varieties was 9.48 shoots (table 2).
The number of tillers in previous investigation of Serbian wheat was less (Zečević *et al*., 2005; Knežević et al., 2009; Branković et al., 2015) as well as for Italian and Spanish wheat cultivars (Álvaro *et al*., 2008).
The analysis of variance established that the differences between the varieties for the trait productive tillering were significant and highly significant. Differences between years for productive tillering in varieties indicate that there is an influence of external environmental factors on the manifestation of productive tillering. The established significant differences in the average values of number of tillers plant^{-1}, indicating genetic divergence of varieties (table 3).

Table 3. Analysis of variance for productive tillering in wheat in two vegetation season

Source of variance		Vegetation season 2015/16							Vegetation season 2016/17						
	df	*SS*	*MS*	*F*	*F crit*	σ^2	$Lsd_{0,05}$	$Lsd_{0,01}$	*SS*	*MS*	*F*	*F crit*	σ^2	$Lsd_{0,05}$	$Lsd_{0,01}$
Genotypes (G)	49	40.613	0.829	86.388	1.4829	0,273	0.040	0.054	36.608	0.747	44.993	1.482944	0,243	0.053	0.071
Repetitions (R)	2	0.046	0.023	2.419	3.0892				0.009	0.005	0.283	3.089203			
Error	98	0.940	0.010			0,010			1.627	0.017			0,017		
Total	149	41.599	149			0,283			38.245				0,260		

Based on the obtained values for productive tillering, five clusters of mutually similar genotypes are distinguished in the first vegetation season (2015/16). The first cluster contain 11 varieties, second-11 varieties, third-six varieties, fourth-16 and fifth cluster contain six mutually similar varieties. Among those five cluster, the highest similarity was between first and second cluster, with which the third cluster is the most similar. The less degree of similarity showed cluster forth with fifth cluster, and this formed cluster manifested the least similarity with cluster formed from first, second with third cluster (Figure 1).



1) 2)

Figure 1. Similarity of wheat genotypes according to productive tillering in two vegetation season: 1) 2015/16 and 2) 2016/17

In second year (2016/17). the six cluster mutually similar varieties were established. The first cluster contain 13 varieties, second-9 varieties, third-six varieties, fourth-17, fifth-2 and sixth cluster contain three mutually similar varieties. Among those six cluster, the highest similarity was between first and second as well between fifth and sixth cluster. Less degree of similarity, but the highest showed third with formed cluster from first and second, and than less but the highest similarity showed fourth cluster with formed cluster from fifth and sixth cluster. The least similarity manifested the between formed cluster from first, second and third and cluster formed from fourth with fifth and sixth cluster (Figure 2).

In these studies, the examined wheat genotypes showed a high potential for productive tillering, which on average varies between 7.99 and 9.76 shoots in two years. In the study of other wheat genotypes, similar values of variation in productive tillering were found (Zečević et al., 1995), and slightly higher values of tillering were found in the study of Dimitrijević et al. (1996). The obtained high values of the number of productive shoots indicate the potential of tillering, which was manifested in the plants grown under well supplied mineral elements of nutrition, the plants had a large vegetation area due to sowing seed with large distance in row and between the raw, and the distribution of precipitation was favorable.

The crop nutrition with nitrogen and phosphorus fertilizers has a high impact on wheat yield (Takahashi and Anwar, 2007; Knežević et al., 2016; Jelic et al., 2017), while phosphorus deficit results in a lower number of tillers (Valle and Calderini, 2010; Fioreze et al. 2012). The greatest contribution to the increase in the number of productive tillers have soil moisture, mineral nutrition and accessibility of mineral elements for absorption, temperature, light and process of photosynthesis and reutilization and translocation of organic matter (Elhani et al., 2007)).

Conclusions

Based on the obtained results, it was established that the number of productive tillers varied in the analyzed wheat genotypes that were grown in two vegetation season which characterized different weather conditions. The variation of tillering in the same variety in two years shows the genotype's response to different environmental conditions. The average value of productive tillering for all genotypes in the second year was 9.48 tillers , which was higher than in the first year, 8.91 tillers. The Zastava varitey had the highest average value of productive budding for both years with 9.78 tillers, although in the second year Zastava and Partizanka had the highest number 10.22 of productive tillers. The differences between genotypes were significant and highly significant for productive tillering. Genetic factors, environmental factors and genotype/environment interaction had an influence on the manifestation of the studied traits.

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PROPAGATION OF ZELKOVA CARPINIFOLIA (PALL.) DIPPEL. BY SOFTWOOD AND HEEL CUTTINGS

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Abstract

Zelkova carpinifolia (Caucasian elm, Caucasian zelkova) is highly valued ornamental tree, with unusually short and wide trunk and attractive leaves that turn golden orange in the autumn. Caucasian elm is native to Armenia, Georgia and Turkey, but it is not invasive in Europe and it is more resistant to Dutch elm disease than native elms. Caucasian zelkova can be propagated by seed or vegetatively by cuttings, but seed germination is low and vegetative propagation is the more suitable propagation method. There are reports regarding *Z. carpinifolia* propagation using softwood cuttings with basal part containing a small section of the previous season wood (heel cuttings). The aim of this study was to evaluate possibility of using softwood cuttings with current season wood only (without heel). The cuttings were treated with 50 ppm IBA (Indole-3-butyric acid) solution for 24 h or with 2500 ppm IBA for 5 seconds (quick-dip method) and rooted under intermittent mist system. The highest rooting rate was obtained with softwood cuttings without heel treated with 2500 ppm IBA (50.5%), while rooting percentage of heel cuttings was lower (44%) after the same quick-dip treatment. However, rooting percentage of heel cuttings was higher (48%) after treatment with 50 ppm IBA compared to softwood cuttings without heel (33%). Control treatment for both types of cuttings resulted in very low germination rate indicating that auxine is necessary for propagation of Caucasian zelkova.

Keywords: *Caucasian zelkova, Caucasian elm, softwood cuttings, heel cuttings, quick-dip method.*

Introduction

Zelkova carpinifolia (Caucasian elm, Caucasian zelkova) is a long-lived, shadow-tolerant and wind-resistant ornamental deciduous tree, with short and wide trunk and attractive leaves that become golden orange in the autumn. Caucasian zelkova can grow on dry stony soils, although it prefers moist humus-rich clay or loamy soils. It is a good timber tree, its wood is rot-resistant and widely used in construction. Caucasian elm is native to Armenia, Georgia, Azerbaijan, Iran and Turkey, but it is cultivated worldwide and it is not recorded as an invasive species (Vukićević, 1996; Glen and van Wyk, 2016). Besides, it is more resistant to Dutch elm disease than native elms and Grbić *et al*. (2012) recommends that Caucasian zelkova should be included in the Green list of alien non-invasive species in Serbia, as a species suitable for wide use in urban spaces in Serbia in the conditions of climate changes. Caucasian zelkova is often propagated by seed but germination rate is low (Kozlowski and Gratzfeld, 2013), and vegetative propagation is the more suitable propagation method enabling to preserve characteristics of mother plants and to obtain uniform plants.

For this reason, selected elite trees of Caucasian zelkova well adapted to a urban conditions in Belgrade were propagated by heel cuttings (softwood cuttings with basal part containing a small section of the previous season wood) placed under low polyethylene tunnel, or directly covered with a thin polyethylene film (20 mm) without any supporting construction (Grbić *et al*., 2012). The aim of our study was to evaluate the possibility of using softwood cuttings with current season wood only (without heel) and to perform rooting under intermittent mist instead of polyethylene cover.

Material and Methods

The cuttings were collected on June 19, from the same Caucasian zelkova trees as in the research conducted by (Grbić *et al*., 2012). Two types of cuttings were taken: softwood cuttings with current season wood only (without heel) and softwood cuttings with heel. The leaf laminas of the cuttings were cut in half to reduce transpiration and the base of cuttings was wounded 0.5 cm deep. The cuttings were treated with Benlate® solution (600 mg/L) and after that, they were treated with 50 ppm IBA (Indole-3-butyric acid) water solution for 24 h or with 2500 ppm IBA (diluted in 2% ethanol) for 5 seconds (quick-dip method). The base of cuttings in the control treatment was immersed in distilled water for 24 h. Three replications of 50 cuttings were used per treatment. The rooting was performed in sand, under intermittent mist (10 seconds every 15 minutes) in the greenhouse of the Faculty of Forestry, Belgrade. The state of cuttings (callused, rooted, necrotic or without change) and rooting percentage were determined 8 weeks after sticking. The number and length of primary roots and percentage of rooted cuttings forming secondary roots was also measured. The significance of differences between the means was determined by the analysis of variance (ANOVA, $p<0.05$) and the least significant difference (LSD) test.

Results and Discussion

Eight weeks after sticking, cuttings were taken from sand, examined and their state (callused, rooted, necrotic or without change) was recorded (Table 1). The cutting type and treatment influenced the rooting percentage. The auxine treatment was necessary and rooting rate in the control treatment was low for both types of cuttings. Softwood cuttings without heel had higher rooting rate (50.5%) after treatment with IBA applied using quick-dip method than after treatment of cuttings in the water solution of 50 ppm IBA (33.0%). However, IBA treatments did not significantly influence the rooting rate of softwood cuttings with heel, because obtained differences were not statistically significant, although 50 ppm IBA water solution treatment resulted in slightly higher rooting, 48.0% compared to 44.0%.

Table 1. State of cuttings 8 weeks after sticking

Treatment	Callusing (%)	Unchanged (%)	Necrotic (%)	Rooted (%)
Without heel – control	72.5^{a}	2.0^{b}	22.0^{ab}	3.5^{c}
Without heel – 50 ppm	43.5^{b}	6.0^{b}	17.5^{b}	33.0^{b}
Without heel – 2500 ppm	32.0^{b}	10.5^{a}	7.0^{c}	50.5^{a}
With heel – control	80.7^{a}	2.0^{b}	2.0^{c}	7.3^{c}
With heel – 50 ppm	39.3^{b}	4.7^{b}	8.0^{c}	48.0^{ab}
With heel – 2500 ppm	26.7^{c}	2.7^{b}	26.7^{a}	44.0^{ab}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test

Cutting type and way of IBA application did not affect the number and the length of primary roots, but primary roots were considerably shorter in control treatments indicating that auxine is important for root development (Table 2). Similarly, cuttings in the control treatments did not form secondary roots, while a large number (69.7-86.1%) of rooted cuttings treated with IBA developed secondary roots.

Table 2. The number and length of primary roots and occurrence of secondary roots

Treatment	No of primary roots	Length of primary roots (cm)	Occurrence of secondary roots* (%)
Without heel – control	1.6^{b}	4.7^{b}	0.0^{c}
Without heel – 50 ppm	2.1^{a}	15.0^{a}	69.7^{b}
Without heel – 2500 ppm	2.0^{ab}	17.2^{a}	74.3^{b}
With heel – control	1.4^{b}	6.0^{b}	0.0^{c}
With heel – 50 ppm	2.2^{a}	18.6^{a}	86.1^{a}
With heel – 2500 ppm	1.9^{ab}	17.0^{a}	81.8^{a}

Values followed by different letters are significantly different at the $P < 0.05$ level according to the LSD test
* the percentage of rooted cuttings forming secondary roots

Obtained results differ from results obtained in the research conducted by Grbić *et al*. (2012) where the rooting rate was higher, even in the control treatments. Considering that the same trees were used as a cutting source in our research and in the research performed by Grbić *et al.* (2012), and also that cuttings with heels were treated with the same IBA treatments in the both studies, we can conclude that rooting conditions significantly influenced rooting rate. Grbić *et al*. (2012) obtained significantly higher rooting (92% and 85.3%) of cuttings covered directly with a thin polyethylene film compared to cuttings rooted in low tunnel (76.7% and 79.3%) while in the our research the rooting rate of the same cuttings type rooted under intermittent mist was 48% and 44%.

Besides, cuttings in our research were rooted in sand, while Grbić *et al*. (2012) used a mixture of peat and sand (2:1). Also, the wounding of cuttings can stimulate cell division and production of root primordia, but also it can promote callus production and excess callusing may hinder rooting in some species (Hartmann *et al*., 2014), and this can explain the high percentage of callused cuttings in our experiment.

The cuttings both with and without heel treated with IBA using quick-dip method had the higher rooting rate in our research compared to cuttings treated with 50 ppm IBA which contradicts the results obtained by Grbić *et al*. (2012). It is possible that time of taking cuttings and endogenous hormone levels in cuttings can also influence response of cuttings to auxine addition and thus the rooting rate was different (Marković *et al*., 2014). Dirr and Frett (1983) propagated *Z. serrata* using semi-hardwood cuttings reporting rooting percentage of 62% after quick-dip treatment with 16000 IBA, and Li *et al*. (2015) propagated also *Z. serrata*, but they used softwood and hardwood cuttings, obtaining the best rooting percentage of 40.6% for softwood cuttings treated with 3000 ppm IBA applied as a powder and also rooting percentage of 40.6% for hardwood cuttings treated with 3000 ppm K-IBA (4-3-Indolyl- butyric acid Potassium salt) using a quick-dip method. This indicates that vegetative propagation of Caucasian zelkova can be further investigated using different cuttings types and different auxine concentrations and application methods.

Conclusions

Z. carpinifolia is an ornamental tree which should be planted in Serbia more often. Caucasian zelkova trees which are well adapted to Serbian conditions can be successfully propagated by softwood cuttings (with or without heel), rooting should be performed in a mixture of peat and sand (2:1) under polyethylene film cover, and auxine treatment is necessary for successful rooting. Wounding of cuttings is not recommended. Propagation by semi-hardwood and hardwood cuttings should be further researched, as well as an effect of different auxine concentrations and application methods on rooting.

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EFFECTS OF DROUGHT STRESS ON PURSLANE (*PORTULACA OLERACEA*)

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Abstract

Purslane (*Portulaca oleracea* L.) is a herbaceous fleshy plant, which is relatively more drought tolerant than other species. Drought is one of the most important environmental stresses that influence the metabolism and growth of plants. The objective of this study was to determine the influence of drought stress on the chemical composition, yield and some quality parameters of purslane (*Portulaca oleracea*). The experiment was conducted in an unheated and 110 m^2 plastic polyethylene (PE) covered greenhouse of the Ege University- Bayındır Vocational Training School (38 ° 12 '09.9 ″ N, 27 ° 40' 20.8 ″ E) in Türkiye during the summer of 2021. The results showed that purslane yield was affected by the treatments, the highest (888 g pot^{-1}) being in the Control (I_1). Findings also indicated that the yield obtained from $I_{0.8}$ irrigation dose was high and statistically in the same group with the Control. However, under water stress conditions ($I_{0.6}$ and $I_{0.4}$), the yield decreased drastically as 607 and 537g pot^{-1} respectively. The drought conditions/treatments in this respect had significant effects on yield, plant height, total plant number, thin stem plant number, and thick stem plant number. Chemical analysis (dry matter basis) of leaves showed insignificant differences among the different drought treatments for all the characteristics measured. Only leaf and stem K contents decreased as the water stress increased.

Key words: *Drought, purslane, yield, stress, plant nutrition.*

Introduction

Purslane (*Portulaca oleracea* L.), a wild edible plant endemic to the Mediterranean area (Gonnella et al.,2010; Ceccanti et al.,2018) has been proposed as a new ready-to-eat vegetable product (Rinaldi et al.,2010). This species shows interesting nutritional traits, with particular reference to its high content of several healthy compounds such as mineral elements and ω-3 fatty acids, especially α-linoleic acid (Ceccanti et al., 2018; Petropoulos et al.,2016). Purslane belongs to the family Portulacaceae, which is widely distributed all over the world, and grows well in diverse geographical environments (D’Andrea et al., 2014).
All of the above characteristics made purslane flourisher more than many other crops. Alam et al. (2014) screened the most salt-tolerant purslane accessions prioritizing the utilization as a resource of vegetable nutrients and commercial cultivation for saline agriculture and sustainable development. Yang et al. (2012) investigated the mechanisms underlying purslane’s tolerance to high temperature and high humidity stresses, and the results suggested that purslane deployed multiple strategies to cope with combined stresses.

Purslane (*Portulaca oleracea* L.) is a herbaceous fleshy plant, which is relatively more tolerant to drought than other species. So far, there is little information on the combined response of this plant to drought (Chauhan and Johnson, 2009).
Drought is defined as the shortage of rain in the plant environment (Rahdari and Hoseini, 2012), which leads to damage to plants. The growth and development of plants were severely inhibited under drought-stress conditions. To date, the effects of drought stress on plants have been well-documented in many plants, but the responses to rehydration after drought stress and related mechanism are still insufficient (Liu et al., 2010; Luo et al., 2014; Xu et al., 2010). The water availability is spatially and temporally heterogeneous, especially in arid and semi-arid ecosystems (Huxman et al., 2004). Consequently, plants are exposed to drought repeatedly during their life cycle. It is important to decipher the mechanisms that trigger off physiological responses to drought stress and rehydration. It is known that the vegetative growth of stressed plants can recover after rewatering (Ortuno˜ et al., 2005). The response of several crops, trees, mosses and cut flowers to drought and rehydration were partially documented (Filippou et al., 2011; Liu et al., 2010, 2013; Luo et al., 2011).
The objectives of the present study were (a) to identify the optimal growth cycle duration for maximum yield performance of purslane with high quality parameters and (b) to assess the effects of water stress strategies on mineral nutrient elements in purslane leaves.

Materials and methods

Plant material and growth conditions

The experiment was conducted in an unheated and 110 m^2 plastic polyethylene (PE) covered greenhouse of the Ege University- Bayındır Vocational Training School (38 ° 12 '09.9 ″ N, 27 ° 40' 20.8 ″ E) in Türkiye during the summer of 2021 (Fig.1).
Purslane was grown at four different drought levels. $I_{1.0}$ -Control (irrigation was done as 100% of the total evaporation from the class A evaporation pan.), $I_{0.8}$ (80% of water given to Control plants), $I_{0.6}$ (60% of water given in Control), $I_{0.4}$ (40% of water given in Control). In the experiment, purslane seeds were sown in 75x25x16 cm balcony-type PE pots filled with soil. Purslane seeds were sown as 0.6 gr m^{-2} and 10-8-10 kg/da NPK fertilizers were applied. Sampling was done 39 days after germination from full-grown leaves and stems in order to examine effects of drought on purslane (Fig.1B)

Greenhouse temperature and humidity values
Greenhouse indoor temperature and relative humidity values were measured with sensors (Delta-T Devices) and recorded with data loggers (HOBO-Datalogger tep/RH/2 ext channels U12-013 manufactured by Onset computer cooperation). The greenhouse temperatures were recorded between 12 -42 °C during the growing period in the greenhouse and the relative humidity between 24-91%.

Average light intensity measurement values
Radiation energy in the greenhouse were measured manually with the Lux Meter HP-8818 device between the dates 21.06.2021 and 30.07.2021 every half an hour from 10:00 hours to 16:00 when the daylight is most efficient. The averages of the obtained values were taken and evaluated with Lux x100 unit. According to measurements (Lux Meter HP-818) the minimum

and maximum values were recorded between 107 and 405 Lux x100 during the purslane vegetation.

Chemical Analysis of Plant Materials

All plants from each pot were harvested and weighed to evaluate the fresh biomass yield (FBY) and results are given as gm^{-2} in Table 2. Some additional parameters were measured such as DW, plant height, thin plant stem number, thick plant number, thin stem diameter, and some nutrient elements (Kacar 1995). Leaf samples were taken at the harvesting time (39 days from planting), dried at 65 °C until constant weight, ground and digested by HNO_3: H_2O_2 (6:3 mL) in a microwave oven and determined by ICP-OES (Inductively Coupled Plasma-Mass Spectrometer) (Chapman and Pratt 1961; Geraldson, Klacan, and Lorenz 1973; Kacar and Inal 2014; Roorda Van Eysinga and Smilde 1981; Rodushkin, Ruth, and Huhtasaari 1999; Zarcinas, Cartwright, and Spouncer 1987).

A B

Figure 1. Purslane development (A-28.07.2021) and harvest period (B-30.07.2021)

Soil Analysis of purslane pot trial

The pH of the soil was slightly alkaline in reaction, loamy sandy in texture, poor in $CaCO_3$, poor in organic matter and N and had no salt problem. Regarding the plant nutrients, Ca and Mg were found medium, K and Na poor, P sufficient (Table 1).

Table 1. Physical and chemical properties of the experimental soil of purslane.

EC(dS/m)	**pH**		**Org. matter (%)**			**Texture**
0.12	7.79		0.45			Loamy Sand
N (%)		**Available (mg kg^{-1})**				**$CaCO_3$(%)**
0.022	**P**	**K**	**Na**	**Ca**	**Mg**	0.12
	15	75	38	2460	89	

P: Water extractable; K, Ca, Mg, Na: NH_4AOC extractable.

Results and discussion

The preliminary one-year results regarding the nutrient status of purslane plants which were studied via leaf analysis and discussed with water stress conditions are given in Tables 2 and 3.

Table 2. Essential leaf nutrients (macro elements) of purslane (*Portulaca oleracea*).

	%											
Irrigation Dose	**N**		**P**		**K**		**Ca**		**Mg**		**Na**	
	Leaf	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**
I_1(Cont.)	3.86	1.64	0.48b	0.36	1.13	0.33b	0.39	0.19	1.76	1.61	0.39	1.39
$I_{0.8}$	3.96	1.67	0.59a	0.38	0.26	0.34b	0.41	0.21	1.47	1.61	0.37	0.46
$I_{0.6}$	4.00	1.97	0.51b	0.40	0.29	0.33b	0.33	0.16	1.81	1.60	0.38	0.35
$I_{0.4}$	3.17	1.43	0.49b	0.39	0.24	0.23a	0.27	0.25	1.83	1.61	0.38	0.35
LSD $^{0.05}$	**ns**	**n.s**	**0.061***	**n.s**	**n.s**	**0.033***	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**	**n.s**

*P<0.05: Means in the same column followed by different letters are significantly different.
ns: not significant

Table 3. Essential leaf nutrients (micro elements) of purslane (*Portulaca oleracea*).

	(mgkg^{-1})							
Irrigation Doses	**Total-Fe**		**Total-Mn**		**Total-Zn**		**Total-Cu**	
	Leaf	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**
I_1(Cont.)	264	92	361	61	27	3.00	19	5.67
$I_{0.8}$	205	85	343	70	26	3.67	18	6.33
$I_{0.6}$	244	97	409	47	35	2.67	17	6.33
$I_{0.4}$	270	104	255	62	34	2.33	20	6.67
LSD $^{0.05}$	**ns**	**Ns**	**ns**	**ns**	**n.s**	**n.s**	**n.s**	**n.s**

ns: not significant

Table 4. Yield and some quality parameters of purslane (*Portulaca oleracea*).

Irrigation Doses	**Yield (g. pot^{-1})**	**Yield (gm^{-2})**	**%DW (65°C)**	**Plant height (cm)**	**Thin plant stem number**	**Thick plant number**	**Thin stem diameter(mm)**
I_1(Cont.)	888a	4736a	7.15	47a	36c	24a	4.62
$I_{0.8}$	853a	4549a	6.69	54a	46bc	20ab	4.14
$I_{0.6}$	607b	3237b	5.71	37b	66a	20ab	4.00
$I_{0.4}$	537b	2864b	5.57	38b	55ab	17b	4.26
LSD $^{0.05}$	**153****	**816**	**ns**	**7.94****	**12****	**4***	**n.s**

*P<0.05: Means in the same column followed by different letters are significantly different.
**P<0.01: Means in the same column followed by different letters are significantly different.
ns: not significant

Chemical analysis (dry matter basis) of leaves showed insignificant differences among the different drought treatments for all the characteristics measured. Only N and K content of leaves and stem decreased as the water stress increased.

Statistical results of the macro and micro elements of purslane leaves showed not much significant differences with respect to irrigation doses. Nitrogen varied between 3.17 and 4.00 % in the leaves, 1.43 and 1.97 in the stems, and the least leaf N was determined in the $I_{0.4}$ irrigation dose. As a result, water scarcity negatively affected by N uptake.

The phosphorus content of the leaves ranged from 0.48 to 0.59%. In this regard, stem P differed significantly ($P<0.05$) and was found between 0.23 and 0.34%. Leaf K was found between 0.24% and 1.13%, and 0.23% and 0.34% in the stem. The lowest K was measured in the $I_{0.4}$ irrigation dose both in the leaf and stem of purslane. Calcium content of the purslane leaves was between 0.27 and the 0.41% and 0.16 and 0.25% in stem; Mg 1.76 to 1.83% in leaf and 1.60 to1.61% in the stem. (Table 2)
Rouphael et al. (2012) reported that drought affected N and P uptakes, its distribution and use within the plant, and therefore suppresses the plant through growth, development and physiological processes.
Many different studies reported that the decrease in soil moisture content causes a decrease in N and P uptake in plants (Ahmad Waraich et al., 2011; Sardans and Peñuelas, 2012).
With respect to the micronutrients, our findings showed that Fe was between 205 and 270 mg kg^{-1} in leaves and 85 and 104 $mgkg^{-1}$ in the stems. Results of the statistical analyses indicated that microelements were not significantly affected by water stress except the Mn content of the leaves and stems which changed between 255 and 409 mg kg^{-1} and 47 and 70 mgkg-1 respectively. In conclusion, water scarcity is negatively affected the Mn uptake. The least amount of Mn (255 $mgkg^{-1}$) was measured in the $I_{0.4}$ irrigation treatment (Table 4).
According to the variance of analysis of the results with respect to irrigation doses, yield, plant height, thin plant stem number and thick plant number of the purslane differed significantly. On the other hand, DW (65℃) and thin stem diameter were not significantly affected. The Yield of the purslane was measured between 537 and 888 g pot^{-1}, DW% 5.57-7.15, plant height 38-54 cm, thin plant stem number 36-66, thick plant number 17-24 and thin stem diameter 4.00-4.62 mm (Table 4). Findings also indicated that the yield obtained from $I_{0.8}$ irrigation dose was high and statistically in the same group with the Control. However, under water stress conditions ($I_{0.6}$ and $I_{0.4}$), the yield decreased drastically as 607 and 537g pot^{-1} respectively. The drought conditions/treatments in this respect had significant effects on yield, plant height, total plant number, thin stem plant number, and thick stem plant number (Table 4).

Conclusions

The statistical results of this study indicated that the decrease in fresh yield, plant height, thin plant stem number, thick plant number and Thin stem diameter in purslane cultivation was in $I_{0.6}$ and $I_{0.4}$ irrigation applications.
Only nitrogen and potassium elements from plant nutrients decreased as water stress increased.
Purslane used in our experiment is an innovative plant with high adaptability under Mediterranean climatic conditions, high utilization efficiency of natural resources, and a nutritious food feature resistant to salinity and water scarcity.

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EFFECTS OF GIBBERELLIC ACID (GA_3) APPLICATION ON SEED EMERGENCE OF F1 HYBRID FIGS

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Abstract

A high seed emergence rate of F1 hybrid individuals obtained after hybridization in fruit species is a desirable feature. This study was carried out to determine the effect of gibberellic acid on the germination of F1 seeds obtained from ‘Bursa Siyahı’ × Osmaniye02 crossing. In the study, 1000 ppm, 2000 ppm, and 3000 ppm GA_3 applications were applied to hybrid seeds to fresh seeds obtained after fruit harvest. The emergence rate, time to 50% (ET_{50}) of emergence, and mean emergence time (MET) were measured. The application of GA_3 increased the emergence of F1 hybrid seeds. The highest emergence rate was determined in the 2000 ppm GA_3 application (98.33%), whereas the emergence rate was 7.78% in the control. In addition, the T_{50} and MET of the F1 hybrid seeds were the shortest in GA_3 applications compared to control. As a result, GA_3 applications to fresh F1 hybrid seeds obtained immediately after fruit harvest was successful application because they increased the emergence rate and shortened the emergence time.

Keywords: *Ficus carica, F1 hybrid seed, gibberellic acid, seed emergence.*

Introduction

The figs are the dioecious plant that has caprifig (male fig) and edible (female) figs. The edible fig trees produce syconia parthenocarpically or by pollination (Caliskan et al., 2017).
Although figs are widely propagated by cuttings, breeding methods such as hybridization and mutation are needed to obtain new individuals in terms of earliness, parthenocarpy, fruit size, fruit color, and rich chemical characteristics (Flaishman et al., 2008; Çalışkan, 2012; Kılıç et al., 2021). In addition, Caglayan et al (2010) indicated that fig seeds can be used for producing mosaic virus-free seedlings. Currently, there is limited study on the seedling emergence of fig seeds.
Hartmann et al. (2002) reported that seeds are important for obtaining hybrid plants, however, the seed emergence can be affected by some factors such as the seed coat, undeveloped embryo, or chemical inhibitors (Luna et al., 2014). Therefore, gibberellins are widely used to counteract the effect of dormancy in the seeds.
Caliskan et al (2012) stated that the application of GA_3 at 500 ppm or 1000 ppm reduced the time to germination and emergence from the seeds of fig cultivars. However, 500 ppm GA_3 and 1000 ppm GA_3 applications were not sufficient to ensure uniform germination in seeds obtained from our controlled crosses.
This study was carried out to determine the effect of gibberellic acid on the emergence of F1 fig seeds obtained from ‘Bursa Siyahı’ × Osmaniye02 crossing. The ‘Bursa Siyahı’ cultivar with black skin color is the most important fresh fig with high quality in Türkiye. This cultivar has to be pollinated (Smyrna fig) with caprifigs for the fruit set. However, increased labor costs and difficulties in the controlled use of caprifig fruits can cause low yield and fruit quality. To

develop parthenocarpic individuals in the ‘Bursa Siyahı’ cultivar, it is necessary to pollinate with parthenocarpic caprifigs. For this purpose, it was pollinated with Osmaniye02, Turkey's first caprifig that sets fruit without pollination. These results can be used to provide seed emergence in the shortest time and to obtain uniform seedlings in crossbreeding studies in fig.

Material and Methods

The study was conducted on F1 fig seeds obtained from crossing ‘Bursa Black’ × Osmaniye02 in 2020. For this purpose, pollen of Osmaniye02 genotype was obtained in June and stored at -20°C until used in hybridization. The hybridization was carried out in the first week of August, when there was no pollen emergence in caprifigs, by applying a mixture of pollen of Osmaniye02 genotype and pure water containing 1% sucrose to receptive fig fruits with a 5 mL injector.

To obtain hybrid seeds, the fruits at the eating stage were harvested in October. The seeds were extracted from the fruit flesh using water and dried under shade conditions for two days (Caliskan et al., 2012).

Four applications were used for emergency tests. The control, GA_3 at 1000 ppm, 2000 ppm, and 3000 ppm were applied for 24h in Petri dishes. In the emergence experiments, the seeds were sown in plastic trays (container number, 12 x 16; volume 15 cc) stuffed with peat (Klasmann, Germany), and placed under laboratory conditions (minimum of 21°C and maximum temperature of 27°C). The seedling emergence was recorded for 30 days. Seedling emergence was recorded when the hypocotyls raised above the surface of the growing media. Emergence percentages were determined as the average three replicates of 50 seeds. The mean emergence time (MET) was calculated according to Ellis and Roberts (1980):

MET= Σ (t.n)/Σn, where: t is the time in days from 0 to the end of the emergence test, and is the number of emergency seeds on the day t.

Besides, time to 50% (T_{50}) of emergence was investigated as described by Coolbear et al. (1984):

$$T_{50} = ti + \left[\frac{\frac{(N+1)}{2} - ni}{nj - ni} \right] (tj - ti)$$

where: N is the final number of seeds germinated, and ni and nj are the total number of seeds emergence by adjacent counts at time ti and tj, where ni, < (N +1)/2 < nj.

The percentage values were transformed by the angle transformation before submitting the data to the analysis of variance. Differences among means were analyzed by the LSD method ($p < 0.05$) using the SAS program (SAS, 2005).

Results and Discussion

The data showed that the effects of GA_3treatments on seedling emergences, MET, and T50 in hybrid seeds were statistically significant ($p < 0.05$). The highest emergence percentage was found in 2000 ppm GA_3 application (98.33%) on the hybrid seeds. The lowest emergence percentage was determined in control seeds (7.78%). These results indicated that GA_3 applications had a positive effect on hybrids fig seeds (Figure 1). The results of the very low germination rate in control seeds were consistent with the findings of Caliskan et al. (2012), who reported that the emergence of fig seeds after sowing without any application was low due to after-ripening. However, the emergence values were lower than those of Caliskan et al. (2012). This result may be due to the higher heterozygous effect of hybrid seeds. Also, similar to our results, the previous studies on other species reported that gibberellin applications can stimulate germination and shorten after-ripening periods (Bojovic, 2010; Hauvermale et al., 2015; Çalışkan et al., 2020).

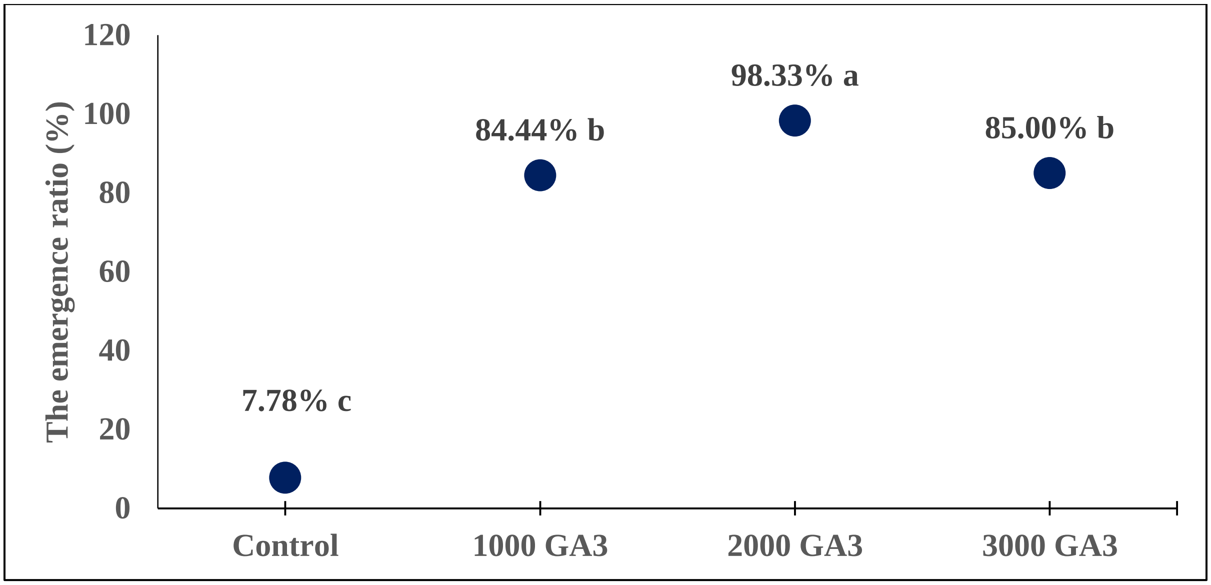

Figure 2. The effect of GA_3 applications on mean emergence percentages of hybrid fig seeds

The MET of hybrid seeds had the shortest in GA_3 applications (Figure 2). The shortest MGT was obtained from 3000 ppm, 2000 ppm, and 1000 ppm GA_3 applications (12.42 days, 12.67 days, and 13.24 days, respectively). The longest MET was found in the control seeds (16.78 days).

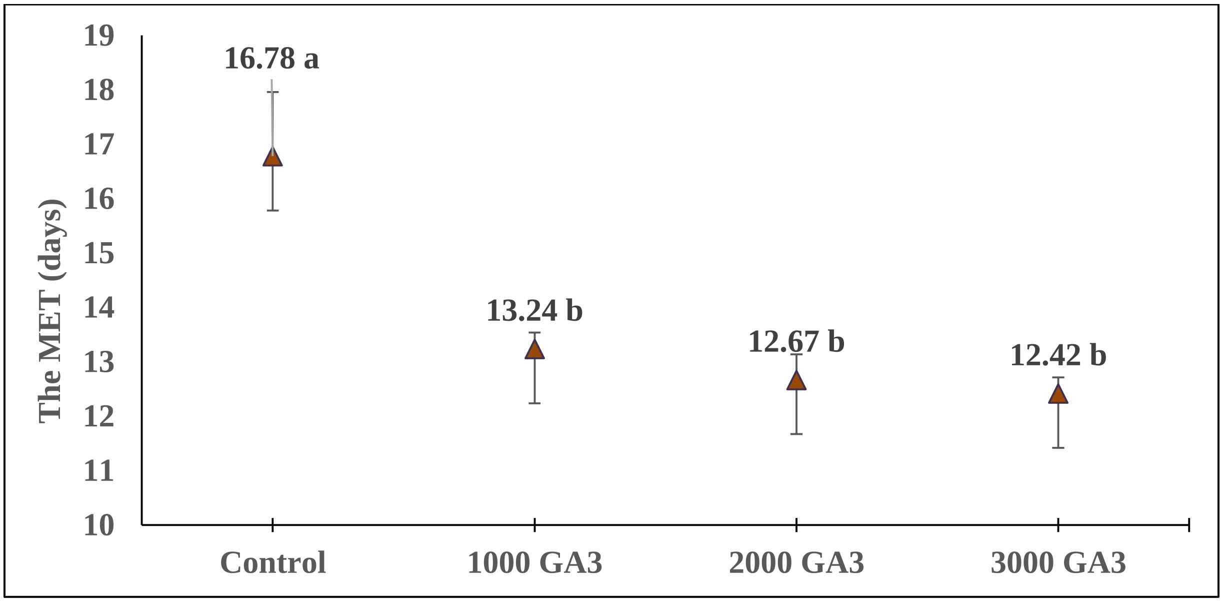

Figure 2. The effects of GA_3 applications on the mean emergence time of hybrid fig seeds

The data showed that the GA_3 applications on hybrid fig seeds reduced MET to 4-5 days compared to the control seeds. These results were similar to those of Caliskan et al (2012), who reported that the MET values of fig seeds were significantly reduced by GA_3 application.
The 50% of emergence time (T_{50}) hybrids seeds were presented in Figure 3. The shortest T_{50} was obtained from 3000 ppm GA_3 (13 days), followed by 1000 ppm GA_3 (15 days) and 2000 ppm GA_3 (16 days). The T_{50} was the longest in control seeds (17 days). These results displayed that 3000 ppm GA_3 application on hybrid fig seeds reduced T_{50} to 4 days compared to the control seeds. Similarly, Caliskan et al. (2012) reported that the shortest 50% of emergence time emergence was found in 500 ppm and 1000 ppm GA_3 applications. In addition, El-Refaey and El-Dengawy (2005) indicated that GA_3 application in loquat seeds increased emergence percentage, and reduced T50.

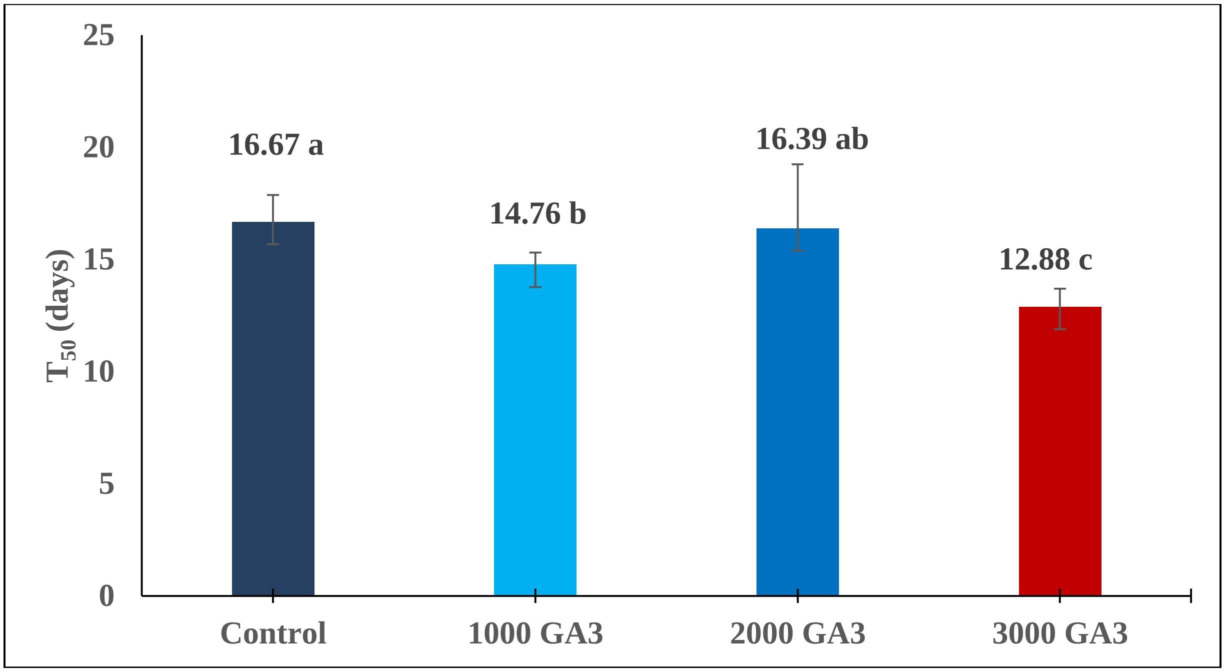

Figure 3. The effects of GA_3 applications on T50 values of hybrid fig seeds

Conclusions

In crossbreeding breeding studies, it is very critical to obtain the desired number and homogeneity of hybrid individuals that make up the starting material. Since the fig is a subtropical fruit species, its seeds do not show a serious dormancy. However, when no application is made to the fig seeds, they germinate in a long time and an inhomogeneous manner. Therefore, this study showed that GA_3 application was very successful for short time and the sufficient number of seed emergence in breeding studies to be done with seed sources such as crossbreeding breeding. The GA_3 applications increased the emergence rate, the MET, and T50 values of the hybrid seedlings, and provided a homogeneous emergency. In this study, 3000 ppm and 2000 ppm GA_3 applications had the highest seed emergence values.

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APPLICATION TECHNIQUES PESTICIDE IN IRAQ

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Abstract

Although the Iraqi economy is mainly based on oil, the fertile lands with the Tigris and Euphrates rivers and irrigation facilities make the agricultural sector important. Only half of the country 8 million hectares of agricultural land can be cultivated. Agriculture is the second main sector in the country, where wheat, barley, rice, apples and dates are grown as the main products. In addition to the climatic and geographical conditions in Iraq, the lack of technical knowledge and experience in the use of agricultural tools and machinery limits agricultural activities. Insufficient agricultural mechanization practices are also known among the reasons for the low yield values in Iraq. In terms of its potential agriculture is seen as an important sector open to development in every field in the country. Especially, studies aimed at increasing efficiency are increasing their importance day by day. Among these research areas, plant protection machines come to the fore. Field and fruit garden sprayers are generally used in fields and fruit gardens in Iraq. However, due to their low cost, knapsack sprayers are preferred intensively especially in fruit and vegetable gardens. In order to increase the yield in agricultural products, researches are carried out to determine pesticide losses in different nozzle types in sprayers. It is seen that there is a great deal of interest in research on the determination of application height spray pressure, volume median diameter in knapsack sprayers used in fruit and vegetable gardens. According to the results of the research conducted in Iraq, it was concluded that detailed and comprehensive studies were needed to determine the applicability of new techniques developed in the field of pesticide application in the world.

Keywords: *Iraq, pesticide applications, sprayer, knapsack sprayer, drift.*

Introduction

Iraq a low middle income oil rich country. Economic growth averaged 7.1 percent over the past five years, compared to 4.5 percent in the Arab world as a whole. Relationships to Education, Health and Higher Life from the Arab Average (World Bank, 2014). However, conflict despite these difficulties of mismanagement, the country has great potential for development (Figure 1).
Actually, Iraq the National Development Plan (NDP) sets out and targets ambitious targets for the period from 2013 to 2017:
1) Diversify the economy and accelerate growth in promising sectors such as industry and energy, agricultural and tourism
2) Make income generation and poverty reduction a central goal for national development
3) Strengthening the capacity of women and youth in particular to contribute to the workforce Market and society (Iraq, Ministry of Planning 2013, 27 Iraqi National Development Plan (2013-2017).

Figure 1. General situation in Iraq

Agriculture is one of the key sectors identified to accelerate non-oil growth, increase incomes and improve income distribution and gender equality (Table 1). Crop yields in Iraq today are low by any international comparison (FAO 2012). This is partly because of protracted wars, internal conflicts, sanctions, droughts and deteriorating infrastructure for input production, research and extension services (Bishay 2003; World Bank 2014b).
Pesticide use has increased many fields over the past few decades.
Approximately 2.54 billion tons of pesticides are used worldwide each year.
The main reasons for pesticide use are to increase efficiency and reduce yield losses through protection.

Table 1. Economic structure of Iraq

Activity	Share in GDP	Labor	Share in exports	Exports intensity	Share in imports	Import penetration
Agriculture	9.7	62.8	0.1	0.2	14.0	12.7
Cereals	4.1	80.7	0.0	0.1	5.1	13.0
Fruits & vegetables	3.3	51.0	0.1	0.8	3.8	11.3
Other crops	1.7	51.3	0.0	0.0	2.4	13.7
Livestock	0.6	36.2	0.0	0.0	2.6	13.3
Industry	46.2	11.0	99.9	53.1	85.9	39.0
Mining	38.1	0.7	98.2	92.0	0.1	0.5
Food processing	0.3	60.1	0.0	0.0	13.3	20.4
Services	44.0	47.4	0.0	0.0	0.1	0.1
Total	**100.0**	**32.0**	**100.00**	**22.9**	**00.**	**16.4**

Based on the table it can be seen that there is an increase in the application of tools and machines in agricultural sector or production in particular in recent years in line with the progress in the throughout the country. on the other hand, the agricultural mechanization and machinery sector in Iraq could not obtain the expected level of development. (Table 2).

Table 2. Distribution of tractors number according to providing sources per province (2015)

No.	Iraqi Province	Total Tractors	Number of Tractors Provided by Government	Number of Tractors Provided by Market
1.	Nineveh	5,629	955	4674
2.	Kirkuk	8,295	1663	6632
3.	Diyala	7,495	2026	5469
4.	Salahaddin	8,400	887	7513
5.	Anbar	4,485	198	4287
6.	Baghdad	5,664	413	5251
7.	Wasit	3,168	452	2716
8.	Babil	5,672	425	5247
9.	Karbala	365	89	276
10.	Alnajaf	3,178	331	2847
11.	Aldiwaniyah	2,237	177	2060
12.	Almuthanna	323	48	275
13.	Dhi Qar	664	60	604
14.	Maysan	1,367	56	1311
15.	Basra	145	29	116
Total		57,087	7809	49278
(%)			14%	86%

The small scale of agricultural enterprises in Iraq affects the development of mechanization negatively as it increases operating costs. The lack of development of the agricultural machinery manufacturing sector and its dependence on imports stand as the biggest obstacle for farmers to use modern tools and machinery. The training of farmers on mechanization could not reach the desired levels (Figure 2). Training should be done in this regard. Farmers should be trained to use modern equipment correctly in agricultural mechanization.

Figure 2. The human labor in Iraq

In particular, there are problems in the use of imported equipment, as well as in its maintenance and repair. In addition to the lack of adequate service, the supply of spare parts through imports affects mechanization in enterprises.

Especially in the country, it is seen that there have been developments in the field of harvest in recent years. The use of combine harvesters is becoming more and more common day by day. It is known that there is an intensive harvesting machine in the region of the Dice and Euphrates Rivers, where agricultural production (Table 3) is intense.

Table 3. Distribution of Harvesters Number According to Providing Sources per Province (2015)

No.	Iraqi Province	Total Harvesters	Number of Harvesters Provided by Government	Number of Harvesters Provided by Market
1.	Nineveh	1,792	358	1434
2.	Kirkuk	612	156	456
3.	Diyala	462	118	344
4.	Salahaddin	673	115	558
5.	Anbar	173	14	159
6.	Baghdad	166	32	134
7.	Wasit	416	58	358
8.	Babil	215	52	163
9.	Karbala	6	2	4
10.	Alnajaf	470	34	436
11.	Aldiwaniyah	307	37	270
12.	Almuthanna	21	-	21
13.	Dhi Qar	96	12	84
14.	Maysan	244	31	213
15.	Basra	13	6	7
Total		5,666	1025	4641
(%)			18%	82%

Pesticide Applications in Iraq

As a result of the diversification of plant production in Iraq, plant protection problems have also started to be seen. In the fight against plant protection problems, it has been used in modern equipment with simple and local equipment in recent years.
There are researches in the field of pesticide application in Iraq in accordance with the developments in the world. These researches are generally carried out on the development and dissemination of new machines according to the products.
Different types of sprayers are used in Iraq, taking into account the size of the land and the pattern of the product. Field, orchard and back sprayers are generally used in Iraq (Figure 3).
However, due to their low cost and small land structure, back sprayers are preferred intensively especially in fruit and vegetable orchards.

Figure 3. Type of sprayer in Iraq

In recent years, researches on pesticide applications have been carried out in Iraq. The increase in mechanization practices, especially with the growth of agricultural areas, has made the use of new techniques mandatory. Due to the problems experienced in pesticide applications, especially in large agricultural areas, researches are carried out on new and technologically new sprayers.

As a result of the survey conducted on pesticide applications, a lack of technical knowledge was determined. The survey also showed that there was no or limited information available that would affect the likelihood of retrying these trials under the same conditions applied.
This study recommended the use of electric sprayers equipped with pumps and spray pressure gauges. In addition, it is recommended to use 110 03 flat fan nozzle type at 3 bars (Ombuki, 2018; Urassa, 2015, and Braimoh and Velk, 2006)
In the survey conducted on pesticide applications, the pesticides and effective substances used by the farmers were determined. Approximately 55% of the surveyed farmers used insecticides, 28% fungicides and 17% herbicides.
In the use of pesticides, it was determined that the farmers only applied with their experience according to the label information. It has been determined that there is a lack of technical knowledge in applications. (Ahmed and Majeed, 2020)
The compilation on pesticide applications, on the other hand, is aimed at determining the norm values of the studies carried out in this field in Iraq and determining the amount of pesticide collection in the target area in practice.
At the same time, it has been determined that the researches on the correct application of the spray pressure and spray height have just begun.
It is suggested that research should be done on new nozzle types that have recently found widespread use in the world in pesticide applications. (Subr et al, 2019)

Type of sprayers Used in Iraq

1-Hand sprayers

Generally, 0.5-to-3.5-liter capacity ones are used in homes and small agricultural araes (Figure 4).

Figure 4. Hand sprayers

2-Backpeck sprayers

Back sprayers with a tank volume of 10-12 liters are used in field and grass areas. (Figure 5). Back sprayers are highly preferred in Iraq due to the small size of the lands.

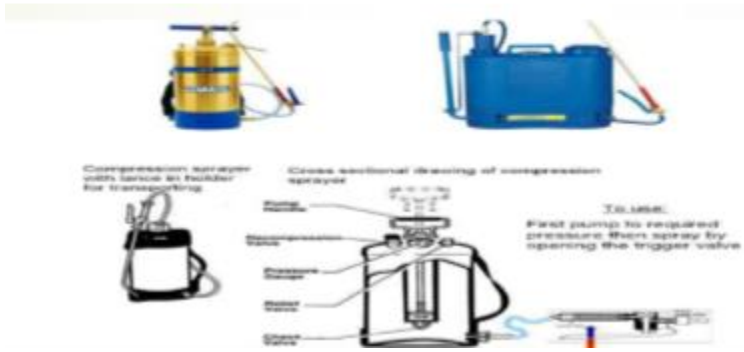

Figure 5. Backpack sprayer

3-Portable sprayers with foot or hand control

Portable sprayers driven by hand or foot pedal are used in gardens and fields with small area (Figure 6).

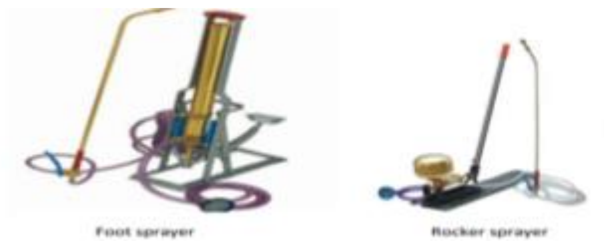

Figure 6. Portable sprayers with foot or hand control

4-Hand operated sprayers

Use of hand sprayers (Figure 7). Hand operated sprayers. This sprayer usage generally in small areas against to harm full. Because most of farmers do not have new type sprayers. Farmers do not have enough purchasing power economically to buy sprayers.

Figure 7. Hand operated sprayers

5-Field and orchard sprayers

In fields and gardens with large areas, tractor-mounted or trailed sprayers are used. (Figure 8).
It is especially used in large agricultural areas in recent years. It is preferred in agricultural areas irrigated by the Tigris and Euphrates rivers.

Figure 8. Field and orchard sprayers.

6-Electrostatic charged sprayers

It has been the subject of research in recent years. (Figure 9). Electrostatic charged sprayers.

Figure 9. Electrostatic charged sprayers.

7-Aerial pesticide applications

Pesticide applications are newly made by using airplanes or UAVs in fields with very large areas. In the last 5-10 years, researches have been carried out on UAV in Iraq. (Figure 10).

Figure 10 Aerial pesticide applications

Conclusions

Technological developments in pesticide applications are not seen in good enough condition. The level of technical knowledge and training on pesticide applications is insufficient.

External dependency on sprayers and spare parts used in pesticide applications should be reduced. The level of technical knowledge and training on pesticide applications is insufficient. External dependency on sprayers and spare parts used in pesticide applications should be reduced. Instead of basic researches, researches should be done on sprayer designs that include technological innovations. Studies on pesticide applications are based on questionnaires and scientific research should be emphasized. Technological innovations in pesticide applications should be researched primarily.

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RECENT ADVANCES IN DIRECT DRILLING TECHNOLOGY FOR SOYBEAN (GLYCINE MAX L.) PRODUCTION

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Abstract

Soybean (*Glycine max* L.) is one of the most important sources of essential protein and due to its capability for nitrogen fixation, also a key part of the crop rotation. However, water is an important limiting factor to growing crops, an increased interest can be seen in soybean global production in recent years. Soil management systems can affect the quality and sustainability of agricultural production since soil tillage modifies surface cover and directly affects the soil structure. However, the shortage of admissible water and higher costs of production due to the increasing costs of energy resources risk the agricultural production which leads to social, economic and environmental difficulties. Direct drilling is widely used as an important agricultural practice since the continuous conservation tillage improves soil properties and modifies impact of weather extremes. Conservation tillage began during the 1960s in the USA and it is expected to be a widespread agricultural practice in the future. The aim of this study was to evaluate the effects of the direct drilling technology on the soybean yield since direct drilling is getting larger year by year. Scientific literature regarding the influence of direct drilling on the soybean yield quality is quite limited, however, direct drilling is an effective strategy for soybean cultivation considering is beneficial to increase the yield and yield stability and has the advantage of high water consumption. To solve the problem of high costs and low production efficiency in soybean production, the preservation of natural resources and environmental stability are crucial.

Keywords: *direct drilling, soybean, Glycine max L., soil tillage, energy saving.*

Introduction

Soybean (*Glycine max* L.) is becoming an increasingly popular crop plant for the high nutritional value of its seeds which contains about 40% of protein and 20% of oils, are used for direct consumption as well as for the production of processed foodstuffs, including infant formulas and edible oil (Gaweda et al. 2014). Soybean is considered a kind of highly efficient nitrogen-fixing crop for improving acidic soil fertility (Yang et al. 2012). Besides fixing the atmospheric nitrogen, this crop has the ability to grow in a range of environments, reduce soil erosion, suppress weeds and to suit inter as well as sequential cropping pattern (Jain et al. 2018). The proper arrangement of plants in appropriate plant density is one of the requirements to achieve high and stable yields during intensive production of soybean. Changing the shape of growing space and row spacing leads to change in microclimate growing conditions (light, relative humidity, aeration) where soybean is very sensitive, especially in the flowering stage (Kolarić et al. 2014). Crop yield is affected by field characteristics and operations as soil strength, compaction, soil water, tillage and residue practices, time of field operations and soil fertility,

which together influence emergence, root development and nutrient availability (Curnoe et al. 2001).
The implementations of farming systems, depending on how they adopted within the agricultural property, may affect crop productivity implemented and physical quality of agricultural soils as density, porosity and the reserves of organic matter (Pereira et al. 2018). The search for a cultivation system which improves the soil structure and makes possible the agricultural management sustainable and the environmental damages are reducing the maximum, is essential to the modern agriculture. In this content, it is necessary to adopt a system of tillage that contributes to improvement the soil quality, increasing the productivity of the cultures and reducing the final cost of production (Queiroz et al. 2011).
In recent years, interest has increased in conservation tillage since this tillage system consists in leaving organic matter on the soil surface or near it and in reducing soil tillage intensity (Ball et al. 1994; Lopez-Fando and Almendros, 1995; Kesik and Blazewicz-Wozniak, 2010). In addition to a significant reduction in production costs, conservation tillage contributes to very beneficial changes in the soil environment (Höppner et al. 1995; Reeves, 1997). Conservation tillage is intended to leave residue on the soil surface, and may include minimum tillage (using disks or chisel plough) or no-tillage (Dam et al. 2005)., minimizes erosion, conserves water within the root zone, and improves soil productivity (Durr et al. 2001). Direct sowing also positively affects the soil structure and water content not only in the topsoil but also at greater depths (Shulan et al. 2009).
This tillage system, which decreases or eliminates tillage operations and maintains greater amount of crop residue on the soil surface, is increasing worldwide and makes possible the quickest and efficient operations. Direct drilling is a type of conservation tillage where the soil is left undisturbed prior to planting, and weed control is accomplished primarily with herbicides (Miller et al. 1998). This practice of management increases the organic matter in the superficial layer, where the crops residues are concentrated by the absence of physical incorporation through soil mobilization (Franzluebbers, 2005; Salvo et al. 2010).
Research has shown that direct drilling system provides greater coverage in surface soil due to crop residue left during the harvesting operation (Diaz-Zorita et al. 2002; Alvarez and Steinbach, 2009). Although its benefit in improving the physical and structural properties of the soil is established (Blanco-Caqui et al. 2012), the question remain about its impact on crop yield (Pereira, 2018).
Conversely, there is few information available about the changes in the physical parameters of the soil and concentration of soil organic carbon affecting crop yield in intensive and non-intensive direct drilling system (Pereira, 2018).One of the options of conservation tillage is direct drilling or sowing which is based on a concept that no tillage is done since the harvest of the previous crop until the sowing of the succeeding crop. Straw of the previous crop remains on the field surface and before sowing is done it is usually necessary to use a non-selective herbicide. Low labour intensity of this system creates, among others, the possibility of growing crops in locations where it is a problem to maintain the optimal sowing time (Gaweda et al. 2014).

Correlation between compaction and soil tillage systems

Soil compaction has serious consequences on crop production and on the environment (Soane and Van Ouwerkerk, 1994). It reduce the productivity of the cultures when the soil reaches high density and low porosity, making difficult the penetration of the roots and the storage of water (Queiroz et al. 2011). The soil compaction affects the soil physics-mechanics properties in bigger or less intensity, most times in the negative form, restricting the development of the roots of the plants, reducing the adsorption of water and nutritious and affecting the yield of the cultures (Blaco-Caqui et al. 2012). Plowing as a basic work, followed by the seedbed preparation and the maintenance works specific to the classical tillage system involve an intense traffic of machines, which leads to soil compaction over time, but it is a significant consumer of energy resources (Serrano et al. 2007; Chetan and Chetan, 2020). As a result of soil compaction, the negative effect on agricultural crop is reflected by reducing the production potential (Rusu et al. 2009; Marchenko et al. 2009; Grigore at al. 2019). Alternative minimum cultivation systems and no cultivation involve reduced invention on the soil, keeping plant debris at the soil surface by at least 50-60% performing the role of soil-protecting mulch. Soil is protected from surface erosion, soil aggregates are stabilized, organic matter and fertility levels will increase, soil compaction and decrease in CO_2 emissions, increased biodiversity (Sabo et al. 2007; Topa et al. 2012; Marin et al. 2015).

Highly compacted soil, particularly in the surface layers, generates inadequate soil physical conditions for seedling emergence. Therefore, the challenge is to attain a suitable seedbed while minimizing traffic-inducted soil compaction, so that the physical properties of the soil do not diminish normal root growth (Botta et al. 2004). Soil strain and compaction degrade soil by decreasing water infiltration and water holding capacity, increasing runoff and erosion, increasing crop production problems, thereby decreasing crop yields and profitability of farming systems (Way et al. 2005).

The choice of the optimal technological variant must take into account the technological properties of the soil: texture, humidity, soil exposure, macro and microclimate, humus content, but also the climatic conditions of the agricultural year and the technological capacity to capitalize on these resources (Stefanic et al. 1997; Szajdak and Rusu, 2016). Direct drilling systems usually have lower traffic intensities than those using conventional tillage, but despite this, after several years of continuous direct drilling yields tend to decrease. This could be the result of increased weed control problems and root diseases as well as gradual increase in soil compaction due to agricultural traffic (Botta et al. 2002).

Correlation between soybean production and direct drilling technology

Scientific literature regarding the influence of direct drilling technology for soybean is quite limited. There are few studies on the effect of direct drilling on yield of soybean cultivars grown worldwide. Rivero et al. (2008) working on soil compaction in Argentina showed that soil under direct sowing is not able to limit subsoil compaction when moderate traffic intensity is applied. Repeated traffic in the same track and using light vehicles produces subsoil compaction. Gaweda et al. (2014) working on investigate the effect of plough tillage and direct seeding on seed yield and some yield components in the climatic conditions of central-eastern Poland showed that soybean seed yield significantly varied depending on the tillage system used. Under conventional tillage, soybean produced higher yield than in the case of direct seeding. Compared to plough tillage, no-tillage significantly reduced plant height and first pod height as well as plant density after emergence and before harvest.

Conclusion

Soil is one of the non-renewable resources that are important to be protected. The quality of soil refers to the capacity in preserving biological fertility and sustainability in plant production.

According to researches, direct seeding mulch-based systems provide an ideal environment for a higher biological activity which leads to increased soybean seed yield. Results showed that by not selecting the appropriate tillage operations that can be carried out in several ways, the compactness of soil can be increased.

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INFLUENCE OF AQUEOUS EXTRACTS FROM BANANA PEEL AND SOYBEAN PLANTS ON SOYBEAN GRAIN YIELD

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Abstract

Foliar aqueous extract application has a positive effect on soybean yield and quality. The aim of this research wos to examine the influence of foliar application of aqueous banana peel and apical soybean plant part extracts on the yield of five soybean varieties that are of different maturity groups. This way, plant material would be used for soybean grain yield increase, and synthetic artificial fertilizer use would be avoided along with environmental polution. The results were processed via tri-factorial experiment variance analysis, and significancy of differences was tested via LSD test. Foliar application of aqueous banana peel extract increased soybean yield in a three-year experiment by 4.07% (annually from 1.33% to 6.62%), and the application of aqueous apical soybean plant part extract by 4.21% (annually from 1.21% to 6.90%). Greater yield increase was recorded during years that were unfavorable for soybean production, with the varieties Rubin (5.69% and 5.90%) and Merkur (4.34% and 4.40%) having a greater yield increase compared to other soybean varieties included in the experiment (NS Kaća 3.73% and 3.30%, NS Maximus 3.37% and 3.76%, NS Apolo 3.04% and 3.38%). The year immensely influences soybean yield, primarily depending on precipitaton distribution and quantity and temperature conditions during the vegetation period. Aqueous banana peel and apical soybean plant part extracts significantly increase soybean yield.

Key words: *aqueous extracts, foliar application, yield, varieties, soybeans.*

Introduction

Foliar applications in soybean crop during the intensive growth phase increases yield (Miladinov *et al*., 2018), especially in adverse years with a distinct drought period, but also in growing seasons with favourable conditions as well (Dozet *et al*., 2013; Dozet *et al*., 2015). Aqueous plant material extracts are being ever more used in flower and vegetable crops, but also in field crop production, both in organic and conventional cultivations (Đukić *et al*., 2021). Considering that soybean grain is being used for obtaining various products used in human consumption, it is very important for a part of total soybean production to come from an organic cultivation system, without mineral fertilizer and pesticide application (Dozet *et al*., 2019). Aqueous plant material extracts, beside macro and trace elements, also contain physiologically active substances which induce plant growth and development, often have a fungicidal and insecticidal effect, are easily prepared at a farmstead, do not require major investments and are favourable for organic production since their use does not have a negative environmental impact.

In recent decades, climate change is being detected in the form of median daily temperature increases during vegetation and on a yearly level, along with ever greater increases in precipitation oscillations, i.e. shifts of pluvial and extremely arid years, and these conditions are highly unfavourable for soybean production (Đukić *et al.,* 2018).

Yield flacuations in certain years confirm that weather conditions during vegetation greatly affect soybean yield (Đukić *et al.,* 2018; Dozet *et al.,* 2019; Dozet *et al.,* 2022).

The aim of this paper was to investigate the effect of aqueous banana peel and apical soybean plant part extracts on soybean grain yield of five varieties which differ in vegetation period length.

Materials and methods

In a three-year experiment, the influence of foliar application of aqueous banana peel and apical soybean plant part extracts on soybean yield was investigated on five varieties of different maturity groups (NS Kaća 000 maturity group, Merkur 00 maturity group, NS Maximus 0 maturity group, NS Apolo I maturity group). The experiment was set up in the Institute of Field and Vegetable Crops' experimental field in Rimski Šančevi, and the experimental treatments were: the control, where the amount of foliarly applied water was identical to the amount of aqueous extracts, aqueous banana peel extract and aqueous apical soybean plant part extract application. The application of aqueous plant material extracts and water on the control variant was being conducted, just before the soybean flowering phase, with an amount of 300 litres of liquid per hectare in which the aqueous extract was diluted in a ratio of 1:15. The experiment had four replications, and the single plot size was 10 m^2 (four soybean rows, 50 cm distance between rows and five meters of length). The aqueous extracts were made by drenching 1 kg of chopped plant material into 10 litres of rain water and, by styrring it daily, the end of fermentation was awaited, after which the aqueous extract was strained by gauze and kept in glass bottles until used. During the vegetation period, standard agronomic practices were applied in soybean production, and harvesting via small operating hold combine was conducted in the harvest maturity phase, grain mass and moisture were measured and yield per hectare with 14% moisture was calculated. The results were processed by trifactorial experiment variance analysis (Program „Statistica 10") and difference significance was tested via LSD test.

Results and discussion

Meteorological parameters for the three year experiments are shown on Table 1. Average temperatures during the vegetation period for the year 2018 (20.9 °C), the year 2019 (19.7 °C) and 2020 (19.1 °C) were higher compared to the perennial average (18.2 °C).

Temperatures in 2018 were high in the first part of plant growth (April and May 5.6 °C and 3.5 °C above the perennial average, respectively) and in August (2.9 °C above average). In 2019, April temperatures were higher than the perennial average by 2.3 °C, May temperatures lower by 2.0 °C, and in the time of blossoming and legume formation (June) and in the time of grain filling (August), temperatures were higher than the perennial average by 2.5 °C and 3.3 °C, respectively.

In 2020, lower temperatures during the intensive plant growth period were recorded, May temperatures were lower than the perennial average by 0.9 °C, while the April temperatures were higher by 1.1 °C, June and July temperatures were higher by 0.6 °C, August temperatures higher by 1.8 °C and September temperatures higher by 2.1 °C compared to the perennial average. The greatest influence that temperature has on soybean yield is during the periods of flowering, pod formation and grain filling (Đukić *et al.*, 2018). Very high temperatures in June and July along with precipitation insufficiency do not favour soybean production (Dozet *et al.*, 2021).

Table 1. Weather conditions in the study years

Month	Mean monthly temperature (°C)				Precipitation (lm^{-2})			
	2018	2019	2020	Long- term average	2018	2019	2020	Long- term average
IV	17.4	14.1	12.9	11.8	50.0	54.0	11.1	47.6
V	20.5	15.0	16.1	17.0	64.0	85.0	47.3	67.6
VI	21.7	22.6	20.7	20.1	164.0	64.0	161.9	88.6
VII	22.1	22.8	22.4	21.8	83.0	22.0	77.3	66.7
VIII	24.3	24.7	23.2	21.4	51.0	80.0	137.5	58.1
IX	19.5	19.2	19.1	17.0	27.2	54.0	31.4	47.8
Average, Total	20.9	19.7	19.1	18.2	439.2	359.0	466.5	376.4

The average amount of precipitation during the soybean vegetation period of 2018 was higher by 62.8 lm^{-2}, and in 2020 by 90.1 lm^{-2} in comparison to the perennial average (376.4 lm^{-2}), while in 2019 there was less precipitation by 17.4 lm^{-2} compared to the perennial values. The precipitation insufficiency in 2019 was expressed in June, July and the first half of August, which, along with high temperatures, led to compulsory plant maturation and significant soybean yield reduction (Đukić *et al.*, 2018). In 2018, precipitation insufficiency occured in August and September, but the distribution was more favourable compared to 2020.

Observing soybean yield by certain years (Table 2), it is noticeable that the achieved yield in 2018 (5.085,1 $kgha^{-1}$) was significantly higher compared to 2019 (3.965,8 $kgha^{-1}$) and 2020 (3.792,6 $kgha^{-1}$). The lowest yield was recorded in 2020 and, compared to this value, soybean yield in 2018 was increased by 34.08%, and in 2019 by 4.57%.

Observing soybean yield by varieties, it is noticed that the highest yield was recorded in relation to the variety Rubin (4.847,0 $kgha^{-1}$), a statistically significant higher value compared to the varieties NS Kaća (3.546,2 $kgha^{-1}$), Merkur (4.165,8 $kgha^{-1}$), NS Maximus (4.370,8 $kgha^{-1}$) and NS Apolo (4.476,1 $kgha^{-1}$). Significantly higher yield was recorded among soybean varieties NS Apolo, NS Maximus and Merkur in comparison to the variety NS Kaća. Compared to the variety NS Kaća, which has the shortest vegetation period, and which achieved the lowest grain yield per surface unit, the variety Merkur's grain yield was increased 17.47%, the variety NS Maximus' by 23.25%, the variety NS Apolo's by 26.22% and the variety Rubin's by 36.68%.

Observing soybean yields by foliar application treatments, it is noticed that the highest yield was recorded at the treatment with a foliar aqueous apical soybean plant part extract application (4.341,7 $kgha^{-1}$), significantly higher value compared to the control (4.166,1 $kgha^{-1}$), while the yield achieved with the aqueous banana peel extract application (4.335,7 $kgha^{-1}$) was also significantly higher compared to the control. In comparison to the control, soybean yield was increased via aqueous banana peel extract application by 4.07%, and via aqueous apical soybean plant part extract application by 4.21%.

Observing the same year and different soybean varieties, it is noticed that in 2018 the highest yield was achieved with the soybean variety Rubin (5.851,7 $kgha^{-1}$), which was highly significant compared to the soybean varieties NS Kaća (3.891,0 $kgha^{-1}$), Merkur (4.802,3 $kgha^{-1}$), NS Maximus (5.415,7 $kgha^{-1}$) and NS Apolo (5.465,0 $kgha^{-1}$).

Table 2. Average soybean grain yield ($kgha^{-1}$)

Year (A)	Variety (B)	Treatments (C)			Average (AxB)	Average (A)
		Control	*AE - Banana peel	*AE - Soybean plants		
2018	NS Kaća	3.867	3.918	3.888	3.891,0	5.085,1
	Merkur	4.729	4.832	4.846	4.802,3	
	NS Maximus	5.385	5.437	5.425	5.415,7	
	NS Apolo	5.437	5.477	5.481	5.465,0	
	Rubin	5.794	5.884	5.877	5.851,7	
	Average (AxC)	5.042,4	5.109,6	5.103,4		
2019	NS Kaća	3.169	3.342	3.316	3.275,7	3.965,8
	Merkur	3.724	3.961	3.953	3.879,3	
	NS Maximus	3.869	4.024	4.107	4.000,0	
	NS Apolo	4.218	4.308	4.320	4.282,0	
	Rubin	4.155	4.506	4.515	4.392,0	
	Average (AxC)	3.827,0	4.028,2	4.042,2		
2020	NS Kaća	3.359	3.523	3.534	3.472,0	3.792,6
	Merkur	3.689	3.876	3.882	3.815,7	
	NS Maximus	3.554	3.779	3.757	3.696,7	
	NS Apolo	3.492	3.762	3.790	3.681,3	
	Rubin	4.051	4.407	4.434	4.297,3	
	Average (AxC)	3.629,0	3.869,4	3.879,4	Average (B)	
Average (BxC)	NS Kaća	3.465,0	3.594,3	3.579,3	3.546,2	
	Merkur	4.047,3	4.223,0	4.227,0	4.165,8	
	NS Maximus	4.269,3	4.413,3	4.429,7	4.370,8	
	NS Apolo	4.382,3	4.515,7	4.530,3	4.476,1	
	Rubin	4.666,7	4.932,3	4.942,0	4.847,0	
Average (C)		4.166,1	4.335,7	4.341,7		
Average 2019-2020					4.281,2	
*AE – aqueous extracts						

LSD	A	B	C	AxB	AxC	BxC	AxBxC
1%	316.9	32.0	173.5	410.6	100.3	178.6	444.8
5%	210.8	216.1	116.5	291.2	70.9	121.5	317.3

Compared to the soybean variety NS Kaća, the variety Merkur’s yield was increased by 23.42%, the variety NS Maximus’ by 39.19%, the variety NS Apolo’s by 40.45% and the variety Rubin’s by 50.39%.
In 2019, the highest yield was achieved by the variety Rubin (4.392,0 $kgha^{-1}$), which was statistically higher yield compared to the soybean varieties NS Kaća (3.275,7 $kgha^{-1}$), Merkur (3.879,3 $kgha^{-1}$) and NS Maximus (4.000,0 $kgha^{-1}$). Statistically very significantly higher yield was also recorded among the soybean varieties Merkur, NS Maximus and NS Apolo (4.282,0 $kgha^{-1}$) compared to the variety NS Kaća, while a statistically significantly higher yield was achieved by the variety NS Apolo compared to the variety Merkur. In comparison to the soybean variety NS Kaća, the yield was increased for the variety Merkur by 18.42%. the variety NS Maximus by 22.11%, the variety NS Apolo by 30.72% and the variety Rubin by 34.08%.
In 2020, the highest yield was achieved by the variety Rubin (4.297,3 $kgha^{-1}$), which is statistically very significantly higher compared to the soybean varieties NS Kaća (3.472,0 $kgha^{-1}$), Merkur (3.815,7 $kgha^{-1}$), NS Maximus (3.696,7 $kgha^{-1}$) i NS Apolo (3.681,3 $kgha^{-1}$). A statistically significantly higher yield was achieved by the soybean variety Merkur in comparisn to the variety NS Kaća. Compared to the soybean variety NS Kaća, the variety Merkur’s yield was increased by 9.90%, the variety NS Maximus’ by 6.47%, the variety NS Apolo’s by 6.03% and the variety Rubin’s by 2.77%.
By observing the same year but different foliar application treatments, it is noticed that soybean yield in 2018 varied from 5.042,4 $kgha^{-1}$ (control variant) to 5.109,6 $kgha^{-1}$ (aqueous banana peel extract application variant), although there was no statistically significant difference between certain treatments. By applying aqueous banana peel extract, the yield was increased by 1.33%, and 1.21% by applying aqueous apical soybean plant part extract. In 2019, statistically very significantly higher soybean yields were recorded when applying aqueous apical soybean plant part extract (4.042,2 $kgha^{-1}$) and aqueous banana peel extract (4.028,2 $kgha^{-1}$) in comparison to the experiment’s control (3.827,0 $kgha^{-1}$). By applying aqueous banana peel extract, the yield was increased by 5.26% and 5.62% by applying aqueous apical soybean plant part extract. In 2020 as well, a statistically significantly higher soybean yield was recorded when applying aqueous apical soybean plant part extract (3.879,4 $kgha^{-1}$) and applying aqueous banana peel extract (3.869,4 $kgha^{-1}$) in comparison to the experiment’s control variant (3.629,0 $kgha^{-1}$). By applying the aqueous banana peel extract, the yield was increased by 6.62% and 6,90% by applying aqueous apical soybean plant part extract. Whilst observing soybean yields of the same varieties, and of different treatments, it is noticed that the variety NS Kaća’s yield was statistically significantly higher among the variant with aqueous banana peel extract application (3.594,3 $kgha^{-1}$) in comparison to the control variant (3.465,0 $kgha^{-1}$). Aqueous banana peel extract application increased the yield by 3.37%, whilst aqueous apical soybean plant part extract increased the yield by 3.30%. Among the variety Merkur, a statistically very significantly higher yield was recorded when aqueous apical soybean plant part extract was applied (4.227,0 $kgha^{-1}$) and a statistically significantly higher yield when aqueous banana peel extract was applied (4.223,0 $kgha^{-1}$) compared to the experiment’s control variant (4.047,3 $kgha^{-1}$). By applying the aqueous bananana peel extract, the yield was increased by 4.34%, and 4.40% by aplying aqueous apical soybean plant extract. The variety NS Maximus achieved a statistically significantly higher yield by applying aqueous soybean plant part extract (4.429,7 $kgha^{-1}$) and aqueous banana peel extract (4.413,3 $kgha^{-1}$) in comparison to the control (4.269,3 $kgha^{-1}$). By applying aqueous banana peel extract, the yield was increased by 3.37% and 3.37% by applying aqueous apical soybean plant part extract. The soybean variety NS Apolo was recorded to give statistically

significantly higher yields when the aqueous apical soybean plant part extract (4.530,3 $kgha^{-1}$) and aqueous banana peel extract (4.515,7 $kgha^{-1}$) were applied, in comparison to the control (4.382,3 $kgha^{-1}$). Applying aqueous banana peel extract increases the yield by 3.04% and 3.38% by applying aqueous apical soybean plant part extract. The Rubin soybean variety’s variants recorded a statistically very significantly higher yield when applying the aqueous apical soybean plant part extract (4.942,0 $kgha^{-1}$) and aqueous banana peel extract (4.932,3 $kgha^{-1}$), in comparison to the experiment’s control varient (4.666,7 $kgha^{-1}$). By applying the aqueous banana peel extract, the yield has increased by 5.69% and 5.90% by applying aqueous apical soybean plant part extract.

Conclusion

The year very significantly affects soybean yield, primarily depending on quantity, precipitation distribution and temperature conditions during the vegetation period. Soybean varieties with a longer vegetation period have a greater yield potential than varieties with a shorter vegetation period. Aqueous banana peel and apical soybean plant part extracts significantly increase soybean yield.

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NUTRITIONAL VALUE OF WILD BERRY SPECIES FROM MOUNTAIN KOPAONIK (SERBIA)

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Abstract

A large number of wild relatives of cultivated fruits in Serbia indicates a significant biological and primarily genetic diversity of wild berry species. Wild berry species are rich in phenols that are usually associated with health benefits due to their antioxidant capacity. The purpose of this work was to investigate the content of total anthocyanins and total phenols of wild berry species fruit extracts originating from an area of mountain Kopaonik (South Serbia): strawberry (*Fragaria vesca* L. and *Fragaria viridis* L.), raspberry (*Rubus ideaeus* L. and *Rubus sahatilis* L.), blackberry (*Rubus fructicosus* L.), red currant (*Ribes petraeum* L.) and blueberry (*Vaccinium myrtillus* L.) as well as their antioxidant capacity. Blueberry and blackberry were characterized by the highest presence of anthocyanins (3.71 and 1.33 g C3G/kg FW). The blueberry extract (*Vaccinium myrtillus* L.) was also the richest in the content of total phenols (4.94 g GAE/kg FW) followed by an extract of strawberry (*Fragaria viridis* L.) and blackberry (*Rubus fructicosus* L.). The antioxidant effects of the sample extracts were evaluated through DPPH (2,2-diphenyl-1-picrylhydrazyl) antiradical assay which is shown that raspberry species *Rubus sahatilis* L. had significantly higher antioxidative activity (244.87 mM TE/mL) compared to other examined wild berry species. However, despite the very high content of anthocyanins and phenols, the antioxidant capacity of blueberry extract was the lowest (211.78 mM TE/mL). In conclusion, the present research increased the knowledge about the nutritive properties of Serbian wild berry species in order to support their conservation and use in the breeding programs of small fruits for choosing berry fruits with a high content of health-promoting properties.

Keywords: *Wild berries, Phenols, Anthocyanins, Antioxidative activity.*

Introduction

Serbia has many different wild plant species, thanks to its favorable geographical and climatic conditions. Kopaonik, situated in the South of Serbia is s the most extensive mountain massif that is a valuable source of wild plant species. Kopaonik is also a prime center of biodiversity in Serbia (Amidžić, 2007). The flora on this mountain consists of numerous types of trees, bushes, and different types of herbaceous plants, many of which are medicinal. Wild fruit species occupy an important place among them. They represent a significant genetic potential that is very important for selection goals and breeding (Mratinić and Kojić, 1998). In addition, wild fruit species produce fruits of exceptional quality and high nutritional value that are used in human nutrition. The population in Serbia has been using the fruits of various wild berry species for fresh consumption or processing in the form of juices, jams, ‘slatko’, etc. Depending on the distribution of the species, wild strawberry, blueberry, blackberry and raspberry fruits are used the most. Despite the fact that wild plants have become a less important food source over time because of the development of agriculture and food industries, the trend of their use is on the increase (Licata *et al*., 2016). Bearing that wild fruit species develop in optimal conditions, they are biologically more valuable, more resistant to diseases and pests, and their fruits are richer in bioactive compounds. Wild berry species are rich in phenols that are usually associated with health benefits due to their antioxidant capacity. Thus, the objective of the work was to evaluate the nutritive composition and promote wild berry species from an area of mountain Kopaonik and make it available to breeding programs, fresh consumption and the food industry.

Materials and Methods

Plant material

Fresh fruits of wild-growing strawberry, raspberry, blueberry, red currant and blackberry were picked up at full maturity from an area of mountain Kopaonik, south Serbia (Table 1). The sites were selected based on the criterion of the richness of the natural wild fruit populations. Samples were kept refrigerated and transferred to laboratory. Two gram of fruits were chopped and extracted in 20 ml of 80% (v/v) methanol in ultrasonic bath for 30 minutes. The extracts were centrifuged at 5,000 rpm for 10 minutes and filtered. Extracts were used for further biochemical analysis.

Table 1. Characteristics of tested wild fruit species

Species	Botanical taxa and family	Local name	Ripening time	Fruit properties and usage
Strawberry	*Fragaria vesca* L. (*Rosaceae*)	Šumska jagoda	June	small, aromatic fruit, egg-shaped to round shape, dark red color; mature fruits are eaten (rich in Vitamin C), fresh or made into juices, syrups, compotes, desserts, preserves called ‘slatko’, jam and cakes
	Fragaria viridis L. (*Rosaceae*)	Turska jagoda	June	small, firm, aromatic fruit, red on top; a less attractive fruit than *F.vesca* and less used in diet
Raspberry	*Rubus ideaeus* L. (*Rosaceae*)	Planinska malina	July	juicy fruit, composed of a large number of small drupe; fresh fruits are eaten raw, or cooked and made into juices, syrups, compotes, jams, and ‘slatko’
	Rubus sahatilis L. (*Rosaceae*)	Kamenjarka	July	sour fruit taste; they are used little in the diet
Blueberry	*Vaccinium myrtillus* L. (*Ericaceae*)	Borovnjača	July	purple-blue fruit, round shape; fresh ripe berries are eaten and made into jams, ‘slatko’, juices and compotes
Red currant	*Ribes petraeum* L. (*Grossulariaceae*)	Pećinska ribizla	July-August	small, round, red berries, sour-refreshing taste; it is not widespread
Blackberry	*Rubus fructicosus* L. (*Rosaceae*)	Kupina, Ostruga	August	dark blue fruit, few drupes, weakly connected; fruits are eaten raw or cooked and prepared as syrup, fruit preserve ‘slatko’, jams, jellies, compotes. Also used to flavour red wine

Total phenolic content

Total phenolic content (TPC), were determined using a modified Folin-Ciocalteau method (Singleton *et al*., 1999; Liu *et al.*, 2002). A 0.2 ml aliquot of the 40-fold water diluted apple extract was added to a 15 ml tube and 0.2 ml of 1:10 Folin-Ciocalteau reagent: water solution was added to the mixture. The tube was allowed to stand at room temperature for 6 min. Then, 2 ml of 7.5% Na_2CO_3 were added to the mixture. After 2 h at room temperature, absorbance was measured at 765 nm by UV/Vis spectrophotometer (Jenway 6300, Cole-Parmer, UK). The results were expressed as g of gallic acid equivalents per kg fresh weight of the sample (g GAE/100 kg FW). Calibration was performed by analyzing the standard gallic acid (Sigma–Aldrich CO., USA) three times at five different concentrations.

Total anthocyanins

Total anthocyanin (TA) content was measured with the pH differential method (Giusti and Wrolstad, 2001). Two dilutions of fruit extractss were prepared by mixing with buffers (pH 1.0 and 4.5). After 15 min, the absorbance of prepared solution was measured at 510 nm and 700 nm. Anthocyanin content was expressed as g equivalents of cyanidin3-glucoside per kg of fresh weight (g C3G/kg FW).

Antioxidant activity

Antioxidant activity (AA) was determined using the free radical scavening DPPH (2,2-diphenyl-1-picrylhydrazyl) method reported by Brand-Williams *et al*. (1995) with modifications (Sánchez-Moreno *et al.*, 1998). The fruit phenol extract was added to DPPH solution in methanol and vortexed. A control sample, containing the same volume of solvent in the place of the extraction, was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 30 min., the absorbance at 515 nm was recorded to determine the concentration of the remaining DPPH. The antiradical activity was calculated from the equation determined from linear regression after plotting known solutions of Trolox with different concentrations (50−250 mM). Calibration was performed by analyzing the Trolox standard three times at four different concentrations. Antiradical activity was expressed as micromol Trolox equivalents per ml (mM TE/mL).

Statistical analysis

Experimental data were processed with one-way analyses of variance (ANOVA). Source of variation was species. The data were analyzed using Statgraphic Centurion 18 program (Manugistics, Inc., Rockville, MD, USA). The analyses were performed in four replications and mean values were compared by Duncan's Multiple Range at the 5% level of probability. Results expressed as the mean ± standard error.

Results and Discussion

Berries are widely recognized fruits for their nutritional quality and potential health benefits. The results of our study show a high concentration of total phenols in all wild berry fruit extracts, which is in accordance with literature data (Radovanović *et al*., 2013; Prvulović *et al*., 2019; Veljković *et al*., 2021). Total phenolic content measured by the Folin Ciocalteu test showed significant variability among wild berry species tested (Figure 1). Wild blueberry (*Vaccinium myrtillus* L.) had a significantly higher TPC (4.94 g GAE/kg FW) compared to other examined wild fruit species. A high phenolic content was also recorded in strawberry species *Fragaria viridis* L. (3.93 g GAE/kg FW) and blackberry *Rubus fructicosus* L. (3.69 g GAE/kg FW). This result can be explained by the high proportion of anthocyanins in the mentioned species, which significantly contribute to the total phenolic content. The presence of anthocyanins in the fruit is manifested by red, blue and purple colored fruits, which have recently been connected with beneficial activities as food ingredients and as promoters of human health (Hosseinian *et al.*, 2008). Significantly higher anthocyanins content was recorded in wild blueberry (*Vaccinium myrtillus* L., 3.71 g C3G/kg FW) and blackberry (*Rubus fructicosus* L., 1.33 g C3G/kg FW) compared to the other examined species. However, no significant differences in TA content were detected between wild blueberry and blackberry. These values were in range with previously reported data of Mitić *et al*. (2014), who examined antioxidant activity and anthocyanin content of selected wild and cultivated small fruit from southern Serbia. They found that wild blackberries contained the highest quantity of anthocyanins expressed as cyanidin-3-glucoside (1063.53 mg/kg FW). The same authors, using 4 different methods for the assessment of antioxidant activity, determined that blackberries proved to possess the highest antioxidant activity, while results for other fruit species varied depending on the applied method. Similarly, Prugar *et al*. (2012) have established that wild blackberry germplasm had a higher total phenolic content than cultivated blackberries. High values of most of the analyzed phenolic compounds contained in *F. vesca* and *R. fruticosus* is confirmed by results of Milivojević *et al*. (2011) who point out that wild species can contribute to the improvement of nutritional quality in cultivars through the selection of genotypes from native populations. The high bioactive potential of wild species Yilmaz *et al*. (2009) explain as an induction in the synthesis of antioxidant enzymes and an increase in polyphenolic concentration brought about due to the greater exposure of the unsheltered wild plants to extremes of temperature, and insult by pests and disease organisms, because phenolic compound synthesis is typically a defensive mechanism.

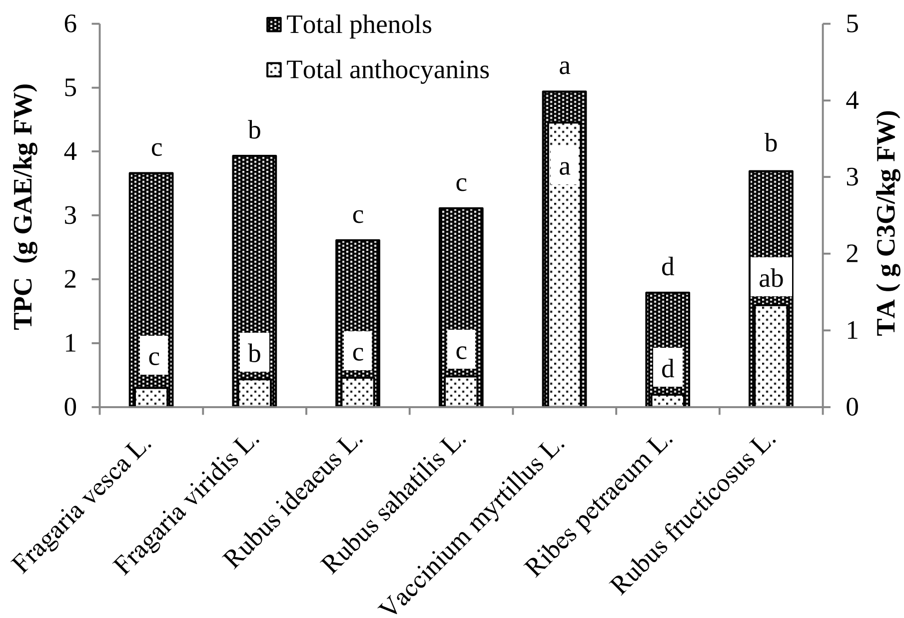

Figure 1. Content of total phenols and anthocyanins in wild berry fruit species. Different small letters at the top of columns indicate significant differences at P $\leq$ 0.05 by Duncan's test.

In our study, the highest AA was detected in samples of wild raspberry *Rubus sahatilis* L. followed by strawberry species *Fragaria viridis* L., whereas wild strawberry *Fragaria vesca* L., raspberry *Rubus ideaus* L., red currant *Ribes petraeum* L. and blackberry *Rubus fructicosus* L. contained the low AA (Figure 2). Interestingly, despite the fact that wild blueberry (*Vaccinium myrtillus* L.) had the highest values of TPC and TA, the lowest AA (211.78 Mm TE/mL) was recorded in this species. It is known that extracts from the leaves and fruit of the *Rubus* species are used in various countries as natural remedies to treat several diseases, such as diabetes, many types of infections, colic and burns (Patel *et al.* 2004). The raspberry is used in traditional medicine in Serbia in a similar way to its use in other European countries (Veljković *et al.*, 2021).

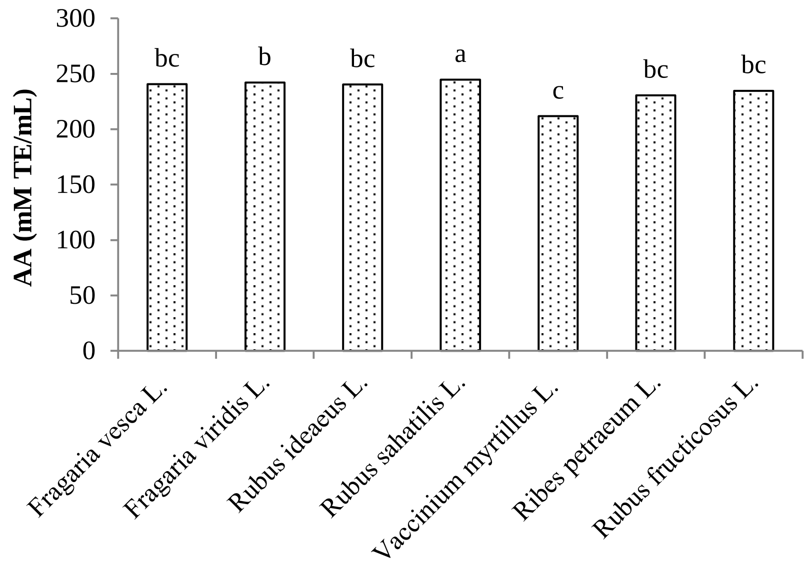

Figure 2. Antioxidative activity of wild berry fruit species. Different small letters at the top of columns indicate significant differences at P $\leq$ 0.05 by Duncan’s test.

Conclusions

Results of our study confirmed that all investigated extracts of wild berry fruits originating from area of mountain Kopaonik contain high phenols and anthocyanins content and show high antioxidant activity. However, wild blueberry and blackberry (*Vaccinium myrtillus* L. and *Rubus fructicosus* L.) showed the highest total phenols and anthocyanins content. Although the five other species have lower anthocyanins and phenolic compounds, they are also a valuable source of healthy compounds. The extracts also show strong antioxidant activity, esspecialy *Rubus sahatilis* L. and *Fragaria viridis* L., species that are less represented in the examined area.
The present research increased the knowledge about the nutritive properties of Serbian wild berry species in order to support their conservation and use in the breeding programs of small fruits for choosing berry with a high content of health-promoting properties as well as their traditional utilization and processing as a functional food that provides health benefits.

Acknowledgement

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FERTILITY PARAMETERS AND GRAIN QUALITY OF WINTER BARLEY

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Abstract

The experiments were performed on samples of biomass and grains of winter barley varieties (Rekord and Zlatnik) for two years. The parameters of fertility and quality (yield, mass of 1000 grains and hectoliter mass, moisture, starch, lipids, ash, cellulose and β-glucan) were monitored. The average grain yield ranged from 3,933 t ha-1 to 5,065 t ha-1. Grain yield differed significantly between years and for all genotypes and on average was higher in the first year compared to the second year of cultivation. Average mass values of 1000 grains ranged from 41.60 to 54.83 g. It was found that there are very significant differences in grain yield compared to the year of testing, while the differences between the studied barley varieties were not significant. The results of the chemical composition of barley grains have shown that the tested parameters deviated between cultivars and years. The starch composition varied from 48.8% to 50.1%, while the composition of the crude protein varied from 10.5% to 11.3%. The average composition of the lipids per dry matter was 1.68% for cultivar Rekord and 1.77% for cultivar Zlatnik, respectively. In all tested samples, the composition of the crude cellulose varied from 3.27% to 3.82%. The obtained results showed that the lowest composition of the crude ashes was recorded in the sample of cultivar Rekord (1.58%), while the highest was detected in the sample of cultivar Zlatnik (1.70%). The composition of moisture ranged from 10.11% to 11.01%, while composition of β-glucan varied from 3.9% to 4.23%.

Key words: *barley, yield, fertility parameters, β-glucan, chemical composition.*

Introduction

The stability of grain yield determined for a certain area is a reliable criterion in the selection and recommendation of varieties because the stability of grain yield established in previous years due to the dynamics of climatic factors does not include risk due to weather conditions in subsequent years. The agronomic value of a variety depends not only on its genetic potential for yield but also on its ability to realize its genetic potential under different production conditions (Malešević et al., 2010). Yield largely depends on genetic potential, which can be defined as the yield of a variety grown in the conditions to which it is adapted, with sufficient water and nutrients and effective control of pests, diseases, weeds and other stresses (Đekić et al., 2012). The mass of 1000 grains in malting barley ranges from 40 to 46 g, while the hectoliter mass ranges from 68 to 75 kg hl^{-1} (Paunovic et al., 2006). The positive impact of fibre on human health from various types of cereals and their functional properties have recently attracted significant attention from the scientific and professional public (Ronda et al., 2015; Sarteshnizi et al., 2015). Grain β-glucan is a soluble fibre that lowers blood cholesterol, postprandial glucose and insulin levels (EFSA, 2011), and affects the technological properties of the drought (Ahmed, 2015; Ahmed and Thomas, 2015). According to the FDA, products having 0.75 g of β-glucan per serving size may be characterized as functional (FDA, 1997 and 2008). The natural grain coating and difficulty in grinding as well as the lack of gluten narrow down the usability of barley in the fabrication of food for humans. The content of β-glucan in barley grain varies depending on the genotype and agroecological growing conditions. Its role in human nutrition is multifunctional - medicinal, which is why there is a trend of its increased market demand (Dickin et al., 2011; Hetherington et al., 2013; Pentikäinen et al., 2014).

Materials and methods

During the two growing seasons, two varieties of winter barley (Rekord and Zlatnik) were tested. The experiments were set up according to a random block system in three replications. The usual technology for barley production was applied. Sowing was done at the optimal time. Before sowing 400 kg ha^{-1} of NPK fertilizer (15:15:15) was added, while 250 kg ha^{-1} (KAN) was added with spring fertilization. The land on which the experiment was set up belongs to the type vertisol in the process of degradation, with heavy mechanical composition and rough unstable structure. Soil fertility is moderate, with low pH values (pH in H_2O = 5.66 and KCl <4.28), with a humus content of about 2.85% and total nitrogen from 0.12 to 0.15%. The easily accessible phosphorus content was low (below 10 mg 100 g^{-1} P_2O_5 soil), while the easily accessible potassium content was high (25.65 mg 100 g^{-1} K_2O soil). The research was conducted during two consecutive seasons in the Kragujevac, region of Šumadija, Republic of Serbia. Data on the average monthly air temperature and precipitation for the studied vegetation period are shown in Table 1.

Table 1. Average monthly air temperatures and amount of precipitation (Kragujevac).

Months	Average monthly air temperatures (^{0}C)			Amount of precipitation (mm)		
	Period I	Period II	Multi-year average	Period I	Period II	Multi-year average
X	10.0	13.5	12.5	33.3	56.2	57.6
XI	3.1	9.5	6.9	1.3	17.7	70.4
XII	4.6	1.7	1.9	43.3	16.4	71.5
I	0.7	2.9	0.5	117.2	62.4	58.5
II	-3.7	4.0	2.4	60.1	84.3	62.7
III	8.1	6.5	7.1	5.7	102.0	45.4
IV	12.9	13.4	11.6	74.5	41.2	48.9
V	16.1	18.2	16.9	87.3	70.8	56.6
VI	23.0	19.9	20.0	57.8	30.3	58.2
VII	25.8	23.1	22.0	35.4	34.4	46.4
VIII	24.0	22.3	22.7	10.1	33.3	32.4
Average/sum	11.37	12.28	10.41	526.0	549.0	608.1

The average air temperature in the second year of the study was higher by 1.87 ^{0}C compared to the multi-year average, while the amount of precipitation was lower by 59.1 mm. In the second year of the research, higher amounts of precipitation were recorded in February and March, which led to a higher grain yield compared to the first year of the research. Based on the obtained results, the parameters of descriptive statistics were calculated. Statistical data processing was performed in the Analyst module of the SAS/STAT program (SAS Institute, 2000). Moisture concentration in the analyzed samples was determined according to the AACC International (2000). Starch, total lipids and total ash were determined according to Kaluđerski and Filipović (1998). For determination of starch concentration, a CARL ZEISS polarimeter of 24/60 Hz was used, with a specific angle of rotation of 181.3. Protein concentration was determined according to the Kjedahl method with a conversion factor of 6.25 (ISO 20483:2006). Crude cellulose (crude fibre) concentration was determined according to Veender, using “Fibertec 2010” and the instructions from the manual: Fibertec 2010 system, User Manual, 1009 9130/rev. 1.3, FOOS Tecator, Sweden. The samples were ground in a laboratory mill (Knifetec 1096). All the samples were tested in three replications, and the results were expressed on a dry matter basis. Nitrogen-free extract (NFE) was calculated as follows: %NFE = 1000 % (crude protein + lipid + ash + crude fibre). The total β-glucan concentration was analysed by the spectroscopic method 32–23.01 (AACC, 2003) using Megazyme β-glucan mixed linkage assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland).

Results and Discussion

The average values of yield and mass of 1000 grains in the studied winter barley cultivars grown during the two growing seasons are shown in Table 2. The yield of the examined barley cultivars differed depending on the year of research. In the first year of research, the Zlatnik variety achieved a slightly higher grain yield (4,100 t ha^{-1}), while the Rekord variety achieved a slightly lower yield (3,933 t ha^{-1}). In the second year of research, the Rekord variety achieved a yield of 5,650 t ha^{-1}, which is 0.530 t ha^{-1} more than the Zlatnik variety. The average grain yield in the observed two-year period was slightly higher in the cultivar Rekord and amounted to 4,792 t ha^{-1}. Đekić et al. (2012b) point out that grain yield and fertility parameters are mainly influenced by climatic and genetic factors. Also, Dumlupinar et al. (2011) state that differences between years occur due to environmental conditions.

Table 2. Average values of the analyzed traits of winter barley.

Variety	**I year**			**II year**			**Average**		
	$\bar{x}$	**S**	$S_{\bar{x}}$	$\bar{x}$	**S**	$S_{\bar{x}}$	$\bar{x}$	**S**	$S_{\bar{x}}$
Grain yield (t ha^{-1})									
Rekord	3.933	0.076	0.44	5.650	0.150	0.087	4.792	0.946	0.386
Zlatnik	4.100	0.218	0.126	5.120	0.207	0.119	4.610	0.590	0.241
Average	4.017	0.172	0.070	5.385	0.332	0.136	4.701	0.758	0.219
1000 grains mass (g)									
Rekord	46.10	1.153	0.666	49.83	1.258	0.726	47.97	2.312	0.944
Zlatnik	54.83	2.255	1.302	51.60	1.442	0.833	53.22	2.450	1.000
Average	50.47	5.044	2.059	50.72	1.550	0.633	50.59	3.560	1.028
Hectolitre mass (kg hl^{-1})									
Rekord	68.47	0.839	0.484	68.20	0.436	0.252	68.33	0.615	0.251

Zlatnik	71.48	1.353	0.781	71.53	0.551	0.318	71.51	0.924	0.377
Average	69.97	1.935	0.790	69.87	1.879	0.767	69.92	1.819	0.525

Starting from the fact that there was a sufficient amount of precipitation in the spring months (Table 1), especially during May, which is very important for the successful production of small grains, it can be concluded that the distribution and amount of precipitation during the second growing season was much more favourable which resulted in a higher yield in this year compared to the first one. In both research years, the Zlatnik variety achieved a higher average mass of 1000 grains (54.83 g and 51.60 g, respectively). A slightly lower average value of 1000 grains mass in both the first and the second year of research was achieved by the variety Rekord (46.10 g and 49.83 g, respectively). Some authors (Jelić et al., 2002; Đekić et al., 2010) point out that the mass of 1000 grains is a varietal characteristic and that there is significantly greater variation between different genotypes than between environmental factors. Hectolitre mass is an indicator of grain quality, especially its monetary value. It is generally considered that grain with a higher hectoliter mass is of better quality compared to one with lower mass values. The value of hectoliter mass in both years of research was slightly higher in the winter barley cultivar Zlatnik (71.48 kg hl^{-1} and 71.53 kg hl^{-1}, respectively).

Table 3. Analysis of variance of the analyzed traits of winter barley.

Effect of years on the traits analyzed				
Traits	**Mean sqr Effect**	**Mean sqr Error**	**F (df1,2) 1.10**	**p-level**
Winter barley				
Grain yield (t ha^{-1})	5.617	0.070	80.233	0.000004
1000 grains mass (g)	0.187	13.924	0.013	0.909916
Hectoliter mass (kg hl^{-1})	0.035	3.637	0.010	0.923569
Effect of cultivar on the traits analyzed				
Trait	**Mean sqr Effect**	**Mean sqr Error**	**F (df1,2) 1.10**	**p-level**
Winter barley				
Grain yield (t ha^{-1})	0.099	0.622	0.159	0.698258
1000 grains mass (g)	82.687	5.674	14.573	0.003388
Hectolitre mass (kg hl^{-1})	30.242	0.616	49.051	0.000037
Effect of the year x cultivar interaction				
Trait	**Mean sqr Effect**	**Mean sqr Error**	**F (df1,2) 1.8**	**p-level**
Winter barley				
Grain yield t ha^{-1})	0.364	0.030	12.284	0.008022
1000 grains mass (g)	36.401	2.519	14.450	0.005227
Hectolitre mass (kg hl^{-1})	0.075	0.757	0.099	0.760656

Based on the analysis of variance, it can be concluded that the interaction of varieties x years very significantly affects the grain yield (Fexp = 12,284 **) and the mass of 1000 grains (Fexp = 14, 450 **). The influence of vegetation year on hectolitre mass in the examined winter barley varieties was not significant, while in the case of yield it was very highly significant. No significant influence of the variety on grain yield was found between the examined barley

genotypes, while significant differences were found at the mass of 1000 grains and hectolitre mass.
The results of the analysis of the chemical composition of the barley are presented in Table 4. Based on the obtained results, it can be seen that there are deviations of the tested parameters between the varieties and the year of production. The starch content varied in the range of 48.8 to 50.1%. The crude protein content in barley grain ranged from 10.5 to 11.3%. This difference can be attributed to the specificity of the variety and agro-ecological conditions of the environment. A previous study (Alijošius et al., 2016) states that the protein content of barley grain has differences depending on growing conditions. The average lipid content is 1.72% on dry matter, with the variety Rekord at 1.68% and Zlatnik at 1.77%. These values in the tested barley grain samples were slightly lower than those established in previous studies (Šterna et al., 2015). Crude cellulose in all tested barley grain samples ranged between 3.27% and 3.82%. The results showed that the lowest content of raw ash was in the sample of the variety Rekord (1.58%), and the highest in Zlatnik (1.7%). The moisture content was in the range between 10.11 and 11.01%.

Table 4. The chemical composition of barley grain.

Variety	**Rekord**		**Zlatnik**	
Year	I	II	I	II
Starch	48.8	49	50	50.1
Proteins	10.8	10.5	11.3	10.9
Lipids	1.66	1.7	1.82	1.73
Cellulose	3.28	3.31	3.82	3.79
Ash	1.58	1.63	1.55	1.7
Moisture	10.11	10.2	11.01	10.82
NFE	23.77	23.66	20.5	20.96
β-glucan	3.91	3.9	4.1	4.23

The content of β-glucan in the seed samples of the cultivar Rekord (3.9%) was slightly lower compared to the samples of the cultivar Zlatnik (4.16%).

Conclusion

Based on the presented results, it can be concluded that the winter barley variety Rekord achieved better results in grain yield, while the variety Zlatnik had a slightly higher mass of 1000 grains and hectolitre mass. Grain yield shows a tendency to increase in years with higher sum and better precipitation distribution during critical phases of plant development. Analysis of variance revealed a very significant effect of the interaction of year x variety on grain yield and mass of 1000 grains in barley, while the influence of the growing season on barley grain yield was statistically justified. Very significant differences were found for the mass of 1000 grains concerning environmental factors. Differences in chemical composition have also been noted, which can be related to the characteristics of varieties.

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FRUIT CHARACTERISTICS OF OF OLD AUTOCHTHONOUS CULTIVARS OF MELONS (*Cucumis melo L.*) AND WATERMELONS (*Citrullus lanatus L.*)

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Abstract

In 2007, the Agricultural Extension Service "Sombor", in cooperation with the Provincial Secretariat for Agriculture and the Ministry of Agriculture, launched the Pilot Project Let's Preserve and Protect Old Varieties of Vegetables. The goal of the project was to preserve old domestic varieties of vegetables traditionally grown in our area, and which survival was endangered with greater commercialization of vegetable production. In the project, 22 primary schools with 3163 participated. In this project 5296 samples were collected over three weeks. After collecting, the samples were each individually determined and sorted by plant species, marked, prepared for sowing and recorded in the sample database. Today, in 2022, over 1000 samples of old cultivars and populations of vegetables and other plant species are being maintained within this program. During 2020 and 2021, in the experimental field of Agriculture Extension Service “Sombor”, biological properties and production characteristics were evaluated from collected samples of watermelon and melon. This paper present the phenotypic and morphological characteristics of interesting varieties of indigenous varieties of watermelon and. These varieties are excellent for further production and testing in laboratory conditions and also for organic production. With this project, forgotten plant species, varieties and food populations have been returned to production.

Keywords: *autochtonous, old cultyvarsprotection, melon, watermelon.*

Introduction

Indigenous/domestic varieties of vegetables are of great importance. They contain various genes and structures that may be the key to the future of agriculture. In the era of climate change and the growing appearance of diseases and pests, the destruction of the natural environment by the use of a large number of pesticides, we have endangered the ecological future of vegetables. For this challenge, we can only choose old indigenous varieties as a fight against the present in order to preserve genetic diversity. Old varieties are also the stamp of local agricultural production and authentic gastronomy. They are also important from the ecological aspect, in the context of traditional agricultural practices and the way of their cultivation that was once practiced. They are adapted to the environment and the people they have lived with for centuries. They have cultural and spiritual significance, they are associated with customs, songs, folk art. The germination of vegetable seeds and cereals decreases with each year of storage, so that after a certain number of years it may not germinate at all, so vegetable seeds are among the most endangered crops. Since 1900, about 75% of agricultural plant varieties have been lost. Until 1949, 10,000 varieties of wheat were used in China, and in 1970 only 1,000 varieties. The seeds of different vegetable crops came to different places through human migration, trade and exchange. Only those seeds that best adapted to local conditions, gave satisfactory yields and

quality served for further reproduction. Spontaneous crossing, natural selection under the influence of the environment in many of our regions have created many valuable ecopopulations of vegetables.
Numerous nations with developed social consciousness have long ago developed programs that protect the most endangered varieties and ecopopulations of plant species from permanent extinction. There are few traditional family farms that already buy vegetables for their own needs or for the market from their own reproduced seeds, and they are mostly of foreign origin, organized seed production has been drastically reduced and preserving the cultivars cultivars of old varieties commercially has not been interesting to anyone.

Materials and Methods

With the commercialization of vegetable production, the disappearance of traditional peasant gardens and the increasing depopulation of rural areas, the genetic fund and the biological diversity of vegetable species in the area of Vojvodina are extremely endangered by permanent extinction. In 2007, Agriculture Extension Service ”Sombor" launched an action to save old varieties of old varieties that are traditionally grown in our area from permanent loss, and their survival is endangered by the increasing commercialization of vegetable production. The task of this Pilot Project was to try to start a program for the preservation and protection of old varieties of vegetables. The action was organized by Agriculture Extension Service "Sombor" in cooperation with the Ministry of Agriculture, the Provincial Secretariat and the School Administration. 22 primary schools and 3163 students took part in the action, collecting seeds from the village from their grandmothers and bringing them as samples to Agriculture Extension Service "Sombor". During the action which lasted 3 weeks, 5296 samples were collected. After the samples were detected in the database, each sample was assigned a separate name, and it was recorded in the database under the code. Over 23 different types of vegetables, fruits and flowers were collected in the action.
In 2008 and 2009, all samples were sown in the experimental field Agriculture Extension Service "Sombor". Experts employed in the service monitored morphological characteristics and parameters during the vegetation in order to have an insight into the characteristics of the species and production ability at the end of the vegetation. In the following years, a number of species were also tested to propagate the seed. From 2007 onwards, Agriculture Extension Service "Sombor" has been planting a number of types of vegetables with the aim of multiplying seeds, spreading knowledge and learning about the morphological characteristics of old vegetable varieties. For this work, we have singled out the most interesting types of watermelons and melons, which are different in color, taste, smell and texture.
Watermelon (*Citrullus lanatus* L.) is an annual herbaceous plant from the Cucurbitaceae family. Watermelon is grown for its ripe, sweet, tasty fruits, pleasant aroma and refreshing taste. The redder or yellower the meat, the higher the value of watermelon (Radić, Đ., 2011). It is mostly used in its fresh state at physiological maturity, but it can be used to make juices, bark syrup is very tasty sweet and oil is extracted from the seeds, which is very rich in vitamin D. The nutritional value of watermelon is high (40% of sugar is sucrose) from the total mass of the edible part, it contains cellulose, hemicellulose and pectin, and from minerals K, Na, Mn, Fe and S. According to the iron content, watermelon is right behind spinach and lettuce. The world's largest producer of watermelon is China. In Serbia, watermelons and melons are grown on about 22,000 ha, of which 85% is under watermelon (Ilić, Z. 2009). Optimal maturity for most varieties

occurs 45 days after flowering and then the total soluble matter reaches a maximum. If harvested carefully and on time, watermelon can be stored for 14-21 day (Ilić Z., 2009).

Results and Discussion

Old varieties of watermelons stood out primarily because of their color. When we think of watermelon, we always think of red, but what stands out here are the watermelons of yellow and orange. Cultivars Cultivars A735, average fruit weight 5.33 kg, oval shape, fruit length 19 cm, width 22.5 cm. Characteristic light green color of the peel with orange flesh, pronounced stripes and a sugar content of 7.5 %.

Cultivars Cultivars A 711 average fruit weight 6.90 kg, oval elongated shape, fruit length 34.0 cm and fruit width 20.0 cm. Interesting light green peel with orange flesh and 6.0 % sugar content.

Cultivars Cultivars A 212 fruit weight 6.62 kg, oval shape, fruit width 25.0 cm and fruit length 20.0 cm. Intensely dark green rind with yellow flesh and sugar content 6.0 %.

Cultivars Cultivars A211 / 1 has an interesting light green bark color, very smooth texture and yellow flesh color. The weight of the fruit is 4.95 kg, oval shape, fruit length 20.0 cm and width 19.9 cm, sugar content 9.0 %.

Table 1. Characteristics of fruits by varieties watermelons

cultivars cultivars	fruit weight / kg	fruit shape	fruit length / cm	fruit width / cm	bark thickness / cm	RSM %	meat color	bark color	seed color
A 211/1	4,95	oval	20,0	19,9	1,0	9,0	yellow	light green	brown
A 735	5,33	oval	19,5	22,5	0,7	7,5	orange	light green	brown
A 212	6,62	oval	20,5	25,0	1,5	6,0	yellow	dark green	black
A 711	6,90	oval elongated	34,0	20,0	1,2	6,0	orange	light green	brown
SOMBORKA	6,50	oval	28,1	20,2	1,6	12,6	red	dark green	dark brown

The most interesting is certainly the most famous old indigenous cultivars cultivars Somborka, weighing 6.50 kg, oval in shape, 28.1 cm long and 20.2 cm wide, dark green bark with red flesh and exceptional sweetness 12.6 %.

Also, during the vegetation, the parameters based on the UPOV descriptor were monitored: leaf color, green intensity, leaf hatching, leaf stalk length and degree of leaf incision.

Table 2. Morphological characteristics according to the UPOV descriptor

cultivars cultivars	A 735	A 212	A 711	A 211/1	SOMBORKA
fox: color	green	green	green	green	green
coot: int.green	medium	medium	medium	medium	medium
coot: hat	weak	weak	small	small	weak
leaf stalk: length	short	medium	medium	medium	small
fox: degree of incision	strong	strong	strong	strong	strong

Watermelons are usually picked at full physiological maturity, because the taste does not improve during storage. The temperature that fruits have in the field (28-35°C) should be reduced below 15°CIlić Z., 2009) as soon as possible. High temperature accelerates rapid ripening, which can be slowed by lowering the temperature.

Melon (*Cucumis melo* L.) is a heat-loving vegetable species that is characterized by gradual ripening and the possibility of fruit ripening after harvest. It originates from Asia and was brought to Serbia via Italy and Greece in the middle of the 15th century (Radić Đ., 2011). It is grown for its aromatic fruits. In addition to being used fresh, jam, jams and marmalades are made from melons at physiological maturity. Green fruits are placed in pickles (Falik E., 2009). The edible part of the melon contains 6.0-18.5% of dry matter, of which sugars make up 4.8-15.0% and 72-74%, respectively, and sucrose predominates, which, depending on the cultivars cultivars and growing conditions, varies from 1.0- 11.2%. Melon fruits have a high content of vitamin C of 30-48 mg %. (Falik E., 2009).

In the world, melons are grown on about 1,000,000 ha. Production is mainly related to the open field and is characterized by the seasonal character of production, outdated assortment, the occurrence of fruit cracking and a number of diseases that reduce yields.

Cultivars Cultivars A 1505 very interesting round elongated shape, fruit weight 1.47 kg, fruit length 14.4 cm and width 15.0 cm with a high percentage of sugar content.

Cultivars Cultivars A 670 round shape, fruit weight 1.15 kg, fruit length 13.0 cm, fruit width 13.5 cm with high sugar content 12.5%. The interesting green color of the meat with mesh stripes makes it recognizable.

Cultivars Cultivars A 505 weighs 2.03 kg, round elongated lemon shape, fruit length 14.5 cm and width 16.8 cm with a high sugar content of 13.0%. Interesting color of white meat with yellow-green color of the crust.

Cultivars Cultivars A 1756 is the most interesting cultivars cultivars among the old varieties, weighing 2.25 kg wide elliptical shape with green stripes, fruit length 17.8 cm, fruit width 16.0 cm and white flesh. What stands out from other varieties is the high sugar content of 16.0%.

Cultivars Cultivars A 995 is an old cultivars cultivars of very interesting shape with large lobes that make it recognizable in a series of melons. Fruit length 13.3 cm and width 14.7 cm, intense orange flesh and bark color. The sugar content is 8.0%.

Table 3. Characteristics of fruits by varieties melons

cultivars cultivars	fruit weight / kg	fruit shape	fruit length / cm	fruit width / cm	bark thickness / cm	mass sacch%	meat color	bark color	seed color
A 1756	2,25	broadly elliptical	17,8	16,0	1,0	16,0	white	yellow-green	brown
A 505	2,03	round	14,5	16,8	1,0	13,0	bela	yellow-green	brown
A 670	1,15	round	13,0	13,5	1,0	12,5	gren	gray	yellow
A 1505	1,47	round	14,4	15,0	8,0	14,0	white	yellow	brown
A 995	2,26	oval	13,3	14,7	1,1	7,9	orange	orange	yellow

Table 4. Morphological characteristics according to the UPOV descriptor

sort	A 995	A 505	A 670	A 1505	A 1756
leaf size	medium	big	little	medium	little
coot: int.green	medium	medium	medium	medium	medium
notch development	medium	medium	medium	strong	weak
hats foxes	weak	medium	medium	small	little
leaf stalk: position	upright	upright	upright	upright	upright
young fruit: shade	white green	green	green	green	green
maturity	late	medium	late	wound	wound

The content of total soluble matter (URM), the share of which is mostly sugars, is the most reliable parameter in determining the maturity of melons. According to EU standards, the refractometric value of fruit flesh must be at least 8% measured in the central equatorial part of the fruit. (Falik E., 2009). One of the parameters for determining the optimal harvest time is the production of ethylene.

Conclusion

And the 1756 has a very interesting shape and appearance, so it can compete with the hybrids currently on the market. We can also produce cultivars A 995, which has a very interesting shape and is certainly more interesting than most standard melons on the market. Compared to hybrids, it can certainly compete in terms of sweetness, because it has a high sugar content. We can see from the attached that watermelons have a very interesting shape and appearance, attractive color, very unusual, which are suitable for growing on the market and for comparison with hybrids. The old varieties that we mentioned in the paper are, in addition to their interesting shape and appearance, very resistant to today's conditions, so that in this respect they are not behind the hybrids that are on the market today. Also, very suitable varieties for organic production. Nowadays, the trend of healthy food gives us the opportunity to use these old varieties, and we hope that this story with the old varieties will continue.

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PLANT PROTECTION AND FOOD SAFETY

THE HARMFULNESS OF APPLE BLOSSOM WEEVIL (*Anthonomus pomorum* Linne) ON DIFFERENT APPLE VARIETIES IN THE REGION OF EAST SARAJEVO IN BOSNIA AND HERZEGOVINA

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Abstract

The apple blossom weevil (*Anthonomus pomorum* L.) is a widespread pest in world. worldwide. It´s present in all apple-growing regions and represents one of the most significant pests of generative organs of the apple. The harmfulness of this species in region of East Sarajevo (entity of Republic of Srpska, Bosnia and Herzegovina) was examined in 2022. in extensive plantation in the locations Kula and Klek. In Kula, examination was done on the varieties: Jonagold, Golden Delicious, Idared, Granny Smith and Melrose. In Klek, the examination included varieties: Jonagold, Golden Delicious, Braeburn, Gloster and Fuji. Using entomological methods such as visual inspections of trees, method of shaking branches and method of sampling of 100 flower buds, the presence and harmfulness of *A. pomorum* on different apple varieties was determined. The higher percentage of damaged flower buds was in the location Kula (27.2). The highest percentage of damaged flower buds was found in the variety Golden Delicious (41), while the fewest damaged flower buds were on the variety Melrose (12). In the location Klek, out of the total number of examined, 22.4 percentage of flower buds were damaged. The highest percentage of damage was determined on the variety Breburn (37), and the least on the variety Fuji (9).

Keywords: *apple blossom weevil, apple, varieties, East Sarajevo.*

Introduction

One of the most important pests of the generative organs of the apple tree is the apple-blossom weevil (*Anthonomus pomorum* Linne) (Coleoptera: Curculionidae). This species feeds on *Malus* spp. and *Pyrus* spp. (Brown *et al*., 1993; Duan *et al*., 1998; Toepfer *et al.,* 1999a), and it is quite common all over Europe (Daniel *et al*., 2005; Knuff1 *et al*., 2017; Miñarro and García, 2018). The apple-blossom weevil is distributed in the Eastern and Western European countries, in the greater part of Asia, Ukraine, European part of the Russian Federation, in Korea, Japan, Northern China, USA, Romania and Greece especially in the gardens located near the forests where wild apple and pear-trees grow (Hull, 1985; Vasiliev, 1988; Niemczyk, 1994).
Overwintering adults make damages on closed flower buds during feeding period and oviposition. In general, the egg-laying by the apple-blossom weevil is noted into the buttons of the apple, pear, quince, cherry, sweet cherry and some other species (Batiashvili, 1959., cit. Zabrodina *et al*., 2020). Larvae feed and development within the buds, provoking their drying and falling (Gratwick 1992; Maceljski 2002, Almaši *et al*., 2004). After leaving the egg the larva feeds first on the anthers, becomes very voracious and secretes a large number of excrement which glues the petals of the button and thus does not allow them to open. If the egg is laid late, namely in the phenophase of the button opening, then the larva does not have time to glue the

petals with its excrement, and the button is opening. In this case the larva is killed by the direct sunbeams. After the larva has glued the petals, it eats away the contents of the buttons causing the petals to deprive of nourishment; they become brown, dry and form the caps. The damaged buttons do not open; they become well visible against the background of the apple tree blossoming (Matson *et al*., 1997; Simon *et al*., 2010).
The harmfulness of the *A. pomorum* has increased in several European countries (Hausmann *et al*., 2005), particularly in organic farming systems because to reductions in the application and the spectrum of pesticides over the last two decades (Brown *et al*.,1993; Cross *et al*. 1999; Danelski *at al*., 2012; Knuff *et al*., 2017). The harmfulness of this species depends on the population size, variety (prefers to attack varieties that bloom early), length of flowering, weather conditions at the time of oviposition, the number of flowers and chemical treatments. The harmfulness increases especially during the years of poor yield, when the fruit buds are killed by the frost; and nowadays it increases especially in the orchards with no protective measures or where the application of the insecticides is limited (Blommers, 1994; Almaši *et al*, 2004; Mody *et al*., 2011). The size of populations of *A. pomorum* might be limited by opportunities for both overwintering and egg-laying. While it is known that adults overwinter sheltered under the bark of apple trees, most modern orchards are grown on dwarfing rootstock that produce small trees with smooth bark and thus provide little opportunity for winter shelter (Toepfer *et al*., 2000). As a result, in young and modern orchards, adult weevils shelter in the trees surrounding the orchard. In some years damages are going up to 70-80% or even more (Maceljski, 2002; Ciglar, 1998; Cosoveanu and Palagesiu, 2010).
Economic consequences can be disastrous because far more than half of the blossom buds can be destroyed, with reports varying between 60% and close to 100% (Toepfer *et al*., 1999a; Bajec *et al*., 2013). The weevil caused the greatest damage in the northern regions of the apple cultivation in the cold spring when the blossoming was delayed and the females had time to lay off their entire stock of the eggs. In such years the apple-blossom weevil can destroy more than 70% of buttons. The damage caused by it is especially dangerous in the years with poor blossoming of the apple trees (Hausmann *et al*., 2005; Mody *et al*., 2011; Knuff *et al*., 2017; Miñarro and García, 2018).
In the years of mass reproduction of the *A. pomorum* the losses can reach up to 80–90% of the crop yield in the centres of damage unless the protection measures from this pest are carried out. The proportion of flowers damaged by the weevil can reach up to 95% as for the apple trees and it can reach up to 4–5% as for the pear trees depending on the intensity of the damage. The apple plantations located near the forests are damaged most often (Gamina, 1991; Kashirskaia, 1991). In Belarus and Moldova, the economic losses caused by the apple-blossom weevil are enormous. According to Yakymchuk and Muten (2008) the decrease in the number of chemical treatments in the apple orchards of Moldova in recent years has led to an increase in the number of the apple-blossom weevil, previously considered a minor pest.
In Bosnia and Herzegovina the apple-blossom weevil damaged up to 100% of the apple buttons (Batinica, 1958).

Materials and Methods

The survey was completed in 2022. in the field, in extensive plantations (locations Klek and Kula), and in the laboratory of the Faculty of Agriculture in East Sarajevo. A field survey was conducted in different apple orchards. In the location of Kula, the orchard is at altitude of 550 m,

19 years old, with an area of 0.2 ha. The examination included varieties: Idared, Jonagold, Golden Delicious, Granny Smith and Melrose.
The other orchard, is at altitude of 862 m, 22 years old, with an area of 0.2 ha, in the location of Klek. In this location, the examination included following varieties: Jonagold, Golden Delicious, Braeburn, Gloster and Fuji.
Methods of visual examination of randomly selected trees was determined the presence of *A. pomorum*, their number and symptoms of damage. Visual examination of the trees was done from the beginning of the vegetation until the beginning of June, at intervals of 7-10 days. The survey included 20 randomly selected trees of each apple variety, in both localities.
By method of shaking branches, the adult stadium of *A. pomorum* were collected. This method involved randomly selected 20 trees of of each variety. One sample was a 100 strokes with 100 branches, at different heights and position. It was used to collect overwintering adult stadium of *A. pomorum*.
By methods of sampling of 100 flower buds with a randomly selected trees, the presence, harmfulness and development stadium of *A. pomorum* was determined. This method involved randomly selected 20 trees of each variety in both localities. All sampled infested flower buds was observed in laboratory. All collected larvae and pupa, were reared in the lab to adult stadium. Determination of species was based on morphological characteristics of the adult and before adult stadium, followed by keys and appropriate entomological literature (Balachowsky, 1966; Hering, 1957).

Results and Discussions

In apple orhards of East Sarajevo in 2022., the apple blossom weevil (*A. pomorum*) on different apple varieties was determined.
By the method of shaking branches, in mid-April in the both localities, the first overwintering adult stadium were determined. This method was applied once (April 18 in the location Kula and April 25 in the location Klek) of each variety in both localities (Tab.1).

Table1. The number of adult stadium *A. pomorum* on different apple varieties

Locations					
	Kula		**Klek**		
	Variety	Number of adults		Variety	Number of adults
1.	Jonagold	5	1.	Jonagold	3
2.	Golden Delicious	7	2.	Golden Delicious	5
3.	Idared	4	3.	Braeburn	6
4.	Granny Smith	3	4.	Gloster	1
5.	Melrose	1	5.	Fuji	1
Total number		20	Total number		16

By this method, a total of 20 overwintering adults *A. pomorum* in location Kula and 16 adults in the location Klek, were collected. The largest number of adults were found on the variety Golden Delicious in both locations (7 in the location Kula and 5 in the location Klek). The lowest number of adults was found on the varieties Melrose (Kula) and Fuji (Klek).

The overwintering adults were found in the second half of April, during feeding period and oviposition what is in agreement with literature data about the time of activation of this adults and biology of this species (Beletskil, 2008; Dovhan, 2009; Yevtushenko, 2005).

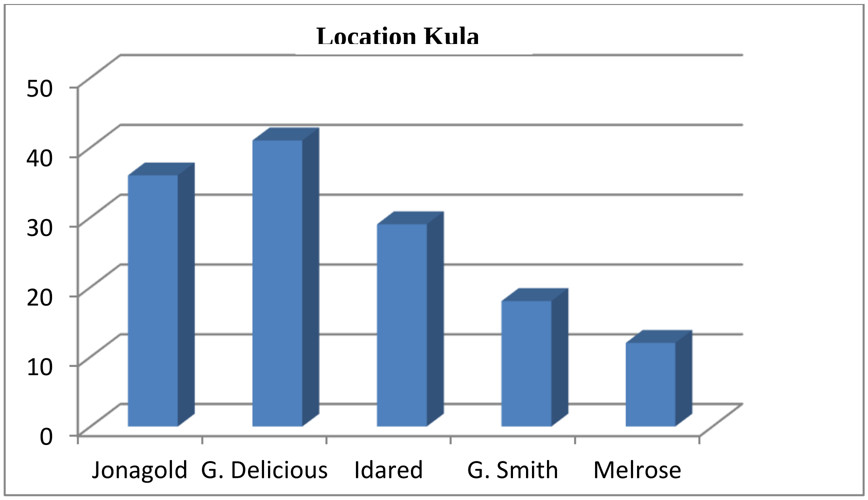
With the appearance of flower buds, a visual examination of the orchards revealed the presence of unopened and rotten flowers that gradually acquired a dark color (Fig. 1). In this period, the method of sampling of 100 flower buds was done of each variety, in both locations (May 6 in the location of Kasindo, May 11 in the location of Klek). By this method, larvae *A. pomorum* were determined (Fig. 2). They were fed on the contents of the buds, destroyed them and prevented their opening.

These results corresponding to the numerous literature data about the symptoms of damage from larvae *A. pomorum* (Maceljski, 2002; Almaši *et al*., 2004; Gratwick, 1992; Duan *et al*., 1998; Zabrodina *et al*., 2020).

Figure 1. The damage flower buds from larvae *A. pomorum* (photo original)

Figure 2. Larva *A. pomorum* (photo original)

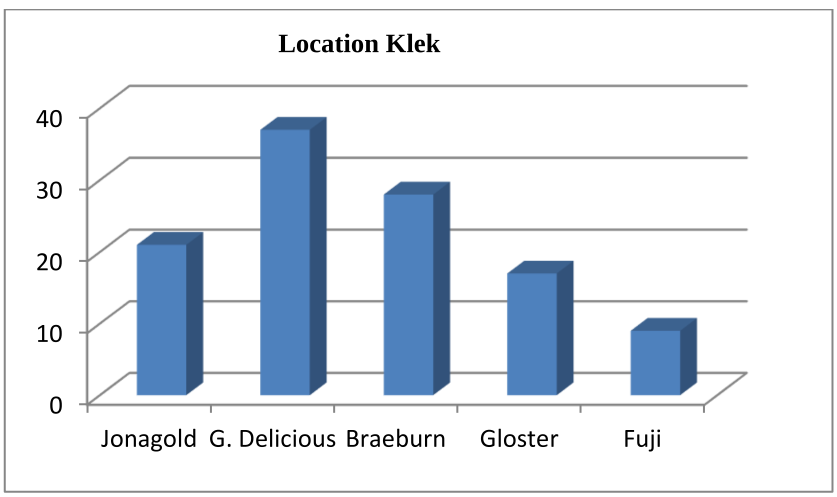
Their number was different in examination locations. In the location Kula, regarding the total number of 500 flower buds (5 varieties x 100 buds), 136 buds were damaged or 27.2%. This percentage indicates a strong attack, in agreement with literature data about attack intensity - attack is weak (up to 5% of damage buds), medium attack (5.1-15%), strong attack (15.1-50%) and very strong attack (over 50 % of damage buds) (Collective of authors, 1983).



Graph. 1. The percentage of damage flower buds from larvae *A. pomorum*

The highest percentage of damaged flower buds was on the variety Golden Delicious (41), then on the varieties Jonagold (36) and Idared (29), while the fewest damaged flower buds were on the variety Melrose (12) (Graph. 1). Literature data about more intense attack *A. pomorum* on early and medium-early flowering varieties (Almaši, 2004; Volkodav and Konverska, 2002) are in correspodence with our results because *A. pomorum* was more represented on the medium-early flowering varieties (Golden Delicious, Jonagold and Idared).

In the location Klek, regarding the total number of 500 flower buds (5 varieties x 100 buds), 112 buds were damaged or 22.4%, which indicates a strong attack, in agreement with literature data about attack intensity (Collective of authors, 1983).



Graph. 2. The percentage of damage flower buds from larvae *A. pomorum*

The highest percentage of damaged buds was on the variety Golden Delicious (37), then on the Braeburn (28), Jonagold (21) and while the fewest damaged flower buds were on the variety Fuji (9) (Graph. 2).

As is evident, a strong attack of A. pomorum was in both localities, but a slightly lower percentage of damaged flower buds was in the location Klek, compared to the location Kula. The lower intensity of attacks in the location Klek can be explained by the higher altitude this apple orchard, which is reflected in the temperature conditions that directly affect the development of all species of insects, including this one.
When it comes to the presence and damage of A. pomorum on different varieties, it is evident that the highest percentage of damage was on the variety Golden Delicious in both locations, and the lowest on the varieties Melrose (12) and Fuji (9).
Furthermore, during the vegetation period (second half of May), by visual examination of randomly selected trees, in damaged flower buds, pupae and newly developed adult stadium of A. pomorum were found, in the location Kula. In the location Klek, the pupae and the developed adult stadium were recorded later (in the second half of May), which is certainly a consequence of the higher altitude of this location and different temperature conditions.

Conclusion

In apple orchards, in the region of East Sarajevo, on different varieties, apple blossom weevil (*Anthonomus pomorum)* was determined.
The number of overwintering adults of *A. pomorum* and the percentage of damaged flower buds differed in localities. Thus, in the location Kula, a total of 20 adult stadium of this species were found, and 16 in the location Klek. The largest number of adult stadium of *A. pomorum* was found on the variety of Golden Delicious, in both localities.
The total percentage of damaged flower buds was also higher in the location Kula (27.2). The highest percentage of damaged flower buds was found in the variety Golden Delicous (41), while the fewest damaged flower buds were on the variety Melrose (12). In the location Klek, 22.4 % of flower buds were damaged, with the highest percentage of damage on the variety Golden Delicous (37), and the least on the variety Fuji (9).

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EFFECT OF VARIOUS SUBSTRATES ON GROWTH AND SPORULATION OF SELECTED ISOLATES *ALTERNARIA* SPP

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Abstract

Six selected isolates of *Alternaria* spp. were used in these studies, as follows: A: 1) FM-15; 2) BM-2; 3) CLA-108 monitoring *A. dauci* isolates; B: 4) Mr-114; 5) IM-14, and 6) Aa-82 control isolate for *A. alternata*. Testing the effect of nutrient substrate on the growth and sporulation of selected isolates of *Alternaria* spp. was conducted on five different agars as follows: potato dextrose agar (PDA), V8 agar (V8A), Water agar (WA), Malt agar (MA), and Carrot leaf agar (CLA). The growth rate was determined after inoculation by measuring the diameter of the colony after 3, 5, 7 and 10 days in five replications, while the intensity of sporulation was expressed through the number of conidia per 1 cm^2 of colony. The largest radial growth of colonies of all three isolates from group A studied was achieved on V8 agar, while the isolates from group B studied achieved a slightly higher growth on PDA. The influence of nutrient substrate on the intensity of sporulation in the isolates from studied group B was abundant and very abundant, while sporulation in isolates of group A was absent on all agar, except for two isolates.

Keywords: *Alternaria alternate isolates, Alternaria dauci isolates, nutrient substrate, radial growth, sporulation.*

Introduction

Numerous pathogenic fungi endanger the cultivation and production of plants from the family Apiaceae Lindl., among them fungi from the genus *Alternaria* Ness are of special importance. Changes that these fungi can cause in carrot plants are spotting and leaf drying, brown root rot, seedling blight, root neck rot and leaf stalk drying (Bulajić and Krstić, 2007; Bulajić et al., 2014; Trkulja et al., 2020). In order to study the breeding traits - the effect of various substrate on the growth and sporulation of selected isolates of *Alternaria* spp., six selected isolates of *Alternaria* spp. were used, as follows: 1) FM-15 isolate from Bosnia and Herzegovina; 2) BM-2 isolate from Serbia; 3) CLA-108 monitoring *A. dauci* isolate; 4) Mr-114 isolate from Bosnia and Herzegovina; 5) IM-14 isolate from Serbia, and 6) Aa-82 monitoring *A. alternata* isolate.

Material and Methods

The study of the effect of nutrient substrate on the growth and sporulation of six selected isolates of *Alternari*a spp. was conducted on five different agars, as follows:

- Potato dextrose agar (PDA) was prepared from 200 g of potatoes, 17–20 g of dextrose, 17–20 g of agar and 1 l of distilled water (Kiraly et al., 1970);

- V8 agar (V8P), was prepared from 200 ml of fresh tomato juice, 3 g of $CaCO_3$ (Calcium carbonate, Analytical Reagent, Mallinckrodt Chemical Works, Saint Louis, New York) and 800 ml of distilled water (Strandberg, 1987).);
- Water agar (WA), prepared from 17 g of agar and 1 l of distilled water (Dhingra et Sinclair, 1986);
- Malt agar (MA) was prepared from 10 g of malt extract, 17–20 g of agar and 1 l of distilled water (Sheppard et Maddox, 2001);
- Carrot leaf agar (CLA) was prepared from carrot leaves that had been air-dried in the laboratory and stored in paper bags until use.

The substrate was prepared from 25 g of dried carrot leaves, which were immersed in 1 l of distilled water for 1 hour, decanted, after which the resulting liquid was made up to a volume of 1 l and 17 g of agar was added (Strandberg, 1987).

Substrate inoculation was performed using a spear needle, with which a section of colonies (∅ 5 mm) of pure cultures of studied *Alternaria* spp isolates grown for seven days on potato-dextrose agar was transferred to the Petri box center on five different test substrates. The inoculated Petri dishes were then incubated in a thermostat at 25 ° C, without the presence of light. The growth rate was determined after inoculation by measuring the diameter of the colony (Pryor et Gilbertson, 2002) after 3, 5, 7, and 10 days in five replications.

The effect of nutrient substrate on the sporulation of the tested isolates was determined by a modified method described by Strandberg (2002), which involves making suspensions of conidia by adding a constant amount of distilled water to the colonies of tested *Alternaria* spp isolates. The sporulation of the tested isolates was determined in such a way that the prepared microscopic preparations were made from fungal cultures grown on different substrates. As a mount in temporary microscopic specimens, a drop of tap water was used in which a fragment of the colony affected by a spear needle from cultures of tested isolates of *Alternaria* spp. was applied. The material thus applied was then covered with a glass slide and directly observed under an Olympus CX 41 optical microscope at magnifications of 40 × to 400 ×. Determination of sporulation was performed 3, 5, 7 and 10 days after inoculation, in five replications.

Results and Discussion

The effect of nutrient substrate on the growth and sporulation of six selected isolates of *Alternaria* spp. was tested on five different agars as follows: 1) potato dextrose agar (PDA); 2) carrot leaf agar (CLA); 3) V8 agar (V8A); 4) malt agar (MA), and 5) water agar (WA). Three selected isolates of representatives of group A and group B were grown on them in parallel, while the radial growth, average daily increment and sporulation were analyzed in all isolates (Table 1).

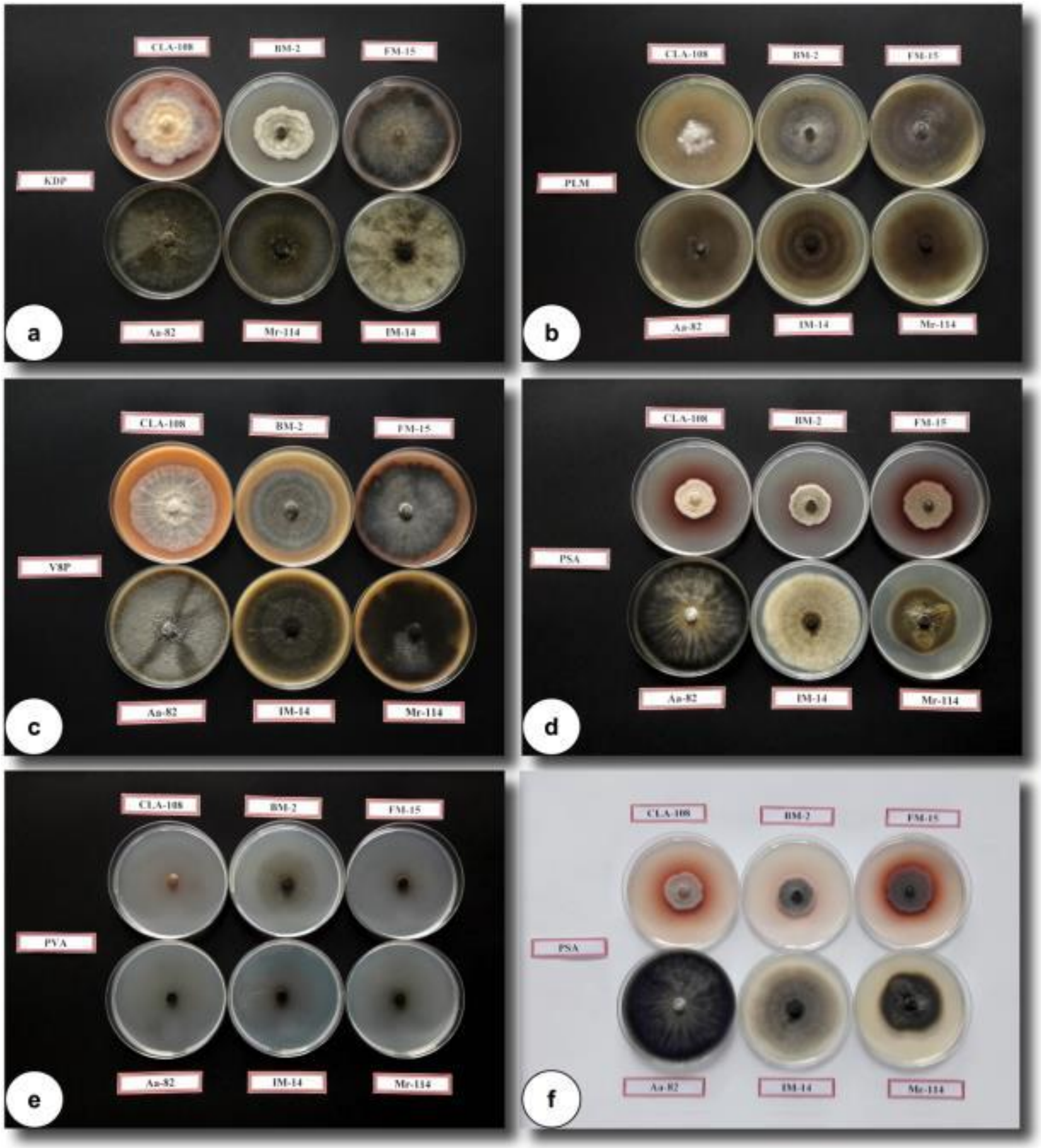

Figure 1. *Alternaria* spp. appearance of colonies of the studied isolates on: a) potato-dextrose agar (PDA); b) carrot leaf agar (CLA); c) V8 agar (V8A); d) malt agar (MA); e) water agar (WA), and f) malt agar (MA) photographed on a white background, in order to more clearly show the secretion of pinkish-red pigment into the substrate in some of the studied isolates.

Table 1 Effect of various substrates on colony growth, average daily growth and sporulation of studied isolates of *Alternaria* spp.

Substrates	Isolates					
	p A			p B		
	CLA-108	BM-2	FM-15	Aa-82	IM-14	Mr-114
Colony diameter (mm)						
PDA	53,50 ±10,95^{b}*	47,17 ± 5,78^{c}	75,00 ± 4,34^{a}	83,00 ± 0,00^{a}	81,83 ± 1,17^{a}	82,50 ± 0,84^{a}
CLA	52,67 ± 2,16^{b}	69,67 ± 7,42^{a}	77,17 ± 1,60^{a}	73,67 ± 1,21^{c}	75,67 ± 0,52^{b}	75,17 ± 1,33^{b}
V8A	67,50 ± 1,64^{a}	70,17 ± 1,60^{a}	77,83 ± 2,32^{a}	81,00 ± 0,89^{b}	79,00 ± 1,55^{a}	77,50 ± 1,76^{b}
MA	33,50 ± 1,05^{c}	33,83 ± 1,47^{d}	38,83 ± 3,71^{c}	82,67 ± 0,52^{a}	75,33 ± 3,39^{b}	56,83 ± 2,14^{d}
WA	40,33 ±10,11^{c}	58,00 ± 2,83^{b}	50,67 ± 4,76^{b}	65,00 ± 1,67^{d}	52,00 ± 3,90^{c}	62,50 ± 3,33^{c}
Average daily increment (mm)**						
PDA	5,40 ± 1,59^{b}	4,81 ± 1,37^{c}	7,67 ± 1,56^{a}	8,89 ± 4,28^{a}	8,58 ± 2,66^{a}	8,74 ± 3,22^{a}
CLA	5,68 ± 3,10^{ab}	7,26 ± 1,82^{a}	7,97 ± 1,87^{a}	7,99 ± 4,11^{a}	7,82 ± 1,77^{a}	7,81 ± 1,73^{ab}
V8A	6,80 ± 0,64^{a}	7,17 ± 1,25^{a}	8,02 ± 2,14^{a}	8,60 ± 2,84^{a}	8,32 ± 2,39^{a}	8,00 ± 1,50^{ab}
MA	3,25 ± 1,36^{c}	3,33 ± 1,06^{d}	3,82 ± 1,17^{c}	8,61 ± 2,31^{a}	7,70 ± 1,23^{a}	5,61 ± 0,67^{c}
WA	4,16 ± 2,33^{c}	6,10 ± 1,76^{b}	5,33 ± 2,00^{b}	7,51 ± 6,17^{a}	5,51 ± 2,92^{b}	7,01 ± 4,58^{bc}
Sporulation ***						
PDA	+	–	+	++++	+++++	+++++
CLA	–	–	–	+++++	+++++	+++++
V8A	–	–	–	+++++	+++++	++++
MA	–	–	–	+++++	+++++	+++++
WA	–	–	–	+++++	++++	+++++

* Values marked with the same letter do not differ statistically (Duncan test; p≤0,05);
* Average daily increment calculated by the formula: (D2 – D1) / (T2 – T1), where D2 and D1 is the diameter of the culture of the last and first measurement, and T2 and T1 time (in days) of the last and first measurement;
*** Sporulation: (-) no sporulation, (+) very weak, (++) weak, (+++) medium, (++++) abundant, and (+++++) very abundant sporulation.

Radial colony growth. The radial growth of colonies of all six studied isolates was statistically significantly affected by different substrates (Table 1).

The largest radial growth of colonies of all three studied isolates from group A was achieved on V8 agar, then on potato-dextrose agar, except for isolate BM-2 where a smaller radial growth was observed compared to other tested isolates, while the studied isolates from group B achieved some greater growth on potato-dextrose agar compared to V8 agar. On the carrot leaf agar, the radial growth of the tested isolates was quite uniform, while on the water agar, the radial growth of all tested isolates varied significantly (Table 1). The obtained results are in accordance with the statements of Simmons (2007) who, in addition to potato dextrose agar recommends V8 agar and carrot leaf agar (CLA) as very favorable for growth rate and sporulation of *Alternaria* spp. Such results are also in accordance with the statements of Pryor et Gilbertson (2002) and Bulajić (2006) who also found that there are isolates such as BM-2 isolate that do not fit into the regularities observed by these authors in their work to distinguish individual species within the genus *Alternaria*.

Average daily increment. Different substrates had a statistically significant effect on the average daily increment of colonies in most of the tested isolates, except for isolates Aa-82 where there was no statistically significant effect of the substrate on this observed property (Table 1).

Sporulation. The effect of nutrient substrate on the intensity of sporulation of the studied isolates from group A (CLA-108, BM-2, FM-15) and group B (Aa-82, IM-14, Mr-114) varied, while sporulation on all substrates in the studied isolates from group B (Aa-82, IM-14 and Mr-114) was abundant and very abundant, but sporulation in isolates from group A (CLA-108, BM-2, FM-15) was absent on all substrates, except for isolates CLA-108 and FM-15 on potato-dextrose agar on which the sporulation of these isolates was assessed as very weak, while in isolates BM-2 sporulation was also absent on this substrate (Table 1).

These results are in agreement with the results of Gupta et al. (1979), Strandberg (1987), Pria et al. (1997) and Bulajić (2006) who studied the growth and sporulation of *Alternaria* spp. on different nutrient substrates. In addition, the obtained results show that the isolates of group A sporulated very poorly, which is also stated in the literature for the species *A. dauci* (Strandberg, 2002; Bulajić, 2006). As in vitro sporulation of fungi of the genus *Alternaria* is a common problem that accompanies their study, Strandberg (2002) also designed a carrot leaf agar (CLA) to encourage their sporulation. However, in this paper it was found that all three isolates of *A. dauci* on this agar did not sporulate, while Krishna et al. (2018) state that this species is well to abundantly sporulated. Abundant sporulation on the CLA agar *A. dauci* is also reported by Strandberg (2002), who in this paper did not prove to be favorable for the sporulation of this pathogen.

Conclusions

Testing the breeding traits of selected isolates of Carrot pathogens *Alternaria* spp. in Bosnia and Herzegovina and Serbia, showed that these fungi have a very wide range of environmental conditions in which they can grow and sporulate. The tested isolates managed to grow and sporulate on all nutrient substrates that were included in the tests in this paper, which indicates the great adaptability of the tested isolates to virtually all environmental conditions that can occur in nature, which may be due to their variability and prevalence in different climatic and ecological conditions. The largest growth in colonies of the studied isolates of group A was on V8 agar, and the isolate of group B on potato-dextrose agar.

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ANALYSIS OF FATTY ACIDS IN SELECTED SAMPLES OF COCONUT OIL

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Abstract

Edible oils of plant origin are a rich source of fatty acids and lipophilic antioxidants. In addition to contributing to a high energy value in the diet, oils contain high nutrients, vitamins, and minerals that are beneficial to human health. Among them are oils obtained from coconut kernel, which include dill oil (CO), virgin coconut oil (VCO), and refined, bleached, and deodorized (RBD) oil. Depending on the method of preparation, their composition and biological effects vary. This study aimed to determine the content and composition of fatty acids in different samples of coconut oils that can be found on the market of the Republic of Serbia. Gas chromatography with a flame ionization detector determined the content and composition of fatty acids in different samples. Statistical processing of the data presents the results as percentages of individual fatty acids concerning the total. In addition, the compared physicochemical and biological properties of coconut oils prepared by different methods were performed. It was observed that there is no significant difference in the percentage of fatty acids in different samples of coconut oil. All samples contained the highest rate of lauric acid, 49.31 ± 0.63 relative to total fatty acids. It is followed by myristic (19.35 ± 0.46), caprylic (7.16 ± 0.68), and palmitic (8.47 ± 0.68). The content of monounsaturated acids, oleic (5.19 ± 0.44) and linoleic (0.84 ± 0.12), is highest in samples of the virgin, cold-pressed oils. As the percentage of monounsaturated fatty acids is small, it can be concluded that the tested oil is relatively stable, i.e., resistant to thermal modifications. Therefore, it is best to use them as edible unrefined vegetable oils due to the preservation of active ingredients with favorable health effects.

Keywords: *coconut oil, saturated and unsaturated fatty acids, gas chromatography*.

Introduction

Lipids are considered to be one of the essential nutrients for humans. Lipid metabolism produces many bioactive molecules, which are the basic mediators of several signaling pathways and are also necessary compounds in the construction of cell membranes. Therefore, changes in lipid metabolism can result in modification of membrane composition and permeability, as well as some pathological conditions, such as cancer, cardiovascular, neurodegenerative, and metabolic diseases.

Lipids consist of fatty acids (FAs) classified mainly according to the presence or absence of double bonds: saturated (SFAs) without double bonds, monounsaturated (MUFAs) with one double bond, and polyunsaturated fatty acids (PUFAs) - with two to six double bonds; further as cis or trans based on the configuration of double bonds and as n-3 or n-6 (Orsavova, 2015). Vegetable oils have been part of the diet since ancient times and are a source of essential fatty

acids. Therefore, they play an important role in determining the physiological and biochemical state of the organism. The oil can be found in plant cells, associated with proteins and carbohydrates such as starch, cellulose, hemicellulose, and pectins (Marina, 2009). Based on the composition, the oils are divided into polyunsaturated (*e.g.*, sunflower oil), monounsaturated (*e.g.*, mustard oil) and saturated (*e.g.*, palm and coconut oils). Among them, oils produced from coconut (*Cocos nucifera* L.) are intensively used for food and industrial purposes (Narayanankutty et al., 2018).
Coconut oil is an edible oil extracted from the kernels of ripe coconuts harvested from the coconut palm, which is grown in humid tropical areas (Health Effects of Coconut Oil-A Narrative Review of Current Evidence, 2019). Mechanical or thermal processes mainly obtain it. Due to a large amount of saturated fatty acids, it is resistant to oxidative modifications such as hardening or thermal oxidation, making them ideal for cooking. Based on the method of processing, coconut oils are divided into copra oil (CO), virgin coconut oil (VCO), and coconut testa oil (CTO) oil. CO and RBD oil are produced from dried coconut kernel, with RBD oil also going through chemical refining and bleaching. Coconut seed oil can be extracted using isopropyl alcohol from coconut seeds. VCO oil is obtained from fresh coconut milk, extracted naturally, and not processed chemically or thermally. This difference in the production itself causes changes in the chemical composition and physiology of different types of oils (Narayanankutty et al., 2018). Based on the production method, there are several types of VCO oils. Using fermented oil and oil obtained by hot extraction has shown more efficient removal of radicals and prevention of lipid peroxidation than oil obtained by cold extraction. Although they have similar fatty acid profiles, VCOs contain higher amounts of some nutrients (*e.g*., vitamin E) and biologically active compounds (*e.g*., polyphenols). The composition of fatty acids is similar in all types of coconut oil. However, there is a relatively higher level of unsaturated fatty acids in CTO oil than in VCO oil and CO, with a simultaneous reduction of medium-chain saturated fatty acids (MCFA). Other oils contain large amounts of MCFA. Among them, lauric (C12:0) acid (45-52%) dominates, followed by myristic acid (15-19%) and palmitic acid (10-11%) (Narayanankutty *et al.*, 2018). Lauric, caprylic, and myristic acid triglycerides are also present in different types of coconut oils (Narayanankutty *et al.*, 2018).
The popularity of coconut oil is growing due to the beneficial health effects of medium-chain fatty acids. However, further human studies are needed to confirm the positive effects of coconut oil, and the current ones are mainly based on in vitro studies and/or the results of animal studies (Wallace, 2019). The aim of this paper is to determine the content of fatty acids in ten different samples of coconut oil present on the market of the Republic of Serbia. The method used in this work is gas chromatography with a flame-ionizing detector. After that, the percentage of fatty acids in all samples was determined.

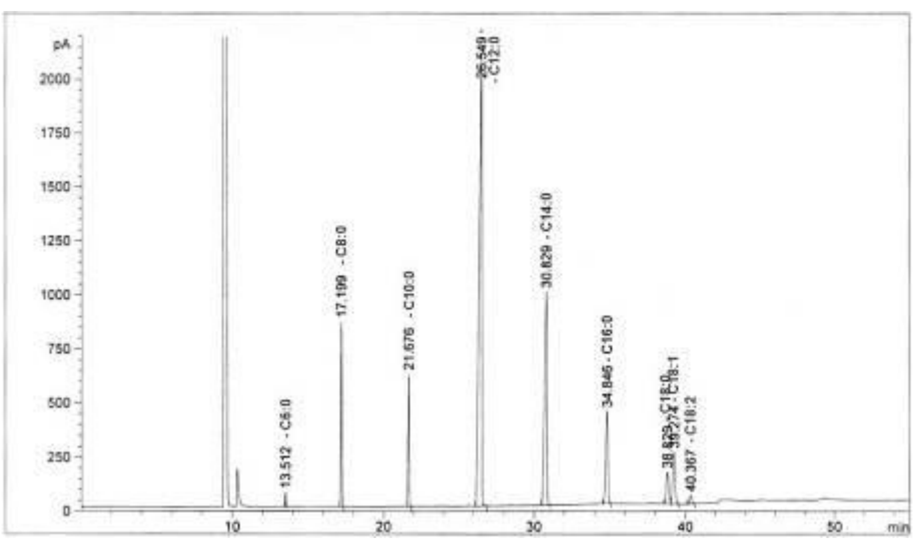
Material and Methods

In the experimental part of this paper, the content of fatty acids in ten different samples of coconut oil available on the market of the Republic of Serbia was examined. The coconut oil preparations used in the test are Lučar-cold-pressed; Lučar-organic cold-pressed; dm Bio coconut native-unrefined coconut oil; DTC-Organic Extra Virgin Coconut Oil; GranumFood - organic coconut oil; Twisted - cold-pressed; Healthy food - "Zegin" - organic; Linum - cold-pressed; Beyond - organic extra virgin coconut oil; Sanaterra long-life - organic cold-pressed.

After the derivatization of fatty acids into volatile methyl esters, the composition and content of fatty acids were determined by gas chromatography with a flame-ionizing detector (FID). Fatty acid methyl esters were obtained by transesterification with a solution of hydrochloric acid (NSĺ) in methanol, as described in the method by Ichihara and Fukubayashi, (2010). Fatty acid methyl esters were tested by gas chromatography on an Agilent Technologies 7890A with an FID detector. A capillary column SR-Sil 88 (100 m x 0.25 mm x 0.2 µm) was used, and helium (chromatographic quality) was an overnight gas with a flow rate of 1 ml/min. Samples were injected at a column temperature of 80 °C, the injector temperature was 250 °C, and the detector temperature was 270 °C. The column temperature increased from 80 °C to 220 °C at a rate of 4 °C/min, and after 5 minutes, at the same rate of 4 °C/ min, it increased to 240 °C, and the column maintained this temperature until the end of the analysis (Ichihara and Fukubayashi, 2010). Fatty acids were identified based on the retention times of a standard mixture of fatty acids (Supelco FAME Míh, Bellefonte, PA). The efficiency of the column is shown through the number of theoretical plateaus of a standard mixture of fatty acids. At the same time, the repeatability of the response was determined as a percentage of the relative standard deviation (RSD%) of successive measurements of the same reference solution. The separation power of the column is estimated based on selectivity factors and resolution factors (Clodoveo *et al*., 2022).

Results and Discussion

The qualitative composition of fatty acids in coconut oil samples was determined by gas chromatography. The first peak that appeared on the obtained chromatograms came from hexane coming out at the 10th minute, and then the fatty acid peaks in the following order: caproic (6:0), caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1 n-9), linoleic (18:2 n-6). An example of the chromatogram of the analyzed samples is shown in Figure 1.



Sample 1. Chromatogram of the analyzed samples

The content of fatty acids in the tested samples of coconut oil is shown in Table 1. The presented results represent the mean value of three repeated determinations for each sample.

Table 1. Content of individual fatty acids (expressed in %) in relation to total fatty acids in the sample

	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
Lučar	0.50	7.21	6.08	49.52	19.25	8.30	3.18	5.17	0.80
Lučar (org)	0.48	6.92	5.88	49.24	19.42	8.74	3.29	5.22	0.82
dm Bio	0.50	7.39	6.34	49.87	18.67	8.35	3.03	5.07	0.78
DTC	0.48	7.45	5.87	49.53	19.80	7.88	3.20	5.03	0.77
GranumFood	0.50	7.44	6.23	50.00	19.04	8.21	3.09	4.68	0.81
Uvita	0.45	6.76	6.03	49.01	19.04	8.99	3.52	5.35	0.85
Zegin	0.31	5.62	5.44	47.89	20.16	9.78	3.27	6.34	1.19
Linum	0.54	8.02	6.33	49.69	18.88	7.76	3.02	4.94	0.81
Beyond	0.46	6.87	5.85	48.75	19.63	9.08	3.49	5.11	0.76
Sanaterra	0.54	7.88	6.01	49.59	19.58	7.61	2.97	5.00	0.84

*Source: authors results

After statistical data processing, the mean values of the percentage of fatty acids (Xmy) and standard deviation (Sd) were calculated. The standard deviation ranged from 0.07% -0.68%. It is noticed that the difference in the percentage of fatty acids in different samples is not significant, which is shown in Figure 2.

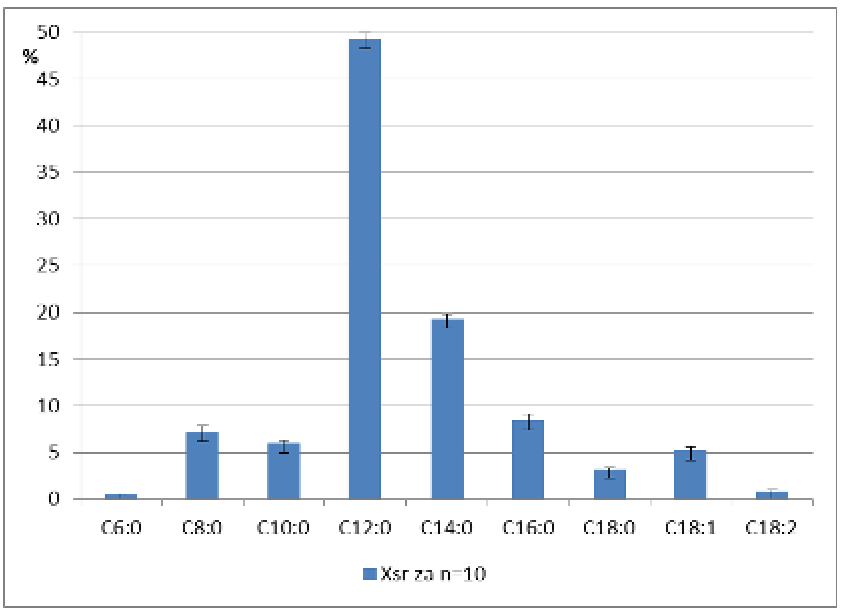


Figure 2. Mean values of the percentage of fatty acids with standard deviations

Studies show that the fatty acid content is relatively consistent with the results obtained in previous studies (Marina, 2009; Dayrit, 2014; Elsayed, 2015; Kappally, 2015; Osavova, 2015; Narayanankutty *et al.*, 2018;). In the examined samples, the most common lauric acid (44.5% - 49.3%), followed by myristic (16.5% - 19.9%), then caprylic (5.62% - 8.02%), which is equivalent to the previous findings. These fatty acids are absorbed and transported by the portal circulation directly to the liver, where they are used to obtain energy (Glick and Fischer, 2013).
In the study of Narayanankuttyi *et al.* (2018), the content of all fatty acids is within acceptable deviations except caprylic (C8:0), where its share in coconut oil in this study was almost

negligible, i.e., 0.8%, while measurements performed in this work showed a significantly higher presence of the same acid, a total of 7.2%.
Analysis of the composition of fatty acids and their effects on the diet (Osavova, 2015) showed the complete absence of palmitic acid (S16:0) in coconut oil. However, the analysis of the samples in this paper showed that palmitic acid is present at 8.4%. Previous studies that followed the importance of coconut oil in the diet and fatty acid content (Marina, 2009; Dayrit, 2014;) showed almost the same proportion of fatty acids in coconut oil as in our study. Minor deviations may result from various interferences and/or human factors or variations in sample preparation. It follows that the fatty acids in coconut oil are relatively stable and have approximately the same percentage in different samples. When comparing the total fatty acid content in different studies, it was found that saturated fatty acids represent a significant proportion of coconut oil. The percentage content of saturated fatty acids ranged from 84.8% (Narayanankutty *et al*., 2018) to 94.3% (Marina, 2009), which coincides with our measurements, whose final result for saturated fatty acids was 94%. The profile of saturated fatty acids is much more diverse, and the most common was lauric acid with 47.5%, followed by myric, caprylic, palmitic, capric, and stearic acid. It was observed that the content of monounsaturated fatty acids, i.e., oleic (C18:1), differing only in comparison with the results obtained in studies conducted by Kappally (2015) and Elsayed (2015), where the content of MUFAs was 2% and 0.06 %, respectively, while the other results are equivalent to our measurements - it was found that monounsaturated fatty acids are present in a proportion of about 5% in coconut oil.
Oleic acid-rich oils are essential in the diet because they have been shown to lower blood lipids, especially cholesterol (Lopez-Huertas, 2009). In addition, it has been proven that oleic acid influences the inflammatory response that characterizes the early stages of atherosclerosis and thus reduces the incidence of cardiovascular diseases. Oleic acid is incorporated into the phospholipid bilayer of the cell membrane at the expense of saturated fatty acids and reduces the expression of adhesion molecules, especially - vascular cell adhesion molecules (VCAM-1). It also reduces mRNA activation for this molecule and interferes with activating the most important transcription factor, the nuclear factor kappa-B (Massaro, 1999). Studies have shown the antioxidant potential of various VCO oil products, especially fermented ones. Among the results of many VCO oil studies, Nevin and Rajamohan (2006) stand out as the first to report the antioxidant activities of the oil in rats. In addition to improving the activity of antioxidant enzymes, fermented VCO oil also increases intracellular reduced glutathione levels in cell culture and the tested animals. The coconut seed oil is the least studied of all coconut oils, although there are indications that it also has good antioxidant properties.
The anti-inflammatory effect of VCO oil was proven several years ago. Studies have shown a protective effect of fermented VCO oil in granuloma formation in chemically induced models of ear and paw edema. Studies have observed that fermented VCO oil effectively reduces acute inflammation, while in chronic inflammation, it is less effective. In addition to anti-inflammatory effects, fermented VCO oil also has anti-analgesic effects. Other VCO oil products have not been found to have anti-inflammatory effects (Narayanankutty *et al*., 2018).
When it comes to the percentage of polyunsaturated fatty acids and refers to linoleic acid (C18:2), our analyzed samples include a share of 0.9% of the total fatty acid content, which was confirmed by previous studies that examined the effects of coconut oil on human health, where the content ranged from 0.2% (Kappally, 2015) to 2.58% (Elsayed, 2015). This low content of unsaturated fatty acids is important in heat treatment because it contributes to better stability.

Conclusions

Based on the obtained results in this paper, it was noticed it was noticed that there is no significant difference in the percentage of fatty acids in different samples of coconut oil present on the market of the Republic of Serbia. coconut oil contains 94% of saturated fatty acids, of which lauric makes 49%, then myristic, which has 19%, and only 6% are unsaturated fatty acids and monounsaturated (oleic and linoleic). As the percentage of monounsaturated fatty acids is tiny, it can be concluded that the tested oil is relatively stable, i.e., resistant to thermal modifications. The popularity of coconut oil is growing due to the observed beneficial health effects of medium-chain fatty acids, and in order to confirm these claims about beneficial health effects, further research on humans is needed, as the current ones are based mainly on in vitro tests or animal studies.

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EFFECTS OF INSECTICIDES APPLICATION, FUNGAL AND ECB INFECTION ON CAROTENOIDS CONTENTS IN MAIZE LEAVES

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Abstract

There are two types of carotenoids in plants which can be further categorized into two classes: the unoxygenated carotenoids (β-carotene and lycopene) and the oxygenated xanthophylls (lutein and zeaxantin). As effective antioxidants carotenoids have an important role in protecting plants from oxidative damage caused by abiotic and biotic stresses. Insecticide application is necessary in maize production, due to high levels of ECB pest pressure causing maize yield losses. The aim of this study was to evaluate effects of two insecticides (deltametrin and chlorantraniliprole), ECB and fungal infection on carotenoids contents in maize leaves of two hybrids. Leaves were collected in reproductive stage two weeks after foliar insecticide applications, three weeks after egg mass infection and 48h after *Aspergillus parasiticus*. The content of carotenoids was determined using high-performance liquid chromatography (HPLC) with an ultraviolet multi-diode detector. Deltametrin application + ECB infection increased content of lutein + zeaxanthin in leaves of hybrid I compared to control (no herbicide application). On the contrary, content of β-carotene decreased after insecticides application, fungal and ECB infection compared to control. In hybrid II the highest content of lutein + zeaxanthin and β-carotene was in control. Also, after deltametrin application + ECB infection content of β-carotene and lutein + zeaxanthin in leaves of hybrid I was higher compare to control + ECB infection. The results indicate that variations in carotenoids content as an antioxidant defense mechanism are caused by abiotic (insecticides) and biotic (fungal and ECB infection) stress.

Keywords: *European corn borer, abiotic and biotic stress, HPLC, Zea mays L.*

Introduction

Maize (*Zea mays* L.) is considered the third most important cereal crop after wheat and rice worldwide. According to the data obtained from the Statistical Office of the Republic of Serbia in 2021, the Republic of Serbia produced a total of 5.9 t/ha of maize. Climate change with increased average air temperatures and the amount of rainfall can cause frequent pest invasion and maize grain yield loss. The two important biotic stresses in different maize growing stages are European corn borer pest attack and infection by fungal parasites. ECB (*Ostrinia nubilalis* Hübn.) may attack the maize plants causing the characteristic symptoms of elongated tunnels and circular holes, that can promote the progression of stalk rot and create points for fungal infection (Razinger et al., 2016). ECB larvae can reduce plant growth and cause stalk and ear damage, leading to an in yield decrease of up to 30% (Bohn et al., 2000). On the other hand ECB can also serve as a vector of different fungal pathogens such as Aspergillus and Fusarium species, and thus favoring high levels of fumonisins in maize kernels (Butrón et al., 2009). *A. parasiticus* has a worldwide distribution and normally occurs as saprophytes in soil and can infest important crops such as maize, with increased average temperatures (Boyd and Cotty, 1998). In addition to direct losses from maize grain infestation, *A. parasiticus* synthesizes aflatoxins, which from the aspect on human and animal health represent the most significant group of mycotoxins (Bennett and Klich, 2003). According to FAO data, about 25% of cereal production in the world is contaminated with mycotoxins (Devegowda et al., 1998).

The most common strategy to control *Ostrinia nubilalis* is direct foliar application of insecticides. In many European countries, ECB conventional control relies on foliar spray of broad-spectrum synthetic insecticides, with well-known side effects including negative impacts on non-target organisms and the risk of resistance development (Vasileiadis et al., 2017).

Combination of insecticides and biotic stresses can cause cell damage and production of free radicals and reactive molecules derived from molecular oxygen commonly named - reactive oxygen species (ROS) (Sies et al., 2022). Increase in ROS production leads to changes in phytochemicals such as photosynthetic pigments of chlorophyll and carotenoids, in order to minimize stress-induced damage. Maize leaves are a source of phytochemicals such as carotenoids (β-carotene and lycopene) and xanthophylls (lutein and zeaxanthin) (Zhao et al., 2003). Carotenoids serve as additional pigments in the photosynthetic apparatus (Niyogi, 2000) forming a key part of the plant non-enzymatic antioxidant defense system (Dumanović et al., 2021).

The aim of this research was to examine physiological response of two maize hybrids based on carotenoids content, to two different insecticides, as well as ECB attack and fungal infection.

Materials and methods

Plant materials

Three maize hybrids (two were chosen for analysis) were sown in randomized complete block design (RCBD) in the experimental field of the Maize Institute “Zemun Polje” (Belgrade, Serbia) during 2021. Chosen hybrids belong to different FAO maturity groups (H1 - 400 and H2 - 500).

Plants were treated with two insecticides chlorantraniliprole (Coragen, Du Pont, concentrated suspension 100 ml ha^{-1}) and deltametrin (Decis 2.5 EC, Bayer, concentrated emulsion in a quantity of 400 ml ha^{-1}). Leaves were collected in the reproductive stage two weeks after foliar insecticide applications, three weeks after egg mass infection and 48h after *Aspergillus parasiticus* infection.

Sample preparation and biochemical analysis

For the extraction of the carotenoids, 0.100 mg of fresh leaf tissue was extracted with 5 mL MeOH for 30 min in an ultrasonic bath. The content of the carotenoids was determined using high-performance liquid chromatography (HPLC) with an ultraviolet multi-diode detector. Chromatographic separation of carotenoids in leaf extract was carried out on a Hypersil GOLD® C18 column, dimensions 150 × 4.6 mm, particle size 3 μm (Thermo Fisher Scientific). The content of carotenoids was presented as a mean value of two replicates as μg/g fresh weight (FW). The Chromeleon 7.2 Chromatography Data System was used for instrument control as well as for data acquisition and analysis.

Result and discussion

Occurrence of pest invasion in maize production can cause significant yield decrease. The chemical treatment of pest control is an important part in cropping practices of maize hybrids

(Saladini et al., 2008). In addition to the biotic stress caused by ECB attack and fungal infection, application of the insecticides, depending on concentration, can induce the abiotic stress in plants. Obtained results of carotenoids contents in maize leaves of the two analyzed maize hybrids with two different insecticides treatment and ECB attack and fungal infections are presented in Table 1.

Table 1. Carotenoids content in maize leaves (µg/g fresh weight)

Hybrid	Insecticides treatment	No infection		*A.parasiticus*		Egg mas	
		ß-carotene	Lutein + zeaxanthin	*ß*-carotene	Lutein + zeaxanthin	*ß*-carotene	Lutein + zeaxanthin
H1	Control	938.37	375.71	652.21	328.55	415.94	333.46
	Decis	820.87	587.11	845.54	581.065	835.48	662.73
	Coragen	803.95	522.29	418.93	486.39	733.87	496.14
H2	Control	938.36	717.21	941.17	691.15	608.05	447.62
	Decis	812.16	503.77	846.70	590.91	721.68	534.34
	Coragen	572.75	428.87	653.58	488.03	528.85	382.19

In H1 leaves after Decis and Coragen application content of lutein + zeaxanthin was higher compared to control (plants without insecticides application). Also, it is observed that the content of lutein + zeaxanthin was slightly lower comparing plants with no insecticides content and no infection, with plants with infection. Similar findings were in work of Perrin et al. (2017) where biotic stress conditions impacted leaves carotenoid contents in a lesser extent, depending on the genotype and the year. On the other hand, content of *ß*-carotene was lower in most combinations of insecticides treatment and infections in both hybrids. In H2 *A. parasiticus* infection indicates the higher content of ß-carotene than control, also plants with Decis application showed higher levels of *ß*-carotene after *A.parasiticus* infection. Same trend was observed with a combination of Coragen and *A.parasiticus*. Higher content of *ß*-carotene was noticed in H1 in combination of Decis and *A.parasiticus* i.e. egg mass. Stronger antioxidant response of H1 can be due to different FAO maturity group (400), which is more tolerant to ECB attack than later maturity groups. Obtained results are consistent with results in previous work Brankov et al. (2019) where herbicide application did not affect carotenoids content. Opposite was found in research on rice with oxadiazon (Langaro et al., 2017). According to our knowledge, there is no research dealing with the effects of ECB infestation on carotenoid content in maize leaves. For that reason it is useful to conduct additional research in the direction of analyzing the effect of ECB attack and fungal infection on the non-enzymatic antioxidant defense system.

Conclusion

Changes in carotenoids content in leaves can be considered as an antioxidant defense mechanism of plants due to the stress caused by insecticides application, as well as *A. parasiticus* and egg mass infection. In order to obtain more precise results of the relative antioxidant response in maize hybrids on the selected insecticides and fungal and ECB infection, it is necessary to conduct additional research with more maize genotypes and analyzing more antioxidant parameters.

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MODERN TECHNOLOGY OF GROWING LATERAL WALNUT VARIETIES IN REPUBLIC SERBIA

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Abstract

The progressive growth of walnut production in Serbia has influenced significant innovations in cultivation technology and the introduction of lateral varieties into the culture. Higher planting density, faster yielding, thus faster return of funds favor them in relation to terminal varieties. Intensive plantations are being built in the image of large plantations in California, Italy and Turkey. Planting distance is 6x4m if machine pruning and cultivation form "hedge" or 7x5m if walnut is grown in cultivation forms pyramidal or boiler crown. Leading lateral varieties are Chandler, Pieral Lara, Tulare, Fernor as well as pollinators Franquette, Fernet and others. The presence and arrangement of pollinators is a very important segment in the establishment of plantations. From the technological and commercial aspect, the mentioned varieties take precedence for the following reasons: higher oil content in the core, higher yield (ratio of shell and fruit core), easy shell fragility and distinct white core with an average weight of about 14g. The production of lateral varieties of walnuts is disrupted by the appearance of the following harmful agents: B*renneria rubrifaciens*, *Xanthomonas arboricola* pv. *juglandis* (bacteria), *Gnomonia leptostyla* (fungi), *Zeuzera pyrina* - leopard moth, *Rhagoletis complete* - walnut fly, *Cydia pomonella* - apple weevil (harmful insects). Field work has shown that adult leopard moth appears quite early, at the end of May, and that the larvae on young seedlings in the first and second year do great damage. Stable yield and high quality fruit is possible with adequate protection (6-8 treatments), regular irrigation and fertilization.

Key words: *walnut, lateral varieties, harmful agents, cultivation technology*.

Introduction

The walnut (*Juglans regia*) belongs to the Juglandaceae family and originates from Central Asia, which is why it is often called the Persian walnut. It belongs to nut fruits (nuts) and until 2013 it was in second place behind almonds in terms of total production, while after the mentioned year, walnuts took the lead (Büyüksolak et al., 2020). Based on FAO data in 2019, the five largest producers of walnuts are China, USA, Iran, Turkey and Mexico, with China producing almost 50% of the world's quantity of walnuts. The walnut has a high caloric value (654 kcal 100 g−1), as well as a significant content of the following substances: proteins (15.2 g/100 g of fruit), lipids (65.2 g/100 g of fruit), carbohydrates (13.7 g/100 g of fruit) (USDA, 2016.) as well as a high content of unsaturated fatty acids (oleic, linoleic and linoleic) (Martinez et al, 2010)Many authors mention the high antioxidant effect of walnuts due to the content of numerous useful substances in the fruit itself (Beyhan, 2009; Polat et al. 2015.; Nunes et al. 2012.). In addition to the high nutritional value, the shell of the walnut can be used as firewood, high-quality walnut oil is obtained from the fruit by cold pressing, and the residues during oil production (oil cakes) can

be used for the production of animal feed (concentrate mixture). In order to obtain varieties with the best possible characteristics and tolerance to various climate changes, harmful agents, etc., in several countries intensive tests of walnut germplasm and work on breeding are carried out (Tamponi et al. 1997.; Aleta & Ninot, 1997.; Atef, 1997.; Molnar et al. 2012.; Poggetti et al., 2017.). In the territory of the Republic of Serbia, walnut cultivation has been in serious expansion for the last 5 years and 90% of the newly formed plantations are lateral varieties of walnut. In contrast to the old, terminal walnut varieties, the lateral varieties are characterized by a higher planting density (larger number of seedlings per unit area), a faster onset of fruiting, thus a faster return of the funds spent on raising the orchard. Intensive plantations are built on the model of large plantations in California, Italy, and Turkey. The planting spacing is 6x4m if mechanical pruning and the "hedge" cultivation form is used, or 7x5m if the walnut is grown in the pyramidal or vase crown cultivation forms. Lateral varieties of walnut produce fruit immediately in the first growing season after planting, however, in order not to disturb the normal development and growth of the plant, these fruits are removed. If it is in good condition so far, a couple of fruits can be left per tree from the third year, and from the fourth year the whole fruits is left. The full fruiting of the mentioned variety is achieved after the tenth year if the orchard is professionally formed and maintained. In full fertility, the mentioned varieties have the potential to produce over 50 kg of fruit in the shell per tree with a yield of about 50% (ratio of shell to core) depending on the variety. In addition to all the mentioned advantages compared to the older assortment of walnuts and the generally more extensive type of cultivation, the lateral varieties are characterized by an extremely white kernel, which, precisely because of this characteristic, achieves significantly higher prices on the market.
The most important varieties of lateral type walnuts are: Chandler, Pieral Lara, Tulare, Fernor as well as pollinators Franquete, Fernet (https://www.orahsadnice.com/vrste-oraha/; https://www.orasi.rs/#). The aims of this study is to present modern techology of walnut production and protection.

Walnut planting and maintenance

Walnut planting is done from mid-November to mid-March, depending on climatic conditions and locality. It is best to plant in autumn if conditions permit. Autumn seedlings will achieve good contact in the rhizosphere zone during winter and intense rainfall, and in spring, as soon as the conditions are established, they will immediately start their physiological processes. The mentioned guarantees a significantly better and easier scarification of seedlings.

Planting material: Before planting, it is most important to obtain healthy and high-quality planting material with all accompanying documentation and certificates. Walnut seedlings should be two years old, with a visible place of grafting, normally developed and distributed buds on the stem, a well-ripened top of the seedling and no changes in the bark (yellow, orange zones, black spots, depressions or cracks caused by batteries, as well as holes caused by woodworm larvae). During intensive nursery production, it is often resorted to the forced end of vegetation in order to carry out the extraction and transport of planting material on time. The procedure itself can cause the seedlings to be insufficiently matured, so after planting, serious damage can occur due to low temperatures. Also, if the buds on the tree are unevenly distributed (on a certain part of the tree close to each other and on a certain part of the tree very far apart) it can indicate a serious stress that the plant experienced during its development. The roots of two-year-old seedlings should be strong, well-developed and without tumorous cancer wounds.

Preparation for planting and planting: Rows and individual places for planting material are marked on the prepared plot. In relation to the direction of flow of dominant winds, a plan for the distribution of pollinators is made. The percentage of pollinators is 5-10%. The role of the pollinator is extremely important for the plant itself, because its good distribution and participation in the varietal structure accelerates the process of pollination and fertilization, and thus significantly affects the yield. Disinfection of planting material is a very important segment and a mandatory step before planting. Disinfection of the root system is carried out with the help of preparations based on copper, fosetyl-aluminum or a combination of two active substances mancozeb + metalaxyl (https://www.fitofarmacija.rs). Preparations based on the mentioned active substances have a bactericidal and fungicidal effect. In addition to the mentioned preparations, the root system of walnut seedlings can also be immersed in solutions of microbiological fertilizers from different manufacturers, fertilizers with an increased content of phosphorus and amino acids, all with the aim of a better and faster healing process. It is important to check the compatibility and miscibility of the mentioned mixtures before the disinfection process itself and the addition of other preparations for scarring.
After marking, dig holes 50-60 cm deep and 50 cm in diameter. 1-2 shovelfuls of “well-burnt” manure is added to the bottom of the hole, depending on the quality. It is simpler and safer to use pelleted manure (200-300g) which is available on the market, simple and easy to manipulate and has good chemical characteristics without admixture of weed seeds and impurities. About 15 cm of soil is added over the manure layer and creates a "buffer zone" between the seedling's root system. After that, the seedling is placed in the hole, if it is absolutely necessary, a minimal shortening of the veins is performed (when extracting the seedlings, the root system is already shortened). A layer of special soil mixtures for flowers or a substrate for vegetable production is added around the root itself, with a suitable pH value, very fine structure, high absorption capacity with the addition of micro and macro nutrients. The mentioned substrates will provide the plant with initial food that is easily accessible and necessary for the first stages of the functioning of the root system. The rest of the hole is filled with loose soil up to the top, and care must be taken to ensure that the grafting point is a few cm above the soil surface. After the planting is complete, a support in the form of stakes or pillars made of wood or PVC material is placed next to each seedling-new plant.

Soil maintenance: During the first few years, it is important to maintain a loose layer of soil in the plantation, especially in the row zone. Several times during the growing season, it is necessary to eliminate weeds and shallow surface cultivation of the soil with rotary tillers, disc harrows or seedbed cultivators. After 2 or 3 years, the orchard can be sown with a grass mixture for orchards if it is currently under an irrigation system. Otherwise, it is not recommended to sow a grass mixture because it would affect the moisture deficit in the soil. The last way to maintain the soil in the plantation is a combination of tillage and the use of total (a.m. glyphosate) and contact (glufosinate-ammonium) herbicides in order to suppress weeds. Application of herbicides in the plantation must be strictly controlled and in accordance with the application instructions. The occurrence of phytotoxic effects must be avoided if there is a drifting of the herbicide spray on the young walnut leaves.

Irrigation: Intensive plantations cannot do without an irrigation system. The drip system is most often applied. The method of irrigation can be surface type, sub-irrigation system or a combination of both mentioned. The most commonly applied line system is drop by drop, which is in a row of walnuts with droppers that are at a certain distance from the tree. In the case of a tree in the first growing season, there are usually two drippers at half a meter on both sides, in the second growing season, one more can be added on both sides at half a meter from the previous ones. As the walnut crown expands, its dynamics is followed by the root system, so the projection of the crown is a mirrored projection of the root system. This means that with age comes an increase in the demand for water, and the most absorptive part of the root is further away from the main stem and the central axis. Therefore, from the fourth growing season, they can be excluded from the use of the first dripper, closest to the tree. The dropper line should not be placed too low or too high. A low-mounted system makes it difficult to tillage the soil, while a high-mounted system with drippers can be subject to the influence of wind, wetting the tree, etc. The optimal height is 50-80 cm. Droppers can release liquid in the form of drops or in the form of a fan with a sprayer that regulates the flow. If droppers with a sprayer are used, care must be taken that the peripheral arc of the liquid fan does not wet the walnut tree because this can cause negative consequences (the appearance of fungal and bacterial diseases of the tree and roots). The most modern system is a combination of subirrigation with surface hoses on which there are drippers, which can expand with the growth of the plant and its roots and make a larger ring around the plant. It is especially important for young plants to have sufficient amounts of water in order to be able to receive and have a normal growth of young plants during the growing season. The most critical period is during the dry months, July and August, when the need for water is significantly higher. The amount of water per tree is highly variable and cannot be universally defined. Irrigation requirements are higher on lighter soils that have a lot of sand in their composition, while the reverse is the case with heavy soils. During the dry period, it is necessary for a young plant (up to 3 years of age) to receive between 25 and 35 l of water on a weekly basis through 2 waterings. The best rhythm of watering can be achieved by regular and detailed monitoring of the plants.
In addition to the amount of water per plant, the watering time is also a very important parameter. It is best to give the water in the late afternoon or during the night, when temperatures are lower, evaporation is reduced, and thus the potential for phytopathogenic pathogens to appear. Watering must not be done in the hottest part of the day.

Walnut pruning: Pruning is the most important operation in intensive plantations, which control the growth of young plants, the correct formation of the growing form and the balanced formation of reproductive buds with the aim of achieving optimal quantity and top quality. In the following text, an example of the formation of the "improved pyramidal crown" form will be shown.
1.vegetation: After planting the two-year-old walnut seedlings, in the spring (after March 15), the first pruning is done. Walnut seedlings are shortened to a height of about 30-45 cm. The height at which plugging is performed cannot be strictly defined because it is important that there are 2-3 well-developed and healthy buds at that height. Due to mechanical or other types of damage, the first good buds can be at 50 cm of tree height, so any lower cutting could affect the percentage of young seedlings. An oblique cut is made on the seedling, 2 cm above the healthy bud with a slant, i.e. falling to the opposite side of the bud so that the water flows to the opposite side of the bud. It is mandatory to coat the cut with high-quality squid wax based on vegetable oils that dries

in the air (https://shop.agromarket.rs/zapi-kalemarski-vosak-200g).During May and June, only the 2 strongest ones, positioned opposite, remain from the chased youths. At the end of June and beginning of July, both saplings should be over 1m tall and that is the moment when the better and stronger ones are chosen and the weaker ones are removed. By the end of the first growing season, the seedlings should have grown a minimum of 1.6 m and have been well compacted.
2.vegetation: Depending on the type of harvest, that is, the collection of walnuts, the first floor is formed higher or lower, i.e. the trunk is shorter or longer. By shortening the seedlings to 1.6 to 1.9 m, it will cause the formation of the first floor at a height of 1.3 - 1.6 m. In both cases, all increments below 1.2 m are removed. During June and July, 6-8 branches are left on the tree, which should have a length of 1.2 m by the end of the growing season.
3rd growing season: Out of 6-8 branches in the previous growing season, the one that has the best position in the center will represent the central leader, its very top is shortened - a few cm. The other joists, 4 of them, will represent the shoulders of the branches of the first floor and are shortened to a length of about 1.2 m. The top bud on the central guide will produce a young shoot that will represent an extension of the first floor. All the buds that are on the leader and are close to the first floor, will give young ones that will be removed. 5-6 young trees are left at the top of the leader, which are at least 50 cm away from the branches of the first floor.
4.vegetation: Young trees of the first floor are cut short in order to form side branches with a crop. Of the 5-6 second floor joists, the 4 best positioned ones are left and shortened to a length of about 1.2 m. The centrally positioned young steam will represent an extension of the leader. From the fourth growing season, all fruits are left and no reduction is done.
5th growing season: Pruning is the same as during the previous growing season, ending with the formation of the third floor of shoulder branches, which created the growing shape of the improved pyramidal crown. In the following growing seasons, the young branches will be thinned and shortened in order to get more lateral branches that bear the more fruits.

Harmful agents to walnuts

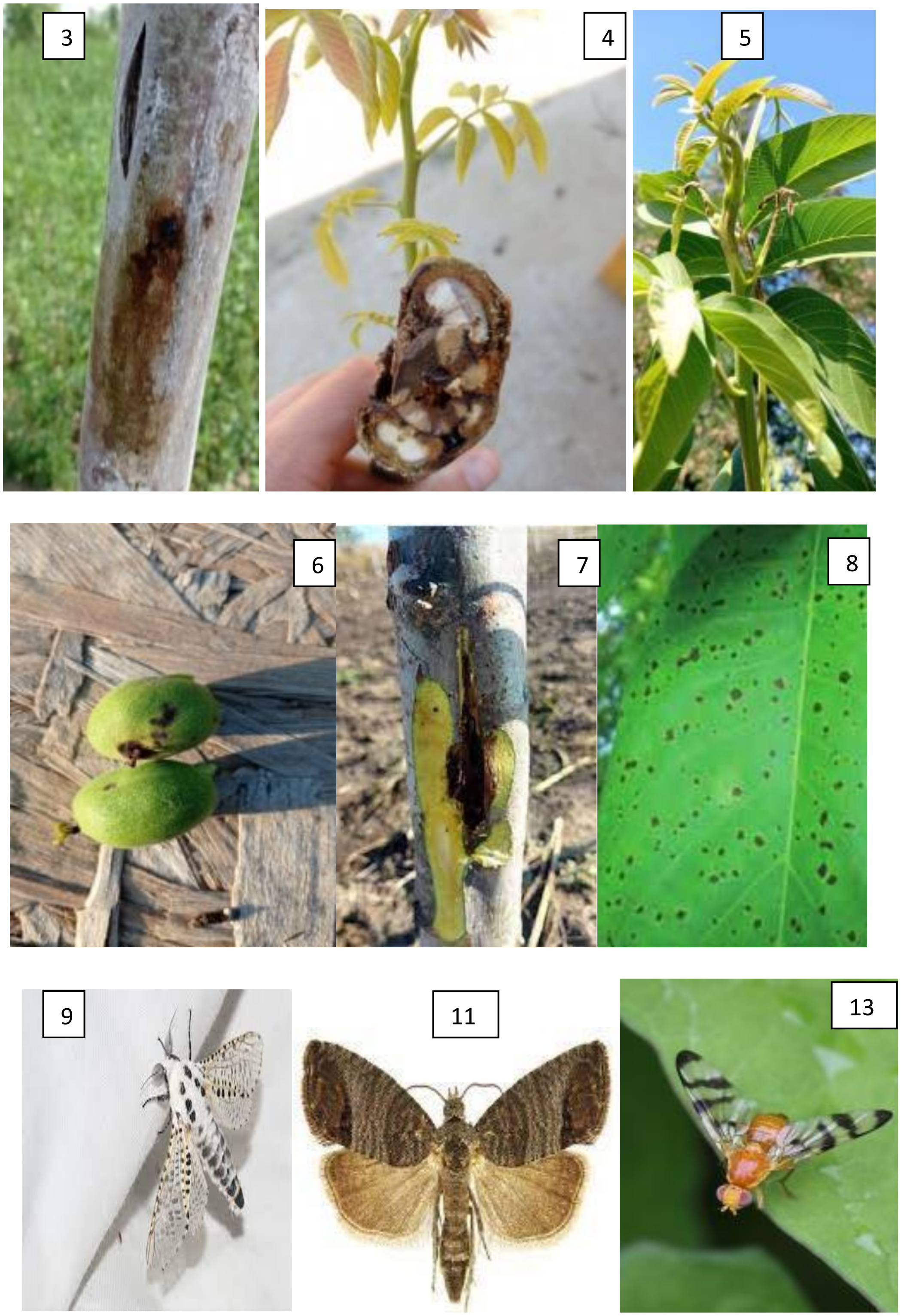
Diseases:

1. *Gnomonia leptostyla* - walnut anthracnose (photo 1): In years with a strong occurrence, the leaves fall prematurely, the shoots do not mature and become sensitive to frost. Symptoms also appear on fruits that fall. Disease symptoms - symptoms appear on walnut leaves, petioles, young shoots and fruits. Small yellow-green spots with a dark edge appear on the face of the leaf. The spots merge, the leaf is deformed and falls off. On the petiole, the spots are oval, sunken. Smaller or larger black spots can be seen on the fruits, but the pith remains healthy (Bagi & Bodnar, 2012.).
2. *Agrobacterium tumefaciens* (photo 2)- cancer of the wound of the root system: Globular formations disrupt the arrangement and function of the roots, and because of this, the above-ground part of the fruit trees does not receive enough water and mineral substances. Diseased roots have fewer hairs, so the ability to absorb water and minerals from the soil is reduced. Diseased plants become stunted and dry over time (https://agronomija.rs/2014/).
3. *Brenneria sp.* (photo 3,4,5)- deep canker of the tree: Bacteria of this genus, which have been present in Serbia for several years, cause serious damage to young walnut plantations. On the tree, black spots first appear, which cover the deeper layers, ring the tree, and bacterial exudate leaks from the wounds due to severe infection. On the young leaves and the tops of young shoots, sunken spots appear that quickly cover the entire top part, so the plant looks burnt due to necrosis, similar to the symptoms of Erwinia amylovora (Cabello et al. 2016.; Iličić et al. 2021.).

4. *Xanthomonas arboricola pv. juglandis* (photo 6,7,8)- bacterial spotting of leaves and fruits: Disease symptoms - angular, watery and black spots appear on walnut leaves along the leaf veins. Over time, the spots merge, their insides break, the leaves curl, but do not fall off. Indented spots are also formed on the shoots. The biggest damage is caused by the symptoms on the fruits: first, small watery black spots are observed on the green skin of the fruit, which grow larger over time. They sink in and rot occurs, as a result of which the contents of the fruit turn black. The fruit shrivels and falls off (Iličić et al. 2021.).

Insects:

1. *Zeuzera pyrina* (photo 9,10)- leopard moth : The imago (butterfly) lays eggs on the saplings near the buds from the end of May to the beginning of August. Damage is caused by the larva, which burrows into the trunk and branches of the walnut tree. If it is not diagnosed in time, it can seriously damage young trees and lead to the breaking of the tree in the place where the larva is located.
2. *Rhagoletis complete* (photo 13,14) - walnut fly: The imago lays eggs in the fruit sheath, in groups of 15-20 pieces. Larvae hatch from the eggs and feed on the green covering of the fruit but do not bore into the fruit. The fruit turns black in the place where the larvae develop, that part decays and bacteria colonize, causing the entire fruit to perish.
3. *Cydia pomonella and Cydia amplana* (photo 11,12) - apple and walnut curler: Both pests lay eggs on young walnut fruits, the eggs hatch into larvae that bore into the fruit and feed on the kernel. Fruits fall prematurely, and those that do not fall lose their quality.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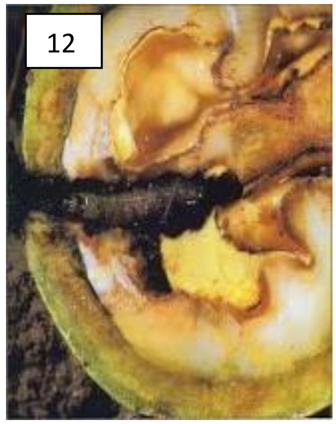

Walnut protection program

Phenophase	Harmful agents	Active substance	Concentration	Quantity per 10 l of water	Fertilizing
Bud swelling	overwintering forms of insects, fruit spider bacteriosis	mineral oil + copper (oxychloride or hydroxide or oxide)	depending on the selected substance	depending on the selected substance	**foliar** / **Irrigation system** NPK 10:40:10
young branches about 10 cm long -beginning of May	bacteriosis leaf spotting harmful insects and mites	fosetyl-aluminium + captan + bifentrin	0,3-0,4 % 0,2 % 0,05 %	30-40 g 20 g 5 ml	**foliar** / **Irrigation system** NPK 10:40:10
young branches about 10 cm long - middle of May	Disinfection after selection branches	copper (oxychloride, hydroxide, oxide)	0,25 % or 0,35%	25 g or 35 ml	**foliar** / **Irrigation system** NPK 10:40:10
end of May - beginning of June	leaf spotting aphids *Cydia* *Zeuzera*	mankozeb+metalaxyl + cipermetrin or deltametrin	0,25 % 0,01-0,04%	25 g 1-4 ml	**foliar** aminoacids, mikroelements **Irrigation system** NPK 20:20:20+me
second half of June	leaf spotting aphids *Cydia* *Zeuzera*	tebukonazol + piriproksifen + acetamiprid	0,07-0,1% 0,15% 0,025%	7-10 ml 15 ml 2,5 g	**foliar** aminoacids, mikroelements **Irrigation system** NPK 20:20:20+me
second half of July	leaf spotting aphids *Cydia*	mankozeb+metalaxyl + hlorantraniliprol	0,25% 0,02%	25 g 2 ml	**foliar** aminoacids + calcium

	Zeuzera				**Irrigation system** NPK+calcium
autumn treatment	Phytopathogenic fungi and bacteria	copper (oxychloride or hydroxide or oxide)	depending on the selected substance	depending on the selected substance	/
					/

Conclusion

In recent years, lateral varieties of walnuts, due to a whole series of favorable features and advantages compared to terminal varieties, have taken precedence in the production of stone fruits. The areas under this fruit grow from year to year, and the plantations are built according to the model of the best world producers. The easy breakability of the fruit shell, good yield, high oil content and the white color of the kernel of the lateral walnut varieties guarantee a leading position on the walnut market. Any intensive production like this one is followed by a whole series of operations that must be done professionally and in a timely manner. Protecting walnuts from harmful agents is an important segment in the production process because the quality and quantity of the crop directly depends on its quality.

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EXTRACTION METHODS OF RESVERATROL FROM GRAPES

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Abstract

Resveratrol became recognizable among scientists after the determination of its multitude of benefits for both human health and the plants that synthesize it. From a medical point of view, it is important in the prevention and treatment of many diseases, and from an agricultural point of view, it is part of the defense mechanism of plants, which synthesize it in significant quantities in stressful conditions. To date, many studies have been conducted to examine this compound and all its advantages and disadvantages. To explore new possibilities of exploiting the benefits of resveratrol, it is necessary first to isolate it from the plants that contain it. One of the major sources of resveratrol is the grapevine, which contains most of it in its fruits. Its isolation can be done by many different extraction methods. Extraction represents one of the first and most important steps towards a further analysis of resveratrol. The extraction efficiency depends on many factors and is determined by chromatographic analysis. The aim is to isolate as much resveratrol as possible so that further research can be carried out. This paper provides an overview of the most commonly applied methods, their advantages, and disadvantages, as well as a comparison of some of the conventional and non-conventional methods.

Keywords: *resveratrol, extraction, grapes.*

Introduction

Secondary metabolites of plants have significant beneficial effects on human and animal health. The grapevine (*Vitis vinifera* L.), is a rich source of these chemicals in the human diet, and over the past decades, all the positive properties of grapes and wine consumption have been studied. From the nutritional aspect, grapes are rich in phenols, stilbenes, flavonoids, phenolic acids, poly-hydro phenols, sugars, vitamins, etc. (Sovak, 2001), which are characterized by antioxidant properties. Compounds belonging to the chemical group of stilbenes, produced by plants in conditions of abiotic or biotic stress, such as ultra-violet radiation (UV), ozone, chemical treatments, mechanical damages, infections caused by various pathogens, and which play a role in their immune response are crucial in many aspects. One of them is resveratrol (3,4',5-trihydroxystilbene). It occurs in nature in two isomeric forms, *cis*- and *trans*-. The *trans*- isomer is synthesized in greater quantity and has greater biological activity considering its antioxidant nature (Soleas et al., 1997), while the *cis*- form is represented in low concentrations, as a result of the transformation of *trans* isomer, most often during UV radiation or the vinification process (Tian and Liu, 2019; Sato et al., 1997; Soleas et al., 1997). Resveratrol and its positive health properties have a long history, as more than a millennium ago it was used in traditional Chinese medicine to treat various health problems (Huang et al., 2019). As a pure compound from the roots of the plant *Veratrum grandiflorum* O. Loes, resveratrol was isolated by Takaoka in 1939, and since then interest in this compound has been growing. Hitherto, the preventive effect of resveratrol in cancer, diabetes, diseases of the cardiovascular system, inflammatory processes, diseases of the nervous system, and many others have been established (Meng et al., 2020). From the aspect of vine production, the benefits of resveratrol have not yet been fully studied, but it is known that its increased synthesis represents the plant's immune response to stressful environmental conditions such as UV radiation, ozone, agrotechnical measures, imbalance in plant nutrition, mechanical damages, etc. (Colica et al., 2018), and biotic factors such as infections caused by various phytopathogenic organisms. From the aspect of plant protection, the potential fungicidal effect of resveratrol is known. The proof lies in the fact that vine synthesizes it to a greater extent in the presence of infections caused by phytopathogenic fungi, especially *Botrytis cinerea* Pers. In that situation, resveratrol is synthesized around the pathogen penetration spot and forms a ring that inhibits the growth and development of the pathogen, slowing down or eliminating the infection (Hasan and Bae, 2017). Resveratrol is most abundant in the grape, and less in the leaves, shoots, and buds of the vine. The highest concentration of resveratrol in berries can be isolated from the skin, and seed, and less from the mesocarp (Hasan and Bae, 2017). When it comes to its content in wine and other products, it depends on the grape variety, mechanical treatments, and vinification process. It is important to point out that the concentration is higher in red than in white wines (Casas et al., 2010). Apart from the grapevine, resveratrol has so far been extracted from over 12 families, 31 subfamilies, and 72 plant species, including mulberry (*Morus rubra* L.), white pine (*Pinus silvestris* L.), peanut (*Arachis hypogea* L.), eucalyptus (*Eucalyptus gunnii* Hook), Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc) and many others (Colica et al., 2018). Today, plant extracts represent the basis in medicine and pharmacy for the production of dietary supplements (Sovak, 2001), whereby grape extracts represent the basis for the production of those whose active component is resveratrol. Also, when it comes to plant protection more and more attention is paid to plant extracts because the need for new, effective active components of plant origin is increasing to achieve ecological agricultural production. The extraction of resveratrol from the grapevine is mainly done from the fruits, and for these purposes, a large number of extraction methods have been established, based on the application of different solvents and apparatus, all with the aim of the most efficient and complete extraction of this specific compound.

Grape sample preparation for resveratrol extraction

The aim of sample preparation for extraction lies in the preservation of biomolecules. Grapes can be extracted fresh or dried. If the extraction is carried out on fresh grapes, it is necessary to know that, depending on the variety, they often contain more than 80% of water. Water in the samples that have to be extracted is undesirable especially if non-polar organic solvents are used. In that case, they do not reach the target compounds. When comes to drying grape samples, it can be carried out in several ways: at room temperature, in an oven, or by lyophilization, and it requires additional time (Azwanida, 2015). Drying the samples at room temperature takes a lot of time, and carries the risk of the appearance of pathogens and the development of mold. When this process is done in the oven, it is necessary to take care of the temperature of drying, considering the fact that resveratrol is a thermolabile compound. The lyophilization process requires expensive equipment. Before the actual process, the grapes have to be cut and frozen. The drying is fast, and the specificity is that the ice evaporates directly under vacuum conditions, without a liquid intermediate phase, and after 12-24 hours completely dry samples are obtained. Regardless of which method of sample preparation is chosen, the aggravating circumstance when grinding the samples is certainly the amount of sugar that the grapes contain. It makes it difficult to achieve the powdery consistency of the samples. This powder consistency is necessary because it increases the contact surface with the solvents during extraction. The finer the sample particles, the more homogeneous the sample is and the extraction efficiency increases (Technical note, 2013; Azwanida, 2015).

Extraction methods

Extraction of any component from plant material is one of the first and fundamental steps toward further investigations. To extract resveratrol from grapes and wine as efficiently as possible, many methods have been tried and established to date, with different effects. Given that resveratrol is considered a photosensitive and thermolabile compound, the most important when choosing an existing or validating a new extraction method is the choice of preparation of samples for extraction, type of solvent, the temperature at which extraction is carried out, and duration of extraction (Romero-Perez et al., 2001). To extract resveratrol (*trans*- and *cis*-isomers), various extraction methods have been used for several decades, and they can be roughly divided into conventional and unconventional. The first group is characterized by the use of large amounts of solvents and manual handling of the entire process and includes maceration, Soxhlet extraction, solid phase extraction (SPE), etc. (Alara et al., 2021). To correct the shortcomings of conventional extraction methods, new, modern extraction methods such as ultrasonication-assisted extraction (UAE), enzyme-assisted extraction, QuEChERS method, and extraction by supercritical fluids (SFE) were created (Mantell et al., 2003; Casas et al., 2010). The maceration method represents an outdated approach to the extraction of resveratrol from grapes because it requires a lot of time and a large amount of solvent. However, it represents one of the simplest methods, which affects the commitment to it. It is carried out in a closed system, by soaking the ground sample in a specific solvent, at room temperature with occasional stirring, after which the solid phase is separated from the liquid phase, usually by filtration (Ćujić et al., 2016). On the other side, a somewhat faster method and more economical when it comes to solvent consumption is Soxhlet extraction. It is carried out in a closed system, the so-called Soxhlet apparatus, and only on powdered samples. Extraction is performed by transferring the sample to the cellulose thimble, which is then placed in the extraction system under reflux. The choice of solvent depends on the solubility level of resveratrol. These are usually organic solvents in which resveratrol is very soluble, such as methanol, ethanol, dimethyl formamide, dimethyl sulfoxide, acetonitrile, acetone, etc. (Liu et al., 2009). The process is based on the evaporation of the solvent from the flask, which condenses, then passes through the sample, and accumulates again in the flask, carrying with it resveratrol molecules. The disadvantage of this method is certainly that it requires a lot of time and still large amounts of solvent, and in addition, higher temperatures represent a risk for resveratrol degradation (Alara et al., 2018). SPE requires the existence of a solid and a liquid phase, and the extraction takes place between them. The solid phase must be such that resveratrol has a greater affinity for it than for the matrix of the grape sample. The extract is finally obtained by washing the solid phase with a solvent in which resveratrol is soluble (Berrueta et al., 1995). Contrary to this, newer methods used today are faster, more economical, require less human involvement, and are more environmentally friendly. One of them is UAE as a simple and easy method, which, unlike the previous ones, does not require large volumes of solvent. Extraction is carried out by forming micro-sized bubbles with the help of ultrasound in the range of 20-2000 kHz. The bubble bursting accelerates the decomposition of plant tissue and facilitates the transition of resveratrol from the matrix to the solvent. As one of the newest methods of extracting resveratrol from grapes and wine, enzyme-assisted extraction has found its place. It is based on the application of certain enzymes that affect the degradation of cell wall components (lignin, pectin, cellulose, etc.) and thus release the cytoplasm, which accelerates extraction (Puri et al., 2012). QuEChERS, as a method established at the beginning of the 21st century (Anastassiades et al., 2003), is most often applied for the determination of pesticide residues in various matrixes such as food, and soil, agricultural and other products. However, with modifications regarding the solvent and applied salts, it is also used for the extraction of phenolic compounds from grapes. It is based on the application of magnesium sulfate and PSA salts for sample purification (Silva et al., 2021). The advantage is in the speed of extraction, small volumes of spent solvent, and the possibility of extracting several samples at the same time. Unlike QuEChERS extraction, SFE requires very expensive equipment and the qualification of the operator. The device itself has special places for entering samples, a pump, as well as a computer where all the details of the extraction are. It is based on the specific properties of carbon dioxide gas, which at increased pressure and a certain temperature (pressure above 72 bar, and temperature above 31˚C) can be transformed into a liquid state, and in this case, serves as a solvent. Changing the pressure and temperature changes its ability to dissolve analytes from the matrix and affects their extraction (Zougagh et al., 2004). This is a method rarely used to extract resveratrol from grapes, as the necessary equipment is difficult to obtain. It is important to mention that when choosing an extraction method to improve its efficiency, it is necessary to carry out its optimization. This involves examining various factors that influence the process, such as sample preparation methods, different solvents and their combinations in different ratios, extraction temperature conditions, process duration, exposure of samples to higher temperatures and light, etc. Each factor change then has to be checked by applying available chromatographic techniques, to determine the yield of the tested extraction and its efficiency in extracting resveratrol.

Advantages of unconventional resveratrol extraction methods

Till today, the extraction of resveratrol has been carried out from different types of matrices when it comes to vines - whole berries, seeds, skins, stalks, wine, etc. Many extraction methods have been tried with different results, which contributes to creating a broader picture when it comes to handling this compound and its behavior under different conditions of extraction and analysis. Isolation of resveratrol was successfully carried out using UAE on samples of grape stalks of Campbell and Gerbong varieties. The extracts were analyzed using the high-performance liquid chromatography technique (HPLC). For the comparison, traditional solvent extraction was also performed in a solvent mixture of ethanol/water at a temperature of 60˚C. The obtained UAE extraction yield was 24-30% higher compared to the traditional one. The maximum concentration of resveratrol extracted from the stem of the Campbell variety was 489.4 µg/g and for the Gerbong variety 194.8 µg/g of dry matter (Cho et al., 2006). Palomino fino grape samples were extracted using the SFE method and then analyzed by high-performance liquid chromatography with UV visible detector (HPLC-UV vis) (Casas et al., 2010). To create a broader picture of the content of resveratrol in different parts of the berries-seeds, the skins and stems were extracted separately. To speed up the extraction process, ethanol was added to the supercritical CO_2, which increased the solubility of resveratrol. For comparison, the samples were extracted by the conventional SPE method, and then the results were compared. The

presence of resveratrol in the seeds could not be detected in the extracts obtained by the SPE, while the concentrations obtained in the stalks and skin were 1.7 and 3.1 mg/100g of dry material, respectively. On the other side, the SFE method showed significantly higher efficiency, and the determined concentrations of resveratrol in the seeds, stalks, and skin were 8.9, 0.9, and 49.1 mg/100 g of dry material, respectively. The results confirmed the assumption that resveratrol is the most abundant in the grape skin. A more recent technique based on a modified QuEChERS method, followed by dispersive solid phase extraction (dSPE) combined with ultra-high pressure liquid chromatography UHPLC, also gave promising results. Thus, Ruiz-Garcia et al. (2016) were the first to use a combination of these methods to determine resveratrol content in grapes. The obtained extraction yields varied from 75.1-99.7%, which indicates the high efficiency of the extraction method.
The use of enzymes is common in wine production and is one of the important steps in grape processing. Their action was also used to improve the efficiency of resveratrol extraction. Averilla et al. (2019) reported the effects of different enzymes available on the market on the efficiency of SPE extraction, and the effect of heating the samples before extraction. In the extract obtained without heating, 20.96 mg/g of dry grape skin was found, while the treatment of heating the sample before extraction at 95 ˚C for 10 min led to a significant increase in the level of resveratrol, which was then 5.4 times higher. The applied enzymes (pectinase, β-glucosidase, β-glucanase, etc.) applied independently gave similar results and without prior heating of the samples were not efficient enough to extract larger amounts of resveratrol from the samples. The results obtained by Averilla et al. (2019) indicate that the structural changes that occur in the skin tissue during short-term heating facilitate the process of extracting resveratrol from the plant material and increase the efficiency of the enzyme in breaking down the cell walls in the extracted tissues. Although high temperatures are considered undesirable when it comes to resveratrol, this research has shown that in a short interval they can have multiple effects on the extraction efficiency.

Conclusion

Previous literature data on resveratrol points to its importance from many aspects. To further study its properties and new ways of application, it is necessary to constantly extract it from natural sources such as grapes. Many methods of extracting this component from grapes are available, but not all of them give results when it comes to resveratrol. Preference should be given to those methods that are simple to implement and do not require expensive and large equipment. The use of solvents during the entire process should be rational, and the chosen solvents should be as less toxic as possible so that the method is acceptable from an environmental point of view. Human involvement should be as little as possible, and the extraction time should be short. After choosing a method, it is necessary to examine all the factors that are part of the method and establish their relationship so that the results are as good as possible, and the method itself as efficient as possible. The extraction is completed only when the analysis of the obtained extracts is carried out, whereby the highest extraction yield is sought. Today, chromatographic techniques such as high-performance liquid chromatography, ultra-high-pressure liquid chromatography, tandem mass liquid chromatography, etc. are used for these purposes. Among them, the most applicable and most accessible is certainly the HPLC technique with the use of different detectors, which is an accurate and precise technique for the analysis of a wide range of analytes, including specific natural compounds such as resveratrol.

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EFFECTS OF PHYTOPATHOGENS ON THE QUALITY PARAMETERS OF CARROTS SEEDS (*DAUCUS CAROTA* L.) IN A THREE-YEAR PERIOD

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Abstract

Carrot (*Daucus carota* L.) is one of the most important root vegetables in the Apiaceae family grown around the world. Production of carrots in Serbia is generated in Vojvodina with the highest yields 27.4 t/ha, while other regions generate lower yields. Current studies on carrots mainly focus on nutrient content and carotenoid synthesis. Seeds can be infected with phytopathogens, which can contaminate other seeds or spread the infection to other crops. Out of the 16% of annual crop losses due to plant diseases, at least 10% are caused by seed-borne diseases. Changes in the quality of Nantes carrot seeds were monitored for three years (2019-2021). Total germination was highest in the first year with 92% (2019). In 2020, there was a significant decrise in total germination of 8%. Accordingly, germination energy was reduced ($p<0.05$). A statistically significant difference in total germination was not obtained between 2020 and 2021 ($p>0.05$). The decrease in total germination from 2019 to 2021 was significant ($p<0.05$%). It has been noticed that the infection of *Alternaria* spp. and *Fusarium* spp. was the lowest in 2019 and increased in 2020 and 2021 (4% and 5%, $p<0.05$), respectively. The results may indicate an association between the percentage of phytopathogens present and germination. In conclusion, pathogen-free seeds are necessary to create a healthy plant population, better germination and higher yields.

Keywords: *seed, carrot, germination, quality, phytopathogens.*

Introduction

Carrot (*Daucus carota* L.) is one of the most important root vegetables in the Apiaceae family grown around the world (Que et al., 2019). The largest producer of carrots in the world is China, which generates 43% (Que et al., 2019; Xiong, 2010) of the total yield of carrots in the world. According to the data published by the Statistical Office of the Republic of Serbia from 2020, carrots were grown on 2,662 ha, with a total production of 52,740 tons. Production of carrots in Serbia is generated in Vojvodina with the highest yields 27.4 t/ha, while other regions generate lower yields (The Statistical Office of the Republic of Serbia, 2021). Current studies on carrots mainly focus on nutrient content and carotenoid synthesis (Sumekar Y., 2019). Seeds can be infected with phytopathogens, which can contaminate other seeds or spread the infection to other crops. Out of the 16% of annual crop losses due to plant diseases, at least 10% are caused by seed-borne diseases. The leaf blight is widely recognized as one of the most common and destructive carrot diseases caused by the pathogen *Alternaria dauci*(le Clerc et al., 2009). The diseases associated with *Alternaria* leaf blight cause root decay, which reduces carrot stands as

well as the quality of root. Diseases associated with alternariosis cause decay aboveground foliage and root which reduces the nutrients of carrots, as well as the quality and market usability of the root (Smoleń et al., 2016; Boedo et al., 2008, Farrar et al., 2004). Fungal infection of seed may occur during seed production, storage and transport. Infection ultimately resulting by decreasing germination and vigor, shortening the storage period, and inducing physiological change (Boedo et al., 2008). According to ISTA Rules, 2020 for selecting high-quality seeds the most common methods are based on physical properties, such as weight and germination as well as biochemical and other physical tests.
The aim of our research was to determine the changes in the seed quality of autochthonous carrot varieties: Nantes and Šantenej over a period of three years.

Material and method

Seed testing of two carrot varieties (*Daucus carota* L.): Nantes and Šantenej was performed by standard methods for assessing the quality and health of the seeds in the laboratory for seed quality testing at Institute for Vegetable Crops, Smederevska Palanka (Serbia). All tested seeds of two varieties of carrots are from the same locality in Smederevska Palanka in the periods from 2019 to 2021. The quality parameters (energy and total germination), seed health and moisture were used to assess the quality of two carrot seed varieties. The seed quality of selected carrot varieties was tested following the Regulations on the quality of agricultural plants (47/87), which is harmonized with the ISTA rules (2020). The seed germination parameters (energy and total germination) are performed using filter paper standard method. Samples of selected carrot varieties consisted of 100 seeds in four replicates were placed in petri dishes with filter paper moistened with 0.2% KNO3. Analysis of germination parameters indicates abnormal germs (damaged, defined, rot) have not developed by the end of the tested time and cannot develop into a normal plant and. The set samples were incubated for 7 and 14 days at 23ºC. The seed health of Nantes and Šantenej was tested on *Alternaria* spp. and *Fusarium* spp. Health testing of two varieties of carrot was performed with the standard method on filter paper. The allowed percentage of infected seeds was 5%. After incubation, the results were scored according to the following formula:

$$\text{Seed health} = \frac{\text{number of infected seeds}}{\text{total number of seeds}} \times 100\ \%$$

Moisture content is defined as the water in the seed and is expressed as a percent. The moisture testing procedure is performed with 5 g of a sample of three pepper varieties on an analytical balance. Moisture determination is performed at a temperature of 105ºC ± 2ºC for 17 h ± 1 h. Dry mass (SM) calculation is performed according to the following formula and is expressed at one decimal place:

$$\text{SM}=\frac{m3-m1}{m2-m1} \times 100\ \%$$

m1 (g) – the mass of a container and lid;
m2 (g) – the mass of a container, lid, and contents before drying;
m3 (g) – the mass of a container, lid, and contents after drying.

Statistical analysis was done with SPSS software (version 23, IBM, USA). Samples (done in triplicates) were compared with one-way ANOVA and Tukey post-hoc test. Statistical significance cutoff was $p < 0.05$.

Results and Discussion

Carrots (*Daucus carota* L.) are an important vegetable that is grown all over the world and represented a source of nutrients in the human diet. The analyses of quality parameter values (total germination, energy, seed health and moisture) were monitored from 2019 to 2021.nSeeds quality is one of the key factors for high yield production. In many crops, fungal infections are responsible for low-quality seeds. Carrot varieties (Nantes and Šantenej) were compared to each other, with a statistically significant difference between varieties and within one group in three years (Table 1).

Table 1. Quality parameters of carrot seeds (total germination energy, and moisture) in the three years period (2019 –2021).

Samples	**Total germination^{a} (%)**			**Germination energy^{a} (%)**			**Moisture content^{a} (%)**		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**	**2019**	**2020**	**2021**
Nantes	92^{b*}	85	84	69^{*}	64^{b}	65	8.6^{b*}	8.4^{*}	8.0^{*}
Šantenej	87^{*b}	82^{*}	79^{*b}	68^{*}	60^{*b}	63	8.1^{b*}	7.8^{b*}	7.5^{b*}

a Qualty parameters were compared on statistical significance (p<0.05) with years and between varieties of carrots but not between parameters.

* Statistical significance between years (p<0.05)

b Statistical significance between varietes

The total germination for Nantes (2019) was 92%, germination energy 69% while *Alternaria* spp was detected 1 % and *Fusarium* spp was not detected (Table 1 and 2). When observing and comparing the total germination and energy of variety Nantes in 2019 and 2020, a statistical difference was observed ($p<0.05$). The total germination between 2020 and 2021 was not statistically significant and amounted to 85% and 84%, respectively. The total germination for Šantenej was 87 % (2019), decreased by 5% and 8%, in 2020 and 2021, respectively ($p< 0.05$). Comparing 2020 and 2021, it was noticed that there was no statistically significant difference between two experimental years ($p>0.05$). The decline in total germination was accompanied by a decrease in germination energy from 2019 to 2021. However, Nantes carrot seeds had better energy and overall germination as compared to Šantenej variety. (Table 1 and Figure 1).

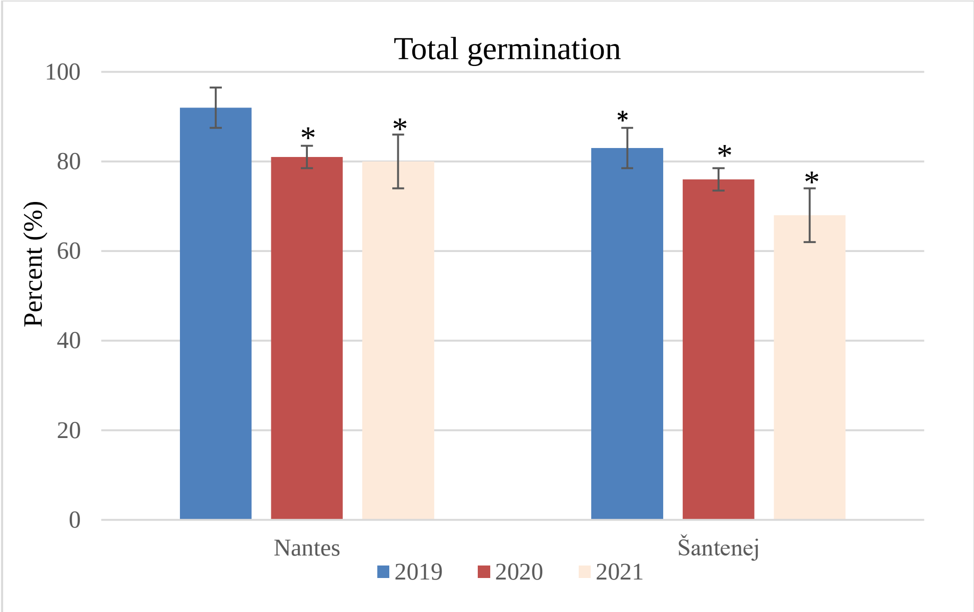

Figure 1. Total germination for two carrot varieties ((Nantes and Šantenej) as observed during three years with statistical significance p <0.05 using ANOVA one-way analysis.

For both varieties phytopathogens were detected in 2020 and 2021 in the higher percent than in 2019 (p<0.05) **(Table 2)**. Fungi can infect seeds by sticking to the surface of the seeds or by penetrating the seeds and causing infection later, resulting in deterioration. One of the factors that determines the effectiveness of chemical seed treatments is how much they can reach and be effective because deep-rooted infections can remain unchanged. For both pathogens during the three years of testing, except for 2021 (detected 5 % *Alternaria* spp), Nantes proved to be a more resistant variety compared to Šantenej (p<0.05) ((**Table 2**).

Table 2. Presence of phytopathogenic fungi in the seeds of selected pepper varieties

Samples	***Fusarium* spp (%)**			***Alternaria* spp (%)**		
	2019	**2020**	**2021**	**2019**	**2020**	**2021**
Nantes	0*b	1b	1	1*b	4	5
Šantenej	1	3*b	2	2*b	5	4

* Statistical significance between years (p<0.05)

b Statistical significance between varietes. (p<0.05)

During seed production, storage, and transport, the seeds are exposed to many factors, ultimately resulting in fungal infections that may adversely affect seeds by decreasing germination, vigor and physiological change. Similar to the study by Zhang et al., 2020, in our study, seed infection rates differ among the tested carrot varieties. Indicating that resistance to seed pathogens could be varietal characteristic. Seed testing is crucial to ensure seed efficiency to determine whether infection levels are at or below economic thresholds. Prevention of carrot diseases caused by *Alternaria* spp is a challenge that aims to achieve or maintain the health of plants and seeds. (Farrar et al., 2004, Li et al., 2017). Since the of these diseases are transmitted by seeds in most areas where carrots are grown, *Alternaria* spp can appear on the leaves and roots as black rot, which causes a large loss of yield (Biswas et al., 2015, Farrar et al., 2004). Selection of carrot varieties uninfected with fungi is a good agronomic practice to minimize the chances of fungal infections (Salim et al., 2022). Currently, there are no varieties of *Daucus carota* L with total resistance for *Alternaria spp*. Several studies with plant extract indicated their antifungal effect of medical plant extracts as treatments can inhibit fungal infection such*Alternaria alternate, Alternaria solani, Fusarium oxysporum, Rhizoctonia solani* on seeds. The ability of the extracts to increase seed germination and seedling germination can be attributed to controlling the infection of fungi found in the seed (Hasan et al., 2005; Tagoe et al., 2011). Baka et al., 2014 and Eltamany et al., 2012 showed that used plant extracts can reduce infection and prolong the quality of natural seeds. This indicates the possibility of reduced infection in Šantenej and Nantes.

Conclusion

Although, seed quality and health depend on agronomic measures such as fertilizer, irrigation, crop rotation, handling, and proper seed storage. These techniques contribute to yield improvement. Future research could be focused on plant extracts as natural disinfections for seeds and alternative to fungicide treatments. Quality parameters for two varieties of carrots were observed for three years and were below the legal maximum. Based on a three-year’s period, it can be classified as a quality seed. The results indicated there was a relationship between germination and the percentage of phytopathogens on the seed. In the future, untreated seeds can be treated with some medicinal plants that could increase and prolong the quality of our seeds, but that remains for future research.

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MICROBIOLOGICAL QUALITY OF COLD PRESSED PUMPKIN AND WALNUT OILS

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Abstract

The aim of this study was to determine the content of crude oil in seeds and the microbiological quality of cold pressed oils of eight genotypes of pumpkin and walnut from Central and Western Serbia. The oil content in pumpkin seeds of different genotypes ranged from 16.44% to 35.13% per dry matter of whole seed, in the walnut kernel was between 50.17% and 60.41% per weight of the walnut kernel. Genotypes of pumpkin seeds differ significantly in oil content - genotype with the lowest oil content contains only 47% crude oil compared to the genotype with the highest oil content. Among the walnut samples, there is less deviation in the oil content of different genotypes. The number of total bacteria in walnut oil was less than 100 cfu/g. The number of yeasts and mold in walnut oil was very low, even in 3 samples the presence of this microorganisms were not detected, in 4 samples the number was lower than 10 and in the 1 sample the number was between 10 and 100 cfu/g. Two samples of pumpkin oil had a number of bacteria more than 100, but in other samples the number of bacteria less than 100 cfu/g. The number of bacteria, yeasts and molds in the oils of all genotypes was lower than the limit values prescribed in the "Guide to the application of microbiological criteria for food". Sulfite-reducing clostridia were not detected in any oil sample.

Keywords: *crude oil, local genotypes, microbiological quality, pumpkin, walnut.*

Introduction

In recent years, consumer interest in non-refined vegetable oils has been increasing worldwide, mainly because of growing evidence these oils have nutritional and health benefits. In the group of non-refined oils, a special place belongs to so-called "cold pressed oils". Cold pressed oils to have a high nutritional value due to the presence of ω-3 and ω-6 fatty acids and non-nutritive components such as tocopherols, sterols, phenolic compounds, squalene end carotenoids. Pumpkin oil and walnut oil belong to the group of edible oils that are produced exclusively by mechanical means and which can be included in the group of functional food products (Patel and Rauf, 2017). In addition to the nutritional and functional properties of walnuts and pumpkin seeds and oil, microbiological quality is also very important. The safety and shelf life of foods depend upon the interaction of chemicals, physical and microbial factors (Falola et al., 2011). Microbial food safety programs for raw and minimally-processed agricultural products have become an essential part of production and processing systems. Identifying sources, mode of contamination, and potential hazards are essential to reduce of foodborne disease (Heaton and Jones, 2008). The microbiological quality of cold pressed oils is directly related to the quality of the raw material. Seed contamination can occur during pre-harvest, harvest and post-harvest processing (CDC, 2006; Heaton and Jones, 2008). During the pre-harvest, growing plants are

susceptible to a wide range of microbial contamination sources. Soil and irrigation water especially those contaminated with industrial and domestic wastes, also animal excrement has been a common sources of microbial contamination (Mapanda et al., 2005). Harvesting includes collecting, classification, packaging and transportation which represent of critical points. In the post-harvest, possible sources of contamination are materials and equipment, lack of hygiene and event handlers and environments (Chitrakar et al., 2019). Bacillus sp., Pseudomonas sp., Penicillium sp., Mucor sp., and Aspergillus sp., were reported as soil/ environmental contaminants, *Staphylococcus aureus* as normal flora of human skin and opportunistic microorganism, and *Escherichia coli* as indicative organisms for fecal contamination/poor sanitary conditions (Ike et al., 2015). The Pseudomonas species (*Pseudomonas oryzihabitans, Pseudomonas putida, Pseudomonas syringae, P. viridiflava* and *Pseudomonas fluorescens)* and Bacillus species (*Bacillus subtilis, Bacillus flexus, Bacillus weihenstephanensis, Bacillus psychrodurans, Bacillus siralis, Bacillus indicus, Bacillus gibsonii and Bacillus firmus)* were detected in different microhabitats of oil pumpkin (Fürnkranz et al., 2012). Literature dates show that several microbes were isolated in pumpkin seed composite flours: Bacillus sp., *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* Penicillium sp., Mucor sp., and Aspergillus sp. (Ike et al., 2020). These isolates could be linked as either environmental contaminants, unhygienic processing contaminants or as inherent microflora.
Pumpkin and walnut are often grown as secondary crops of cultivation in rural areas of Central and Western Serbia, for that reason, the goal of this study was to investigate crude oil content in seeds and the microbiological quality of pumpkin and walnut oil.

Material and Methods

Plant material

The walnut fruits and pumpkin of local genotypes were collected in Central and Western Serbia, which were harvested during the 2021 crop year. The walnut fruits were dried and stored in the shell at room temperature until the beginning of the analysis. Samples of different genotypes of whole pumpkin seeds were stored at room temperature also.

Cold pressed oil

Cold pressed pumpkin and walnut oils were obtained by oil press (OP650W, Gorenje Group, Slovenia) with a temperature below 50 °C during the pressing. Whole pumpkin seeds were partially chopped before pressing. The color of obtained pumpkin oils varied depending on the genotype of light, dark green, with shades of brown and red, while the color of walnut oil did not vary significantly in different genotypes and was golden yellow. The samples of obtained oils were stored at -18 °C until analysis. These samples were used for microbiological analysis of the oil.

Determination crude oil content

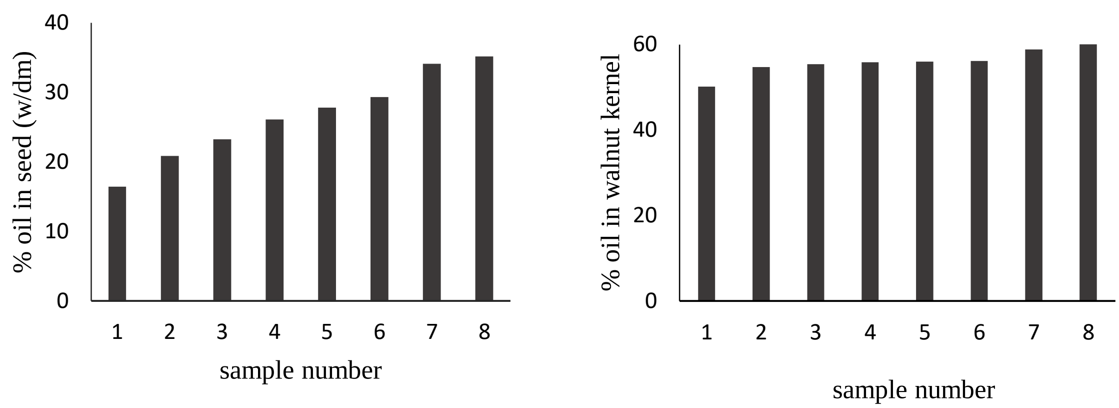
The crude oil content in pumpkin seed and walnut kernel was determined by extraction with petroleum ether in a Solvent extractor (Velp Scientifica ser 148, Italy). The plate temperature was 110 °C. Whole pumpkin seeds/walnut kernels were thoroughly chopped before extraction. Extraction oil from pumpkin seed implied the following conditions: immersion for 30 min and washing for 60 min; for oil from walnut kernel there were 90 minutes of immersion and 30 minutes of washing. The residual amount of petroleum ether evaporated in an oven at 105 °C.

Microbial Evaluation of oil

The number of aerobic mesophilic bacteria, the total number of molds and yeasts and the presence of sulfite-reducing bacteria were determined in the microbiological analysis of the oil. Enumeration of aerobic microorganisms and enumeration of molds and yeasts was determined using the standard microbiological plating method (ISO methods 4833 and 21527-1). An aliquot of 1 mL from each sample was inoculated aseptically into labeled agar plates of the media (agars: Nutrient Agar was used for the total number of aerobic mesophyll bacteria; and Sabouraud Dextrose Agar for cultivation and isolation of yeasts and molds). For the determination of the presence of sulphite-reducing clostridia, test tubes with 1 mL of basic dilution were heated in a water bath for 10 min at 80 °C, and then the Sulphite agar was poured into the tubes (Đukić et al., 2017). The height of the agar in the tubes was > 14 cm and the agar distance from the closure <1 cm. Petri dishes were incubated at 37 °C ± 2 °C for 24 to 48 hours, except for molds where the incubation period was 3 to 5 days, at 28 °C ± 2 °C. At the end of the incubation period, colonies were numerated. All media (agars) were prepared according to the manufacturer's instructions and autoclaved at 121 °C for 20 minutes.

Results and Discussion

The oil content in pumpkin seeds of different genotypes ranged from 16.44% to 35.13% per dry matter of whole seed (Figure 1 a). It is clear that genotypes differ significantly in oil content and the genotype with the lowest oil content contains only 47% crude oil compared to the genotype with the highest oil content. It is also known that the oil content in pumpkin seeds varies depending on the variety and growing conditions. Devi et al. (2018) showed that the average oil content in whole pumpkin seeds was 31.75%.



a b

Fig.1. The oil content in: a- whole pumpkin seeds and b-walnut kernels

Among the walnut samples, there is less deviation in the oil content of different genotypes. The oil content ranged from 50.17% to 60.41% per weight of the walnut kernel. Walnut kernels with the lowest oil content contain 83% oil from walnut kernels with the highest oil content. Beyhan et al. (2016) showed that contein of crude oil varide from 55.38% to 65.15% in different genotypes of walnuts.

Microbiological evaluation of walnut and pumpkin oils

The results of the microbiological analysis of pumpkin and walnut oils are presented in Tables 1 and 2. In 5 of the 8 tested samples of walnut oils, the number of bacteria was less than 10 cfu/g, and in the remaining samples was between 10 and 100 cfu/g (Table 1).

Table 1. Microbial evaluation in walnut oil

Samle of walnut oil	Bacteria cfu/g	Yeasts and molds cfu/g	Sulphite-reducing clostridia
1	10–100	non detected	non detected
2	<10	non detected	non detected
3	<10	10–100	non detected
4	<10	<10	non detected
5	10–100	<10	non detected
6	<10	non detected	non detected
7	<10	<10	non detected
8	10–100	<10	non detected

Generally, the number of aerobic mesophilic bacteria was low. The number of yeasts and mold in the walnut oils was very low, even in 3 samples the presence of these microorganisms was not detected, in 4 samples the number was lower than 10 and in the 1 sample the number was between 10 and 100 (Table 1). In the tested walnut oil variants, the number of yeasts and molds was lower than the limit values prescribed in the “Guide to the application of microbiological criteria for food”. Oil is an inhibitory substance that limits the growth of aerobic microorganisms. It functions by sealing up the air pores through which air could flow in to support the growth of aerobic microorganisms. Microbiological analyzes showed that the presence sulphite-reducing clostridia were not detected in any of the examined samples of walnut oil.

The number of aerobic mesophilic bacteria in pumpkin oil was low, two samples had a number more than 100, and other samples the number of bacteria was between 10 and 100 cfu/g (Table 2). According to this agency, the maximum acceptable limit of bacteria count in food products is 10^3 cfu (ICMSF, 1995). The number of molds and yeasts was very small. Sulphite-reducing clostridia were not detected in any of the examined samples pumpkin oil.

Table 2. Microbial evaluation in pumpkin oil

Samle of pumpkin oil	Bacteria cfu/g	Yeasts and molds cfu/g	Sulphite-reducing clostridia
1	>100	10–100	non detected
2	10–100	10–100	non detected
3	10–100	<10	non detected
4	10–100	<10	non detected
5	10–100	<10	non detected
6	10–100	<10	non detected
7	>100	<10	non detected
8	10–100	non detected	non detected

Traditional pumpkin seed oil is obtained by pressing previously treated seeds at 110–130 °C for 30–60 minutes, these temperatures are sufficient to inactivate a large number of microorganisms, but in this work, no pre-treatment was performed by high temperatures, but the oil was directly squeezed from seeds. The seeds were not peeled before grinding. The shell can be a source of microbiological contamination. The microbiological quality of cold pressed oils is directly related to the microbiological quality of the raw material. Also, microbial populations present in raw nuts depend on production, harvesting and handling practices. Tree nuts often come in contact with the soil during harvest their microbiota may be influenced by the microorganisms present in the soil. Although it is unknown how or when orchard soils become contaminated with pathogenic organisms. Inadequately treated compost, unsanitary irrigation water, wildlife or domestic animals have previously been addressed as potential sources of introduction into these environments (Duffy et al., 2005). The number of microorganisms on the seed can be very different. Silva et al. (2022) state that the total number of bacteria on pumpkin seeds was between 3.6×10^2 and 6.8×10^5 cfu/g. In the case of seed peeling and thermal treatment, the microbiological analysis of the obtained oil in this experiment would be different. It is known that temperatures higher than 80 °C impaired the seeds quality, using the temperature of the 60 °C for several minutes, which would be required to ensure a significant reduction in the numbers of pathogenic organisms. For example, at 65 °C, it has been demonstrated that numbers of pathogenic microorganisms like Salmonella can be reduced by 1 log/min (Adams and Moss, 1995). The general improvements in production and hygiene practices during the production /processing chain are necessary to prevent and reduce microbial contamination of the final product (Ike et al., 2015).

Conclusion

The content of crude oil in pumpkin and walnut seeds depends on the genotype. The total number of aerobic mesophilic bacteria and the number of yeasts and molds in oil were within the eligibility criteria. Sulfite-reduction clostridia were not detected in any of the tested samples of pumpkin and walnut oil, although the fact that neither raw materials (seeds) nor oils were thermally treated at any stage of production obtaining cold-pressed oils.

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STRONTIUM-90 IN MILK AND SOME DAIRY PRODUCTS IN THE REPUBLIC OF SERBIA

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Abstract

Radiostrontium is released to the environment during the testing and use of nuclear weapons mainly in the fifties and sixties of XX century, in addition to nuclear power plant accidents (to some extent in 1986 owing to the Chernobyl accident) and the nuclear fuel reprocessing industry. Strontium is a chemical analogue of calcium (both are earth-alkaline metals) and accordingly when entering a human or animal body, it behaves similar to calcium. A large portion of the strontium will accumulate in bone and teeth and then is included in the metabolism along with Ca, and like calcium, it transfers to milk. Since the strontium uptake by the human body from milk is an important pathway for radiostrontium incorporation, milk and dairy products are good indicators of strontium-90 (Sr-90) content in human diet. Based on the mentioned, the knowledge about Sr-90 content in milk and dairy products is of extreme importance to prevent and control contamination of the food chain. The present study was conducted in order to radiologically control for Sr-90 content in raw milk and some dairy products samples composed of representative locally purchased milk. The analyses are performed using radiochemical analytical oxalate precipitation method, whereby interfering radionuclides are removed by precipitation scavenging. Yttrium-90 (Y-90) is generated from the beta decay of Sr-90. After the ingrowth Y-90 is separated and equilibrium is established, the samples are measured by gas flow proportional counter. The results of investigation showed that Sr-90 activity concentration in many investigated samples was at a lower level than minimum detectable activity (MDA) of the method.

Keywords: *Radiostrontium, radiochemical method, milk and dairy products, Serbia.*

Introduction

The biologically hazardous radionuclide Sr-90 is an artificial radionuclide, produced essentially by the U-235 and Pu-239 fission reaction. This radionuclide is presented in our environment still, given that it is produced during the previous atmospheric nuclear weapons testing and nuclear reactor accidents (Saraygord-Afshari *et al*., 2011). Immediately following a nuclear accident, the fresh fallout contains other radionuclides together with high radiostrontium activity ratio (Sr-89/Sr-90). The old fallout (several years after the release) contains only beta emitter Sr-90 (half-life of 28.5 years) and its daughter Y-90 (half-life of 64.4 hours). For these reasons, the strontium levels in the environmental samples, with particular emphasis on foodstuffs, are of particular concern (Lopes *et al*., 2014).
The radionuclide Sr-90 belongs to the group of extreme toxic radionuclides which affect human health (Grahek *et al*., 1999). The Sr-90 is also commonly regarded as the most hazardous long

lived nuclear fission product because of its high yield, relatively high solubility and similarity in behavior to the essential nutrient and bone constituent calcium. This radionuclide may cause damage to the bone marrow. Its chemical equivalence to calcium makes its incorporation into the trophic chain and, finally, into humans, a relatively easy process (Tarancón *et al*., 2002). Once this radionuclide is discharged into the environment, its accumulation is related to its adsorption. Due to its biochemical similarities with calcium, more than 99% of strontium is efficiently incorporated into the bone tissue and teeth (Saraygord-Afshari *et al*., 2011). Since Sr-90 becomes bound up in the skeleton instead of Ca, it is a potential cause of bone cancer and damage to blood forming tissue (Acar and Acar, 2004).
A great number of analytical methods have been developed and applied for the determination of Sr-90 contamination levels in the environment, biota, foodstuffs including milk, human organisms to calculate transfer factors, following the migration and uptake processes and estimation of the dose impact to man (Vajda and Kim, 2010). Thus, for environmental and nuclear samples various measuring techniques are used, i.e. beta counting, liquid scintillation spectrometry and mass spectrometry.
Since Sr-90 transfers into humans mainly via foodstuffs, and milk is the principle source of chemical analogue of calcium in human diet, thus strontium transfer by cow milk is efficient and rapid. Level of milk contamination can give an indication of Sr-90 deposition over a wide area (Brun *et al*., 2002). Therefore, Sr-90 measurement, especially in milk, has became considered in environmental monitoring programs (Saraygord-Afshari *et al*., 2011). Due to this, this paper is intended to give an overview of the results of Sr-90 activity analysis in the milk and dairy product samples consumed in the Republic of Serbia, followed by the estimation of its annual effective dose, in order to assess the toxic effects of this radionuclide in the consumers.

Material and Method

Milk and dairy products: sour cream, cheese, yogurt and cream were obtained from local markets in Belgrade (Republic of Serbia) or from the control of import/export in the Republic of Serbia. Total number of 40 samples were collected during the years of investigation (from 2015 to 2022): 22 milk samples, 9 sour cream samples, 6 cheese samples, 2 yogurt samples and 1 cream sample. All the samples were prepared before analysis. First, approximately 5 L of milk, sour cream or yogurt sample or approximately 3 kg of investigated cheese or cream sample were dried to a constant weight in a porcelain crucible in an oven at 80 ^{o}C. After the drying of the samples, complete ashing in a muffle furnace at 800 ^{o}C was carried out for about 17 hours (the temperature should be increased gradually). As a result of this step, a large amount of organic materials such as fats and proteins will be decomposed. Approximately 20 g of the ash was used for the analysis of Sr-90.
The applied validated radiochemical analytical method which is the modification of classic method, was based on the direct determination of Y-90 that is in secular equilibrium with Sr-90 (Sarap *et al.*, 2014). The method was tested and confirmed using certified reference milk sample (IAEA-152) received by AQCS (Analytical Quality Control Services) laboratory of the International Atomic Energy Agency (IAEA), Vienna, Austria. Beta counting was performed using low lever gas flow proportional counter Thermo Eberline FHT 770 T (ESM Eberline Instruments GmbH, Erlangen, Germany). The calibration of the beta radiation counting system was performed using a certified radioactive point source of Sr-90, produced by Czech Metrology

Institute from Prague. Each sample was counted once for 5400 s. The efficiency of the counter for beta counting was approximately 35 %.

After sample counting, the activity concentration of Sr-90, *A* (Bq/L or Bq/kg) was calculated using the following equation:

$$A_{Sr} = \frac{(R - R_0) \cdot e^{\frac{\ln 2}{T_{1/2}} \cdot t}}{\varepsilon \cdot \eta_{Al} \cdot \eta \cdot U}$$

where *R* is count rate of the measured sample (1/s), R_o denotes background count rate (1/s), *t* is time elapsed from Y-90 separation (h), $T_{1/2}$ is half-life of ^{90}Y (h), ε is efficiency of the detector, η_{Al} is yield factor of aluminium for the measured sample determined by gravimetry, η is yield factor of the used method and *U* is the initial amount of sample (volume of milk, sour cream or yogurt sample given in L and mass of cheese or cream sample given in kg).

According to the Currie criteria, the minimum detectable activity, *MDA* (Bq/L or Bq/kg), is defined so that, if an amount of radionuclide equal to the MDA exits in the sample, it will be detected with 95 % probability (Alvarez *et al*., 1995). In the conditions of the present research, the minimum detectable activity was evaluated using formula:

$$MDA = \frac{LD \cdot e^{\frac{\ln 2}{T_{1/2}} \cdot t}}{U}$$

where *LD* is detection limit of beta radiation (1/s) which is automaticly calculated by a software used.

In order to assess the annual effective dose rate, due to ingestion of artificial radionuclide Sr-90 by milk and some dairy products *D* (Sv), the following equation was applied (Saraygord-Afshari *et al*., 2011):

$$D = A \cdot \eta \cdot U$$

where *A* is the activity concentration of Sr-90 in samples (Bq/L or Bq/kg), η is the age dependent effective dose conversion factor of Sr-90, which amounts to 28 nSv/Bq for adults, and *U* is the annual consumption of particular milk or dairy products.

Results and Discussion

The results of determination of Sr-90 activity concentration in milk and some dairy products samples are summarized in Table 1. The samples are marked as follow: milk samples (M-1 to M-22), sour cream samples (SC-1 to SC-9), cheese samples (C-1 to C-6), yogurt samples (Y-1 and Y-2), and cream sample (CR-1). The average measured Sr-90 activity concentration in the investigated milk samples was (0.031 ± 0.009) Bq/L, in the sour cream samples (0.049 ± 0.014) Bq/L and in the cheese samples (0.105 ± 0.033) Bq/kg. The percentage of samples with activity concentration of Sr-90 below the detection limit are the following: 59 % for milk samples, and 67 % for sour cream and cheese samples. The radionuclide Sr-90 is not detected in yogurt and cream samples. Comparison of the determined activity concentrations and the minimum detectable activities for investigated milk and dairy products samples shows that the results are mostly close to, or below the detection limit. The obtained values in this study are typical for this type of samples and are comparable with literature values (Saraygord-Afshari *et al*., 2011; Lopes *et al*., 2014). The Rulebook published in the Official Gazette of the Republic of Serbia (2018) stipulates upper limits of radiostrontium isotopes, particurarly Sr-90 activity in milk and dairy

products at 125 Bq/L or Bq/kg. The limit value of Sr-90 content given by the regulation is applied in emergency situations, such as after nuclear accident.
Based on the obtained activity concentrations of Sr-90 in investigated samples, the annual effective dose rate was estimated (Table 1). To estimate biological hazard from this radionuclide, which can occur due to milk or some dairy products consumption, the effective dose is calculated using the above-mentioned equation. Based on this estimation, it can be said that the annual effective dose rate for population due to ingestion Sr-90 by milk and dairy products is significantly below recommended annual dose limit for an individual from the population.

Table 1. The activity concentration of Sr-90 (Bq/L or Bg/kg of fresh weight) in the milk and some dairy products samples and the estimated of annual effective dose rate values for adults (nSv)

Sample ID	Min value	Max value	Average	D_{min}	D_{max}	$D_{average}$
M-1 to M-22	< 0.008	0.08	0.031	22.4	224	86.8
SC-1 to SC-9	< 0.02	0.17	0.049	8.9	76.2	21.9
C-1 to C-6	< 0.05	0.158	0.105	13.4	42.5	28.2
Y-1 and Y-2	< 0.017	< 0.065	< 0.041	9.9	38.2	24.1
CR-1	< 0.053			4.4		

Conclusion

In this research, useful information of the amount of this artificial radionuclide in milk and some dairy products at the area of the Republic of Serbia, by applying the suitable method for Sr-90, was obtained. Due to its long half-life and its radiotoxicity, this radionuclide has a special importance in the food cycle from the point of view of radiation hygiene. Beside this, the speciation of Sr-90 in contaminated milk or dairy products is of interest with respect to the bioavailability to the human organism, processing and decontamination of milk and radiochemical analysis. Therefore, Sr-90 measurement, especially in milk, has acquired considerable attention in environmental monitoring programs.
Data obtained by the conducted study can also provide an opportunity to verify any impact from the ingestion of Sr-90 by the population due to consumption milk and some dairy products. The calculated average annual effective dose rates for adults (from 4.4 nSv to 86.8 nSv) show that these doses stillrepresent a very small fraction of the natural background annual dose received by human, which amounts 1 mSv, so it is sufficiently low to pose a risk to human health.

Acknowledgement

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COMPARISON OF DIFFERENT EXTRACTION METHODS FOR QUANTIFICATION OF INDIVIDUAL PHENOLIC COMPOUNDS IN WINE BY LIQUID CHROMATOGRAPHY

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Abstract

Two different methods for sample preparation as a preliminary phase for the quantification of individual phenolic compounds were compared with the aim to establish the best conditions for the determination of these compounds in wine samples by ultra-high performance liquid chromatography (UHPLC). Wine of the variety Vozd (newly acquired variety from Faculty of Agriculture in Zemun) was the subject of this study. Grapes were harvested in optimal enological maturity which originated from vineyards belonging to winery "Draskovic" in Vrsac (Serbia). Three different vinification techniques were applied (cold maceration, thermomaceration and carbonic maceration). Cold maceration (C) was conducted at temperature of 4°C (four days) and thermomaceration (T) at temperature of 60°C (heated one hour). For carbonic maceration (CM) it was necessary to use dry ice and that maceration lasted four days. After maceration, pomace was separated and obtained wine samples were bottled and stored until analyses. The analysis of individual phenolic compounds (caffeic acid, *p*- coumaric acid, *p*- hydroxybenzoic acid, rutin and quercetin) in wines was performed by using a coupled with a diode array detector and a triple quadruple mass spectrometer (UHPLC DAD–MS/MS). Quantification of investigated phenolic compounds after solid phase extraction have showed higher concentrations of these compounds in wine, than in samples in analysed without any pretreatment (direct injection).

Keywords: *Phenolic compounds, Wine, Solid phase extraction, Liquid chromatography.*

Introduction

Chromatographic analysis of wine and other complex matrices presents an analytical challenge in terms of effective analyte resolution, compound identification and quantification. The quantification of phenolic compounds in wine is very important because of their biologically properties and also due to this group of substances being responsible for colour, astringency, flavour and hardness of wine. The determination of this group of compounds is important since variations in wine types and styles are largely due to the concentration and composition of wine phenols. Different variables, such as type of organic solvent, time of extraction, pH of the extraction, solvent for the extract have different impact to obtaining chromatograms of phenols that could be easy interpreted (Malovaná et al., 2001). Many reported methods lean toward simplicity in sample manipulation and suggest direct injection. Other methods illustrate the advantages of sample fractionation via solid-phase extraction (Manns and Mansfield, 2012). SPE is capable of reducing interference from the high molecular weight phenolics eluting as a broad hump, while at the same time retaining most of the information on the low molecular weight compounds (de Villiers et al., 2004). While some authors favor direct injection of wine samples

(Bonerz et al., 2008; Lamuela-Raventós and Waterhouse, 1994), newer studies support adequate sample preparation required for effective HPLC analysis such as SPE (Malovaná et al., 2001; de Villiers et al., 2004; Manns and Mansfield, 2012). The main goal of pretreatment procedures is to eliminate possible interferences from ballast substances and to simplify the final determination and identification of individual substances. The focus of this work, therefore, was to investigate the use of a SPE procedure for the isolation of phenolic components from red wine and to compare these results with those obtained after direct injection of wine, prior to HPLC analysis.

Materials and methods

The grape variety Vozd (vintage 2020) was harvested in optimal enological matutity which originated from vineyards belonging to the “Draskovic” winery in Vrsac (Serbia). Total sugar content was 24.4% and titratable acidity (determinate with NaOH) was 6.7 g/L as tartaric acid.
After grapes crashing and destemming, the samples of crushed grapes sulfited with 10 g of $K_2S_2O_5$ per 100 kg and yeast strain *Saccharomyces cerevisiae* in the amount of 20 g/hL (BDX, Lallemand, Canada) was inoculated. Alcoholic fermentation with maceration lasted 14 days at temperature of 25±3°C using the “pigeage” system (mechanically punching down).
Three different vinification techniques were applied (cold maceration, thermomaceration and carbonic maceration). Cold maceration (C) was conducted at of 4°C (four days) and thermomaceration (T) at temperature of 60°C (heated one hour). For carbonic maceration (CM) dry ice was used and that maceration lasted four days. After maceration, pomace was separated and obtained wine samples were bottled and stored until analyses.
Quantification of individual phenolic compounds was achieved using ultra-high performance liquid chromatography coupled with a diode array detector and a triple quadruple mass spectrometer (Dionex Ultimate 3000 UHPLC system equipped with a diode array detector (DAD) that was connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer (UHPLC DAD–MS/MS) (ThermoFisher Scientific, Basel, Switzerland). Immediately prior to injection into the HPLC system, a preliminary preparation with the solid phase extraction (SPE) was provided. The Oasis HLB 6cc/200 µm SPE cartridges (Waters, Milford, MA, USA) were used for the solid phase extraction of samples. The cartridge was conditioned with 5 ml of methanol followed by 5 ml of distilled water. A wine sample (5 ml) was passed through the cartridge, washed with 2 ml of water and eluted with 2 ml of methanol. The samples were collected and analysed by UHPLC DAD–MS/MS. Second set of wine samples were directly injected (DI) into the HPLC system and after filtering through a 0.45 mm syringe cellulose filter.

Results and discussion

The results of UHPLC separation and quantification of caffeic acid, *p*- coumaric acid, *p*-hydroxybenzoic acid, rutin and quercetin are listed in Table 1. According to obtained results certain observations on the influence of the type of sample preparation and maceration procedure can be made.
The highest concentration of *p*-hydroxybenzoic acid was noticed in sample obtained by cold maceration and prepared for UHPLC analysis using SPE (1.784 mg/l) and the lowest was measured in wine sample where carbonic maceration (CM SPE) applied (0.614 mg/l). There was no statistically significant difference in amount of *p*-hydroxybenzoic acid comparing direct injection (DI) and SPE ($p > 0.05$). Paired samples T-test was used (IMB SPSS Statistics).

Content of caffeic acid and rutin were generally higher in SPE prepared samples than in samples quantified after direct injection (Table 1), but it was not found statistical significant difference ($p > 0.05$). Wine obtained by cold maceration and prepared for quantification using SPE had a highest value of caffeic acid (25.220 mg/L) (Table 1).

Table 1. Concentrations of quantified phenolic compounds after two different preparation techniques

Wine sample	*p*-hydroxybenzoic acid (mg/l)	Caffeic acid (mg/l)	Rutin (mg/l)	*p*-coumaric acid (mg/l)	Quercetin (mg/l)
T (SPE)	1.120	10.963	0.183	2.519	3.189
T (DI)	0.730	7.591	0.010	2.983	0.174
C (SPE)	1.784	25.220	0.213	3.754	0.239
C (DI)	0.815	16.818	0.012	3.009	0.063
CM (SPE)	0.614	11.114	0.033	4.108	0.116
CM (DI)	0.967	8.024	0.005	3.667	0.042

Contrary to results for other analyzed phenolic compounds, the carbonic maceration (CM) has showed the highest concentration of *p*-coumaric acid (4.108 mg/L) with regard to other applied maceration techniques. For quantification of *p*-coumaric acid, SPE technique gave higher concentrations comparing with direct injected wine sample. Similar to our results for analyzed components in wine, gallic acid in particular often behaved poorly when analyzed via direct injection (Manns and Mansfield, 2012). From all applied sort of maceration during producing and later preparing wine samples for quantification of quercetin, the best combination was thermomaceration with solid phase extraction technique (Tabela 1).
Samples analyzed by direct injection exhibit a great amount of chromatographic interference due to condensed tannins and polymeric anthocyanins (Manns and Mansfield, 2012). According to Malovaná et al. (2001) direct injection into the HPLC system of the wine samples results in very complex chromatograms that do not allow the identification and quantitation of peaks. Except preparing technique of wine samples prior to HPLC injection, the high impact on concentration of quantified phenolic compounds also have maceration time (Budic Leto et al., 2008; Plavša et al., 2012) and applied maceration technique (Sacchi et al., 2005; Borazan and Bozan, 2013).

Conslusions

Solid-phase extraction (SPE) technique appears to be more suited to the isolation of analysed phenolic components from a red wine compared to the direct injection. According to our results and literature, best results were obtained using solid phase extraction because of fractionation/clean-up of wine samples. It should be noticed that solid phase extraction has been more suitable for obtaining higher concentration identified phenolic compounds. Nowadays, SPE is becoming more used because it is rapid, economical, and sensitive and because different cartridges with a great variety of sorbents can be used.

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PENICILLIUM EXPANSUM AS A POSTHARVEST PATHOGEN OF TOMATO FRUIT IN SERBIA

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Abstract

Tomato (*Solanum lycopersicum*, L.) is one of the most widely cultivated crops with high content of vitamins and antioxidant lycopene, which are very important for human health. During the growing season and postharvest storage tomato is susceptible to various diseases caused by pathogenic fungi. In July 2019, tomato (cv. Balkan) with symptoms of blue mold decay were collected from market in Belgrade, Serbia. Macroscopic morphology of three obtained monosporic isolates were observed after growth on Czapek yeast autolysate agar (CYA), creatine sucrose agar (CREA), and malt extract agar (MEA) for seven days at 25ºC. Also, selected isolates were incubated at 5, 25, and 37°C for one week on CYA to monitor the effect of different temperature incubation conditions. Colony characteristics and micromorphology of the fungi agreed with the literature descriptions of *Penicillium expansum*. The conidiophores of isolates were hyaline, mainly terverticillate; stipes usually smooth-walled; metulae and phialides cylindrical; conidia ellipsoidal (3-3,86-4 × 3-3,13-4 µm), greenish, smooth-walled. Total DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and partial β-tubulin (*BenA*) sequence was amplified with primers Bt2a/Bt2b. *BenA* sequence of representative isolate ParP/1 was deposited in NCBI GenBank (Accession No. ON186699). Phylogenetic analysis clustered our isolate with other isolates of *P. expansum*. Pathogenicity test was conducted on symptomless, detached tomato fruits. All tested isolates caused typical blue mold symptoms on tomato fruits after seven days of incubation. To our knowledge, this is the first report of *P. expansum* causing postharvest fruit decay on tomato in Serbia.

Keywords: *Tomato, Postharvest decay, Penicillium expansum, Identification*

Introduction

Tomato (*Solanum lycopersicum*, L.) is one of the most widely cultivated crops in the world. Tomato fruits are a great source of vitamins C and K, potassium, and antioxidant lycopene which are very important for human health. Water-soluble nutrients and high moisture content in tomato fruits make them perishable and susceptible to a number of postharvest fungal pathogens, such as *Alternaria alternata*, *Cladosporium cladospirioides*, *Geotrichum candidum*, *Botrytis cinerea*, *Fusarium acuminatum*, *Rhizopus stolonifer*, *Talaromyces miniolutes*, *Penicillium polonicum*, *P. solitum* and *P. olsonii* (Chatterton *et al.*, 2012; Singh *et al.*, 2017; Petrasch *et al.*, 2019; Ma *et al.*, 2020; Stošić *et al.*, 2020; Slathia *et al.*, 2021, Živković *et al.*, 2021).
Blue mold decay caused by *P. expansum*, is one of the most economically important postharvest plant disease, globally. Cosmopolitan distributions of *P. expansum*, decay-producing capability coupled with a strong virulence are properties that characterize this species as a broad spectrum pathogen (Pitt and Hocking, 2009). Wounds such as punctures created at harvest and during postharvest handling are the primary avenue for infection of fruits by this fungus. Economic

losses caused by the disease are mainly attributed to lower fruit quality and marketability. Also, it is important to note that *P. expansum* is a producer of mycotoxin patulin and other secondary metabolites (citrinin, chaetoglobosins, communesins, roquefortine C, and expansolides A and B) which can compromise human health (Andersen *et al.*, 2004).
In Serbia, postharvest decay caused by *P. expansum* are detected on apple, pear, quince, and medlar fruit, and onion bulbs (Vico *et al.*, 2014; Duduk *et al.*, 2017; Stošić *et al.*, 2021; Žebeljan *et al.*, 2021). To our knowledge, there are no literature data about blue mold fruit decay caused by *P. expansum* on tomato in Serbia. Therefore, the objective of this study was identifying the causal agent of this disease on tomato using morphological, physiological and molecular methods.

Material and methods

Fungal isolation

In July 2019, tomato fruits (cv. Balkan) with symptoms of blue mold decay were collected from market in Belgrade (Serbia). The decayed area of the fruits was pale yellow and tissue was soft and watery. Blue sporulation was abundant on the fruits surface. Small pieces on the margin of diseased and healthy tissue were surface sterilized in 3% NaOCl for 3 min, followed by several rinses with sterile distilled water, and placed on malt extract agar (MEA) for seven days at 25°C. Three monosporial isolates were obtained and characterized using morpho-physiological and molecular methods.

Morphological and physiological characterization

Macromorphology of the isolates were examined on three media: MEA, Czapek Yeast Autolysate agar (CYA), and Creatine sucrose agar (CREA). Cultures were three-point inoculated with 1 µl of conidial suspension and incubated for seven days at 25°C (Visagie *et al.*, 2014). After the incubation, colony growth and texture were noted. Also, the isolates were inoculated on CYA plates and incubated for seven days in the dark at 5, 25, and 37°C to monitor the effect of different temperature incubation conditions. The experiments were performed in three replicates, and basic descriptive values (average and standard deviation) were done in Microsoft Excel 2007 (Microsoft Corporation, U.S.A.). Microscopic slides were prepared from ten day old MEA cultures with 60% lactic acid. Morphological features of conidiophores, phialides and conidia (shape, cell wall ornamentation) were observed using Olympus microscope (BX51, Japan). Measurements of conidia (length and width) were done in Quick Photo Camera software program (Promicra, s.r.o., Czech Republic).

DNA extraction, PCR amplification and phylogenetic analysis

Genomic DNA was isolated from mycelium scraped from the surface of a seven days old MEA culture with DNeasy Plant Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Partial sequence of *BenA* gene was amplified in a polymerase chain reaction (PCR) using Bt2a/Bt2b primer pair (Glass and Donaldson, 1995). Thermal cycle conditions set up as recommended by Visagie *et al.* (2014) - an initial denaturation 5 min at 94 °C; 35 cycles of 45 s at 94 °C, 45 s at 55 °C, 1 min at 72°C; and a final denaturation step of 7 min at 72 °C. Reaction mixture contained 20 µl of 2×PCR Master Mix (TaqNova-RED, DNA Gdansk, Poland), 4 µl of each primer (Metabion International AG, Germany), 10.4 µl of sterile nuclease-free water (Thermo Fisher Scientific, U.S.A.), and 1.6 µl of fungal DNA. Amplified products were analyzed by 1% agarose gel electrophoresis, stained with Midori Green DNA Stain (Nippon Genetics), and visualized under a UV transilluminator. The products of PCR were purified and sequenced by the Macrogen's Europe commercial sequencing service (Amsterdam, the Netherlands). Clustal W algorhitm (Thomson *et al.*, 1994) implemented in MEGA7 software (Kumar et al, 2016) was employed to assembly sequence contigs. Maximum likelihood (ML) phylogenetic tree was constructed in the same software, using reference and reliable *BenA* sequences from NCBI GenBank database.

Pathogenicity assay

To confirm the pathogenicity of our isolates, symptomless, detached tomato fruits (cv. Balkan) were first surface-sterilized in 70% ethanol, and then inoculated with 50 µl of a spore suspension (1×10^6 conidia/ml) from the cultures grown seven days on MEA. The control fruits were inoculated with 50 µl of sterile distilled water. All inoculations were done in three replicates. The fruits were placed in a sterile plastic container at 25°C, and >95% relative humidity. After seven days reisolation of the pathogenic fungi was performed, and obtained cultures were checked for colony and spore morphology to confirm Koch’s postulates.

Results and discussion

Identification and characterization of the causal agent of blue mold decay on tomato is essential to establish the basis of studies on epidemiology and disease control. Morphology and physiology of isolates from tomato fruits (ParP/1, ParP/3 and ParP/4) were uniform. After seven days of incubation at 25°C, all isolates had radially sulcate colonies on CYA (Figure 1B), and plane to moderately radially sulcate on MEA. Cultures manifested different textures on tested media – velvety to loosely floccose on CYA, fasciculate on CREA, plane and weak fasciculate on MEA. Reverse, colonies had light brown colour with brighter margin (CYA), pale yellow with a hint of greenish (MEA), and purple with a yellow margin (CREA). Intensive conidiogenesis was present, with blue-green spores on CYA, and green on MEA and CREA. Isolates had white mycelia on all inoculated media, present as the margin of the culture, broad on CYA (5 mm width), and moderate on CREA (2 mm) and MEA (1.5 mm). Clear exudate droplets were noticed on CREA, while there were no exudate formation on CYA and MEA. All tested isolates manifested intensive growth across all tested media, with CYA being the most stimulative medium (Figure 2A). Isolates cultivation at different temperatures on CYA showed that the optimal temperature for their development was 25°C, and the smallest average diameter was measured at 5°C. Fungal growth was not recorded at 37°C (Figure 2B). Microscopic observations revealed that all isolates formed hyaline, mainly terverticillate conidiophores; stipes usually were smooth-walled; metulae and phialides were cylindrical; and conidia were ellipsoidal (3-3,86-4 × 3-3,13-4 µm), greenish, and smooth-walled. Colony characteristics, micromorphology and physiology of our isolates agreed with the literature descriptions of *P. expansum* (Frisvad and Samson, 2004; Pitt and Hocking, 2009; Visagie *et al.,* 2014; Vico *et al.*, 2014).

All tested isolates caused typical *Penicillium*-like decay on tomato fruits after seven days of incubation. No symptoms were observed on any of the control fruits (Figure 1A). Isolates recovered from inoculated tomato fruits showed the same morphological characteristics as the original isolates, thus completing Koch's postulates.

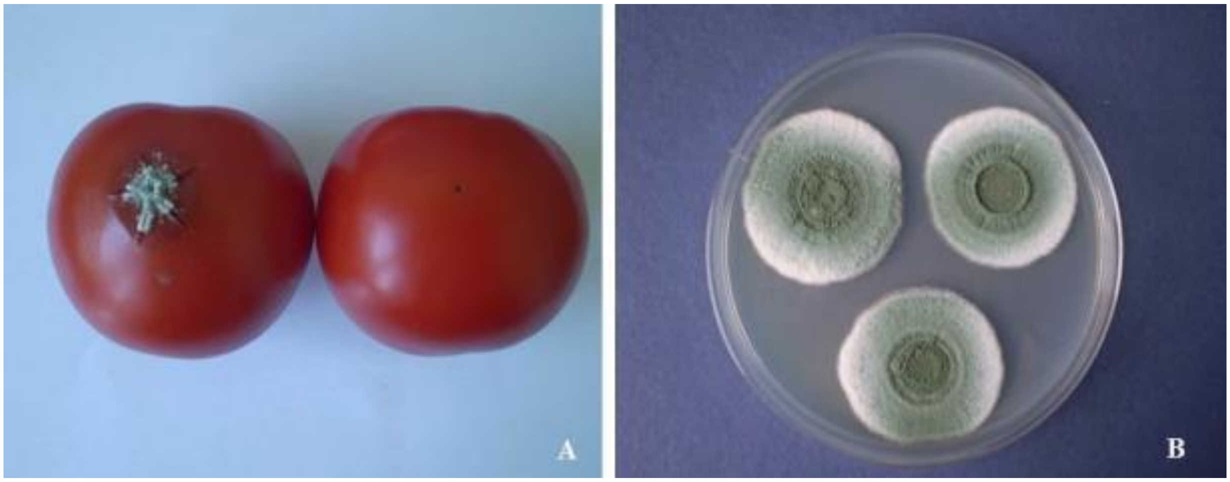

Figure 1. A. Pathogenicity of isolate ParP/1: tomato fruit inoculated with pathogen (left) and control fruit without symptoms (right). B. Colony characteristics of isolate ParP/1 after seven days of incubation at 25°C on CYA.

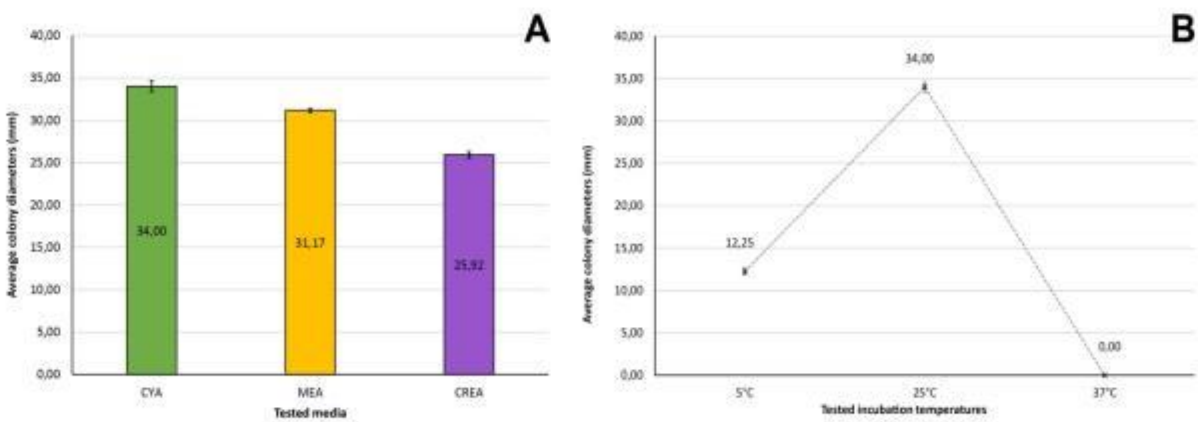

Figure 2. A. The average colony growth of *P. expansum* isolates (mm) on three tested solid media after seven days of incubation at 25°C. B. The average colony growth of *P. expansum* isolates (mm) on CYA after seven days of incubation at three tested temperatures. Vertical error bars represent standard deviation (SD) in both graphs.

Molecular analyses of genomic DNA from tomato isolates confirm the identity of the pathogen. The sequence of representative isolate ParP/1 was submitted to the NCBI GenBank database (Accession No. ON186699). Phylogenetic analysis for selected *BenA* sequences of *Penicillium* revealed that all *P. expansum* isolates (including Serbian) formed a separate clade with a high bootstrap support (98%), (Figure 3). Within that clade, Serbian sequence was separated in one subclade while other *P. expansum* sequences were grouped into the second subcluster. This separation had relatively high node support (71%). Sequences of the ITS region have been used before for resolving *Penicillium* phylogenies (Skouboe *et al.*, 1999). Because its resolution is limited at species level in *Penicillium*, *BenA* is proposed as the secondary molecular marker, especially in a routine identification procedure (Visagie *et al.*, 2014).

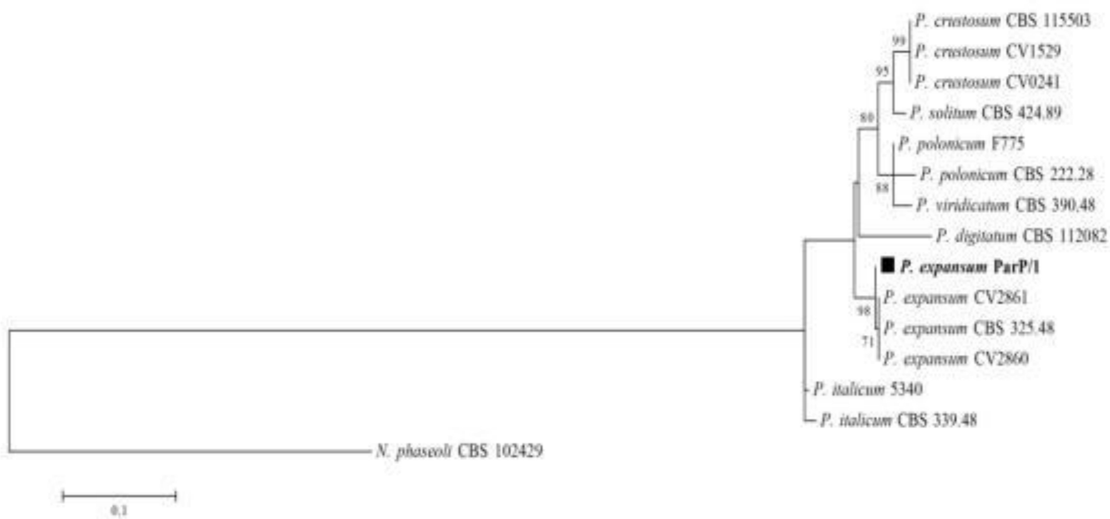

Figure 3. Maximum likelihood (ML) phylogenetic tree based on *BenA* sequences of selected *Penicillium* species. The isolate of *Neocosmospora phaseoli* (CBS 102429) represented an outgroup sequence. Bootstraping was performed in 1,000 replications and bootstrap values <70% are omitted. The scale depicts the number of substitutions per site. Isolate in bold font and with black square is from this study.

Conclusion

Based on morphological and physiological characterization, pathogenicity test, and molecular and phylogenetic analyses, the isolates from this study were identified as *P. expansum*. To our knowledge, this is the first report of *P. expansum* associated with fruit decay on tomato in Serbia. Knowledge of the populations of *Penicillium* species of tomato fruits in Serbia is of great importance due to their ability to reduce quality and shelf life. Also, obtained results provide the base for development of effective disease management strategies.

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APPLICATION OF MEDICAL AND SPICE HERBS IN FOOD PRODUCTS IN ORDER TO ACHIEVE GREATER MICROBIOLOGICAL SAFETY AND QUALITY

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Abstract

There is concern around the world about foodborne diseases caused by pathogenic microorganisms. In order to prevent microbiological contamination of food, various methods of preservation are used, with recent efforts focusing on the elimination of synthetic additives and the use of natural antimicrobial agents. Thus, the use of medicinal and spicy herbs has been introduced in order to extend the shelf life and improve the quality of certain food products. Medicinal and spicy plants can be added to food products in their fresh or dried state or in the form of their derivatives such as extracts and essential oils (EO). EO are good sources of bioactive compounds with antioxidant, antibacterial and antifungal activity. Phenolic compounds in essential oils have been identified as the dominant and major antimicrobial compounds. In order to avoid altering the sensory characteristics of food, research is moving in the direction of combining different medicinal and spice herbs with the aim of improving their efficacy, by reducing the effective doses of individual plants. Moreover, research shows that, in addition to the direct use of essential oils in food, there is an increasing discussion about active packaging based on the use of combined materials that can be carriers of essential oils and provide the possibility of their effective release into already packaged food. This paper reviews individual food groups and medicinal and spice plants that have shown to be effective in improving microbiological safety of food products with extended shelf life, without altering the sensory properties in food products.

Key words: *medicinal and spice herbs, microbiological safety, quality, food, active packaging.*

Introduction

Food safety and quality are a constant concern for the food industry and are the most important item in the choice of food by consumers. Although food safety is achieved by various physical, chemical or biological processes, with the use of food additives, there is still concern among producers and consumers, due to food poisoning caused by pathogenic microorganisms. It has been shown that certain bacteria adapt after the treatment process and show resistance, especially the bacteria *Esherichia coli O157:H7, Listeria monocytogenes, Salmonella enteritidis* (Papadochristopoulos et al., 2021). Of particular concern are new pathogens that have not been registered as such so far. In addition, WHO has called for reducing salt consumption in order to reduce the incidence of cardiovascular disease, which has prompted the food industry to find other additives to ensure food safety. In the last decade, consumer interest in low-processed, high-quality, nutritionally improved food products, while maintaining safety, has increased, using natural extracts as an alternative to synthetic preservatives. Their advantage is synergy with other conservation methods, they are considered safe and possess properties such as antioxidant, antimutagenic, antitoxic, antifungal and antibacterial effect (Augustin and

Sanguansri, 2015). For this reason, today, in addition to new technological processes in food processing and new ways of packaging, new antimicrobial and antioxidant additives as natural food additives, based on medicinal plants and spice herbs, occupy a significant place. Plant isolates such as essential oils (EO) have been used, which, in addition to having an antimicrobial effect, can also favorably affect the quality of the food product during processing, to prevent or delay some undesirable chemical reactions. For example, being present in the marinade, they can reduce the formation of heterocyclic amines during the heat treatment of grilled meat (204°C). The pimento extract, in which eugenol is the main component, reduces the production of acrylamide by 50%, in a potato frying matrix at 180°C, 20 min (Ferreira et al., 2021; Pietrysiak et al., 2019; Mercanoglu Taban et al., 2022). An example is grape tea extract that is rich in the natural antioxidant dihydromyricetin (DHM), which reduces oxidation in various oil systems and meat products, as well as the formation of acrylamide and malonaldehyde in bakery products (Carneiro et al., 2021). The addition of coriander during the fermentation of Korean soy sauce reduces the level of biogenic amines and the bacteria that produce them (Manna and Park, 2020). A limiting factor in the use of plant extracts as a preservatives is the creation of undesirable aromas and flavors.

The aim of this review is to present the antimicrobial activity of natural ingredients derived from medicinal and aromatic plants and their application in increasing microbiological safety during storage as well as preservation of the most sensitive food products, such as meat and meat products and fresh fruits and vegetables.

Medicinal and spicy herbs as food preservatives

Derivatives of medicinal and spice plants inhibit the growth of microorganisms in food due to the presence of EO and other secondary plant metabolites. Several studies have investigated the in vitro and in vivo antibacterial and antifungal activities of medicinal and aromatic plants (Giarratana et al., 2016; Šojić et al., 2021). Aromatic plants used to isolate essential oils are most commonly found in the genera *Apiaceae, Lamiaceae, Lauraceae, Myrtaceae, Pinaceae, Piperaceae, Rutaceae and Zingiberaceae*. EOs are natural, complex volatile aromatic, hydrophobic, oily liquids composed of several related compounds synthesized in aromatic plants as secondary metabolites. The antimicrobial properties of EOs come from components such as phenolic acids, terpenes, aldehydes and flavonoids, and the antimicrobial action of EO is based on the mechanism of altering fatty acids profiles and cell membrane structure and increasing cell permeability, as well as affecting membrane proteins and inhibiting the cell wall (Yousefi et al., 2020; Mercanoglu Taban et al., 2022; Manna and Park, 2020).

Numerous EOs are registered as safe (GRAS) and approved as food additives. EOs can be extracted from plants by distillation, the use of solvents or by squeezing. Around 3000 EOs have been produced from at least 2000 plant species, of which 300 are commercially important (Sakkas et al., 2017). There are more than 500 different known constituents of essential oils, only a few of them are present in large quantities, and they determine the sensory, physical and chemical properties and pharmacological activity. Phenolic compounds in essential oils have been identified as the dominant and most important antimicrobial compounds and show a great variety of structures, from simple molecules (eg ferulic acid, vanillin, gallic acid and caffeic acid) to polyphenols such as tannins and flavonoids. Some phenolic compounds, are not only the main phytochemicals with antioxidant and antimicrobial activity, but also influence the taste, color and texture of food products. At lower concentrations, they act on enzymes in the cell of the microorganism, and at higher concentrations, they denature proteins. Phenolic content decreases during food processing because they are sensitive to heat, oxygen and light, so microencapsulation as a solution has been imposed, ensuring their bioavailability and stability in food matrices. They are known to have antimicrobial effect against *Salmonella spp., Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli.* (Di Santo et al., 2021; Cutrim et al., 2019).

A lower sensitivity of gram-negative bacteria to theeffect of essential oils was observed, given that gram-negative bacteria have a hydrophilic lipopolysaccharide coating that limits the diffusion of hydrophobic compounds. In molds, the structure of the cell membrane is disturbed, its assembly is blocked, and the germination of spores, mycelial growth and cell respiration are inhibited, which leads to cell death. Also, essential oils can denature viral structural proteins or glycoproteins (Latgé and Chamilosa, 2020). Unlike antibiotics, which have only one active ingredient to which the bacterium must develop resistance, essential oils are mixtures of substances with different mechanisms of action. Therefore, bacteria have not yet been able to develop resistance to essential oils (Sakkas et al., 2017). When using aromatic plant derivatives as natural food preservatives, it is very important to know the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) and their interaction with the matrix and sensory properties of food, as well as the biological mode of action and spectrum of target microorganisms. It has been foud that the essential oils of the following spices and medicinal herbs oregano, mint, cinnamon, sage, cumin, thyme and cloves are among the oils with the strongest antimicrobial action. In vitro studies have shown that EOs isolated from oregano and thyme are effective against microorganisms isolated from fermented meat products and cheeses, such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.* and *S. aureus* (Liu et al., 2017). EOs with good antifungal activity are: cineole, limonene, α-pinene, β-myrcene, camphor and β-pinene.

Recent research on some plants that have not been used in the food industry and have potential are interesting, such as the *Lovage* plant (Spréa et al. 2020).

Nowadays, many studies show that in addition to the direct use of EO in food, active packaging can be used, which is based on the use of packaging materials that can be carriers of EO, with the possibility of effective controlled release within pre-packaged food. Edible films containing EO are used in food packaging and contribute to a new dimension of packaging in food protection (Salgado et al., 2021). An example is carvacrol, which is incorporated into edible films of gelatin and nanofibers of potato starch in order to preserve food. The study by Salević (2020) reported that the investigated formulations of films with incorporated sage extract exhibit a complete bactericidal effect against S. aureus, while the effect on E. coli increases with increasing extract content in the film. The application of nanotechnology is increasingly in the focus of obtaining packaging materials, because nanoemulsification of EO can increase their antimicrobial activity while reducing the required amount, i.e., a tastier product can be obtained while extending shelf life and preserving food quality. A special attention is given to the analysis of the influence of the extract incorporation on the physical, chemical and functional properties of films, as well as on their biodegradability.

Application of medicinal and herbs in fruit and vegetable technology

In the last two decades, the number of cases of food-borne diseases that are related to the consumption of fresh or minimally processed fruits and vegetables has increased. The most common cases of disease outbreaks were contamination with pathogens, including *E. coli O157:H7, L. monocytogenes, S. enteritidis, Shigella* and *S. aureus*. When it comes to fruit, the main reason for fruit decay is the presence of molds such as *B. cinerea, Rhizopus spp., Penicillium spp.* and *Aspergillus spp*. The results of studies show that the use of EO can be an excellent substitute for synthetic preservatives in fruits and vegetables after harvest. An additional advantage is that it was found that fruit treated with EO also has higher amounts of sugars, flavonoids, anthocyanins, organic acids and phenolic compounds (Chen et al., 2019, Tagahavi et al., 2018). There is a growing interest in green tea extracts, as many studies have shown that these extracts have antibacterial, antiviral, antifungal and free radical scavenging activity associated with the polyphenols present in them (Othman et al., 2019, Górniak et al., 2019). A study conducted by Fernandez et al (2018) showed an exceptional antimicrobial effect of green tea on beet leaves, against *E. coli*, which is important because it is one of the main microorganisms associated with epidemics caused by salads and minimally processed vegetables.

In the study of Veloso et al. (2019) the successful application of thyme EO (0.2%) on processed rocket was confirmed, because the growth rate of the E.coli population over time was low, although the complete elimination of inoculated E .coli did not occur. Other products of EO distillation are rich in phenolic compounds, so the aqueous extract of oregano with a combination of vinegar and lactic acid has a bactericidal effect on E. coli O157:H7 and total mesophilic microbiota on fresh-cut spinach and lettuce while maintaining acceptable sensory properties (Poimenidou et al., 2016). EO oregano incorporated into edible pectin coatings increases the antioxidant activity in coated tomatoes without adversely affecting aroma acceptability. Biodegradable polyester nets with cinnamon were also observed to maintain the quality of tomatoes during storage (Black-Solis et al., 2019). Xu et al. (2014), in an in vivo test with cherry tomatoes, showed the effectiveness of laurel oil against *Alternaria alternata* infection. Edible coatings and on fruit, in combination with EO, have found wide application in order to extend the shelf life, or delay the ripening process. An example is gum arabic with lemongrass and and/or pomegranate peel extract, or in combination with cinnamon EO, on apple fruits (Pandey et al., 2022).

When it comes to small fruits (strawberries, raspberries, blueberries, blackberries and grapes) that have a very short shelf life, they are trying to find ways to extend storage while preserving quality. An example is the application of chitosan coatings with the addition of different EOs (carvacrol, cinnamaldehyde and trans-cinnamaldehyde) which applied to fresh blueberries, have antimicrobial activity (Sun et al., 2014). Strawberry decay can be reduced by treatments with thymol, eugenol and menthol, and p-cinnamon, linalool, carvacrol, anethole and perialdehyde have effectively slowed down the formation of blueberry mold (Sánchez-González et al., 2011). According to the study by Maghenzani et al. (2018), the shelf life of cherries can be extended to 28 days (1 °C) and 3 days (20 °C) without loss of weight and vitamin C content, using EO thyme and savory (steam treatment).

When storing nectarines and peaches, EO savory and thyme show high antifungal activity against the mold *Monilinia fructigena* (Santoro et al., 2018).El Quadi et al. (2017) confirmed the antifungal action of royal jelly EO on the mold that causes apple decay: *B. cinerea*, *P. expansum* and *Rhizopus stolonifer*. Thyme EO has also been shown to be very effective in controlling *B. cinerea* and *P. expansum* in some apple varieties (Banani et al., 2018).

Zhao et al. (2021) showed the antifungal effect of EO oregano (thymol and carvacrol at 125mg/L) on *B. cinerea* grape mold. In a study by Laranjo et al. (2019), EO of cinnamon, sage, and thyme were effective in controlling the mold population up to the 21 st day, in table grapes. In addition, Oh et al (2017) confirmed a marked inhibition of *Salmonella* contamination in grape berries by fumigation with EO lemongrass. The in vivo antifungal activity of EO mint against the yeasts *C. albicans, C. tropicalis, Pichia anomala* and especially *S. cerevisiae* in the juices of cashew, guava, mango and pineapple juices has been demonstrated (Almeida et al., 2019). Coating whey protein concentrate with 4000 ppm sage extract on pistachio grains completely inhibited the growth of *A. flavus* (Poimenidou et al., 2016). Perez-Alfonso et al. (2012) proved the antifungal effect of thymol, carvacrol and a mixture of both pure essential oils against *P. digitatum* and *P. italicum*, using in vitro tests and in vivo on lemon, whit thymol being the most effective.

Application of medicinal and spice herbs in meat technology

Meat and meat products are food products that must be safely stored under strict sanitary conditions, because they are subject to rapid chemical and microbiological deterioration, under the influence of various pathogens such as *Clostridium spp., Salmonella spp., Campylobacter jejuni, E. coli O157:H7, Aeromonas hydrophila* and *L. monocytogenes.* Of particular concern is the presence of *L. monocytogenes* in meat. *Salmonella spp*. is the second most common cause of reported zoonoses in Europe, and *S. tiphimurium* is one of the most common serovars isolated from pork.

Many studies have confirmed that EOs from medicinal and spice plants have antimicrobial activity against *L.monocytogenes* (EFSA-ECDC, 2018, Kurpas et al., 2018). Thymol, carvacrol, eugenol, carvone, cinnamaldehyde, limonene, α- and β-pinene and p-cymene are examples of major EO compounds that have activity against *L.monocytogenes* (Yousefi et al., 2020). The study conducted by Nedić et al. (2021) showed that EOs from oregano and thyme were effective against *L. monocytogenes* in fermented sausages, preserving the sensory characteristics of 0.3% EO of oregano or thyme and reducing the number of *L. monocytogenes* to below the detection threshold on the 21st day of ripening. Gavriil et al. (2021) confirmed the activities of aqueous plant extracts against S. tiphimurium and their application to improve the safety of pork. Industrial oregano hydrolate had the greatest antimicrobial potential, and in general oregano, thyme, calendula and basil were bactericidal, whereas mint, rosemary and corn silk were bacteriostatic. Furthermore, prepared marinades and edible coatings of industrial oregano hydrolate with the addition of EO oregano that treated pork stored at 4 °C were the most effective in inhibiting pathogens.

In the Mediterranean area, oregano and savory leaves are added to meat, fish and other food products because they show strong antimicrobial activity against various microorganisms (Ozkan et al., 2017). Selim (2011), investigated the effect of the addition of 11 different essential oils in ground beef stored at 7°C against enterococci resistant to vancomycin and against *E.coli 0157:H7* and the best effect was shown to be EO of thyme and sage. The results of Giarratan et al (2016) indicate that a mixture of EOs rosemary and thyme has a bacteriostatic activity against *L. monocytogenes* and that both 0.025 and 0.05% of tested EOs significantly inhibited the growth of *L. monocytogenes*, in mortadella. Sage has the potential to affect microorganisms such as *P. aeruginosa* and *B. cereus* strains. EO sage (0.05-0.1 mL/g) showed activity against microbial growth in fresh pork sausages with no negative effects on the sensory properties of this meat product

Hernández-Ochoa et al. (2014) found that EO cumin and cloves inhibit the growth of total bacteria by 3.78 log cfu/g when used on meat samples for 15 days at 2 °C. The antimicrobial activity of various spice extracts in raw chicken meat during 15 days of storage at 4 °C was also studied. It was found that treatment with extracts of cloves, oregano, cinnamon and black mustard was effective against microbial growth (Radha et al., 2014). Treatment of fresh Tuscan sausages with EO bay leaf reduced the population of total coliforms (a decrease of 2.8 log cfu/g) and extended the shelf life by 2 days (Da Silveira et al., 2014). A study with EO basil (50 ppm) in fermented traditional Nahm pork sausage showed a reduction in *Salmonella enteritidis* from 5 to 2 log cfu/g after 3 days at 4 °C, with a satisfactory sensory impression (Rattanachaikunsopon and Phumkhachorn, 2010). Gomez-Estaca et al. (2010) studied the effects of different EOs against 18 bacterial strains in fish muscle extract and concluded that cloves EO has the best antimicrobial effect; followed by EO rosemary, thyme and lavender. These EOs were able to inhibit *P.fluorescens* and *P.aeruginosa*.
Due to their functional properties, seaweeds are becoming more and more interesting to consumers, and so are the ways to extend their shelf life and maintain good quality. Dolea et al. (2018) found that by using EO thyme and oregano, in various combinations, it is possible to apply low concentrations of EO to achieve microbial inhibition without compromising the sensory quality of salmon burgers and seaweed during storage. However, a satisfactory extension of storage time cannot be achieved.

Conclusion

Herbal extracts are increasingly being used as natural antimicrobial agents to prolong shelf life and maintain food quality. Herbal essential oils of oregano, mint, cinnamon, sage, thyme and cloves are among the best natural antimicrobials. Additional studies are needed to determine the stability, safe doses, better uniformity of distribution in food matrices due to their hydrophobicity, as well as optimal amounts that will not impair the characteristic sensory properties of the food product.

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16S rRNA VS BIOCHEMICAL TESTS: CHALLENGE TO IDENTIFY PINK PIGMENTED *ERWINIA* SPECIES

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Abstract

Genus *Erwinia* has many important and destructive plant pathogenic species in wide host range. The species within the Genus show different morphologic structures in different culture media. Some of the species characterized typical pigmentation on different solid media such as Nutrient Agar and Yeast Extract $CaCO_3$ Agar. Phytopathogenic pink pigmented species of the Genus are known as *Erwinia persicina* and *Erwinia rhapondici*. Besides colony morphology, biochemical tests are still a unique and indispensable part of bacterial taxonomy. Today, molecular methods are applied as an integral part of the definitive characterization of an organism together with morphological and biochemical characteristics. The sequence of 16S rRNA gene region is the most widely used molecular technique for the identification of prokaryotes. In this study five pathogenic, pink pigment producer lettuce strains showing 16S rRNA gene sequence similarity were clarified by using some discriminative biochemical tests. Methyl red and Voges-Proskoer tests and dulcitol, glycerol, melezitoze, and D-xylitol usage of the strains were assessed as discriminative biochemical tests. The strains were recorded as negative on methyl red test, positive on Voges-Proskauer, negative for the usage of dulcitol, glycerol, melezitoze and D-xylitol. The results revealed that, although the sequence of 16S rRNA sequence results provides very high accuracy, a combination of discriminative biochemical tests beside 16S rRNA gene sequence provides a confident identification of the pink pigmented *Erwinia* species.

Keywords: *Erwinia, pink pigmentation, 16S rRNA, methyl red, Voges Proskauer.*

Introduction

Genus *Erwinia* is a member of *Enterobacteriace* family which has many destructive and well-known plant pathogenic bacteria all over the world. The members of the Genus are Gram negative, rod shapes and causes different types of symptoms in different plant hosts. As many of the plant related bacteria, colony features are important morphological identification criteria for *Erwinia* spp. Some of the species differentiate with their pigment production on the culture media. *Erwinia persicina* (*Epersicina*), *Erwinia rhapondici* (*E. rhapontici*) and *Erwinia rubrifaciens* (*E.rubrifaciens*) are the known pink pigmented plant pathogenic members of the Genus (Wilson et al., 1967; Hao et al., 1990; Hauben et al., 1998).It was revived the suggestion to support the proposal by adding evidence from the 16S ribosomal DNA sequence analysis of various plant-associated members of the *Enterobacteriaceae* and as a result, *E.rubrifaciens* was transferred to Genus *Brenneria* by Hauben *et al*.(1999). Molecular methods are the most widely used techniques to certain identification of the organisms.

16S ribosomal RNA sequences have been used extensively in the classification and identification of Bacteria and Archaea (Kim and Chun, 2014; Raina *et al.* 2019). The similarity threshold of

98,7% for species and 95% for genera are currently recognized for delignating taxonomic relationships between prokaryotic strains (Stackebrandt and Ebers, 2006). Although the advantages of the 16SrRNA sequence analysis, sometimes it can be obtained closely related sequence data with in the same genus which makes it confusing a clear classify. Today, in classical bacteriology, biochemical tests and the carbohydrate usage profiles of the bacterial strains still have a key role for the discrimination of the Genus and the strains in the same Genus. There are several reports of on different host plants of the damages caused by pink pigmented *E. persicina* (Hao et al., 1990, Euzéby, 1998; Diánez *et al.,* 2005; González *et al*., 2005, 2007; Zhang and Nan, 2012, 2013, Nechwatal and Theil, 2018; Canik Orel, 2020). *E. rhapontici* has been reported more than 20 plant species and as well as *E.persicina* isolated from soil, seed and plant tissues (Huang *et al*., 2003). Previously, *E.persicina* was reported in Turkey as the soft rot causing bacterium of lettuce (Canik Orel, 2020). In this study, pink pigmented lettuce strains obtained in 2021 from a lettuce field showing necrosis and mild soft rot were examined to identified the causal pathogen by using biochemical tests and 16SrRNA gene sequence. It was revealed the importance of biochemical tests beside molecular techniques in terms of 16SrRNA sequence data to discriminate the pink pigmented *E.persicina* from other pink pigment producer *Erwinia* species.

Material and Methods

During the survey of a lettuce field in the central Anatolia region, infected lettuce showing mild soft rot and leaf necrosis symptoms was collected. After surface sterilization, to obtain pure bacterial culture, bacterial suspension was streaked onto nutrient agar (NA) and sucrose peptone agar (SPA) media. According to morphological characteristics on NA and SPA, strains were subjected to some biological tests for identification. As differentiative tests, methyl red, Voges-Proskauer, dulcitol, glycerol, melezitoze and xylitol reactions of the isolated strains were determined (Hao *et al*., 1993). *E. persicina* strains 15/244/2a (Nechwatal and Theil, 2019) and Ep_Tr1 (Canik Orel, 2020) were used as the reference strains for all biochemical analysis. All tests were performed as four replicates. DNA of the strains was isolated according to Cubero *et al*. (1998) manually. 50 ng of DNA was used as template for the PCR and 16SrRNA gene of the strains was amplified with the universal primer pair 63f/1387r (Marchezi *et al*., 1998). Sterile distilled water was used as negative control. Amplification products were purified and sequenced from both directions. The sequence data was assessed by using MEGA X (Stecher *et al*., 2020) software and the results were blasted on GeneBank with the deposited data.

Results and Discussion

After 48h incubation at 28°C, strains were observed as pinkish on NA (Figure 1). Hao *et al*., (1990) reports that although all three *Erwinia* species produce pink pigment on NA, there are differences between the reactions on some chemicals. None of the strains produced pink pigment on SPA. In a previous study, Adesemoye et al. (2016) was reported *E.rhapontici* strains are not able to produce pink pigment on SPA medium. This colonony morphology features also confirmed the strains are *E.persicina*.

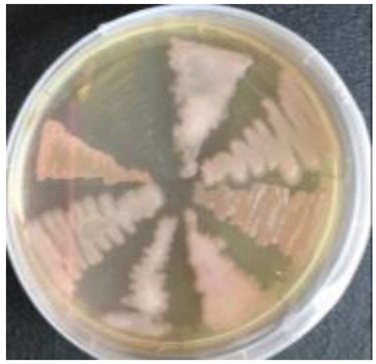

Figure 1. Pink pigmentation of the lettuce strains on NA medium.

As discriminative tests of pink pigment produced species of Genus *Erwinia*, test results of the strains were recorded as negative on methyl red test, positive on Voges-Proskauer, negative for the usage of dulcitol, glycerol, melezitoze and D-xylitol (Figure 2). As reported by Hao *et al*. (1990), *E.persicina* is negative to methyl red and positive to VG although *E.rhapontici* is opposite in both tests, positive to methyl red and negative to VG. The test results in this study are congruent with their results. Sorbitol and D-xylitol fermentation tests have been reported as the useful confirmation tests for identification of *E. persicina* (Gonzalez 2005; Moretti et al. 2011; Canik Orel, 2020).

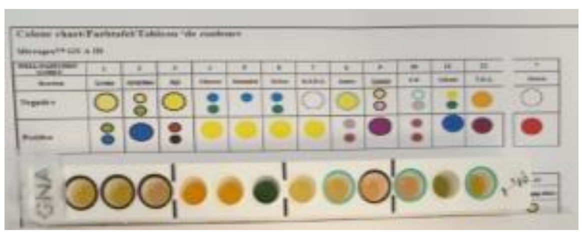

Figure 2. Some biochemical test results of the pink pigmented *Erwinia* strains on the Gram-negative ID panel

Blast analysis shows that the similarity rate between the 16SrRNA gene is 99.4% 5 out of 21 strains. Although the 16SrRNA sequence analysis are used widely for prokaryotic species identification, sometimes it can be obtained closely related sequence data with in the same genus as our case. If there are confirmed tests for the identification, there must be examined to identify a strain. Housekeeping genes have been used to have more data about genome of the organisms as molecular tools but working with more gene regions also increases the costs.

In this study it was used 16SrRNA gene results and biochemical tests to identify pink pigmented strains of Genus *Erwinia* and the results showed that biochemical tests and molecular techniques must be assessed together to have more reliable identification results.

Conclusions

In today’s world, molecular techniques are convenient and rapid for identification, but in some cases, as was mentioned in this case, more than one analysis is necessary to have a certain result of a strain. In a conclusion, in this study, it was revealed that biochemical tests are indispensable for bacterial identification although they are time-consuming and need to support with other tests, molecular and biochemical features of a bacterial strain should be evaluated together for more reliable results.

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DRY STRAWBERRY SNACK

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Abstract

It is important for today's consumers to consume high nutritional value, low calorie, and healthy food. For these reasons, the consumer tends toward the most natural and the healthiest. Fruits have a great place in the human diet. Strawberry is one of the most popular fruits preferred by consumers. It is known for its high nutritional value and organoleptic properties. Strawberry has high levels of bioactive compounds, which has a very short shelf life due to the softening of the texture which makes it more difficult to be consumed as fresh fruit. For these reasons, different applications have been developed to process fresh fruit and extend its shelf life. One of these applications is the osmotic dehydration process. Osmotic dehydration is the process of removing water from the food that involves heating food in a hypertonic solution. By osmotic dehydration, the water activity of the food decreases. The removal of water from the food is important to protect it from spoilage. The osmotic dehydration process can be done with concentrated sugar as well as a natural sweetener such as stevia. The purpose of using stevia in osmotic dehydration is to minimize the energy coming from sugar and to obtain a natural energy source. The result is to produce low-calorie snacks. The aim of this study is to evaluate the effect of stevia used in combination with sucrose on some quality parameters of dried strawberries.

Keywords: *Strawberry, Osmotic dehydration, Stevia, Quality Parameters.*

Introduction

In recent years, consumers have been changing their eating habits and prefer healthier foods since they are more aware of the importance of healthy and natural nutrition in order to maintain their wellbeing and prevent diseases. Moreover, the importance of consuming nutritious foods is better understood during the Covid-19 pandemic. The aim is not to consume too many foods, but to consume foods with high nutritional value, natural, and low in calories.

Strawberries are among the most popular fruits preferred by consumers worldwide and are known for their nutritious qualities. Because strawberries contain high levels of bioactive compounds, their benefits for human health have been proven. They are rich in phenolic acids, vitamin C, flavonoids, and minerals (Kelly *et al.*, 2021). The presence of flavonoids and bioactive compounds in strawberries makes the consumption of strawberries important for its potential health benefits. Dietary intake of flavonoids has reduced the risk of cancer and cardiovascular diseases due to the antioxidant capacity of these compounds. According to the FAO, strawberries are one of the most preferred fruits, as well as one of the most delicate and perishable fruits. On the other hand, strawberry has a short shelf life due to their sensitivity to attacks and softening of the texture. For these reasons, strawberries are consumed not only as fresh fruit but also as processed. One of these methods is to protect by drying. As a result of this process, the properties and nutritional value of the strawberry are preserved (Abdalla *et al.*, 2013).

Quality properties of foods can be classified differently as physical, chemical, microbiological and sensory properties. While preserving the quality characteristics of the foods, attention is paid to the preservation of the nutritional value. Osmotic dehydration is one of the methods used to preserve fruits and vegetables. Osmotic dehydration is a method of removing water from food. It facilitates the processing of foods by preserving their color, aroma, and texture, starting properties (Chavan and Amarowicz, 2012). In osmotic dehydration, the cell is placed in a solution (hypertonic) with a higher osmotic pressure than the cell's osmotic pressure loses water. With the permeability of the cell wall, the difference between the cell wall and the plasmalemma is filled into the hypertonic solution. Meanwhile, the cell is dehydrated until the process reaches equilibrium (Nuñez-Mancilla *et al.*, 2013). Due to this process, the water activity of the food decreases, the food is protected from spoilage and the shelf life is extended. Osmotic dehydration can be done with concentrated sugar as well as with stevia, a natural sweetener. The purpose of using stevia in osmotic dehydration is to present the food produced by using the sweetener, which is more natural, low in calorie content, and has a higher nutritional feature, to the consumer. Stevia is a general term for food ingredients and is derived from the *Stevia rebaudiana* (Bertoni) plant. Steviol glycoside is used for concentrated compounds extracted and purified from *S. rebaudiana*. There has been great interest in steviol glycoside sweeteners in the food industry (Carakostas *et al.*, 2008). This plant is also known as the honey leaf or sugar leaf. Stevia has been used as a sweetener and therapeutic for many years in Paraguay and Brazil. It has been used as a sweetener and food additive in Japan for about thirty years (Dinçel *et al.*, 2018). Stevia's heat resistance, chemical-free, high fiber content, not leaving a bitter taste in the mouth, and being low in calories are among the main features that distinguish it from others. Stevia is approximately 300 times sweeter than sucrose, has high heat and pH stability, and is more natural, making it preferable (İnanç and Çınar, 2009). One of the purposes is to ensure and protect food quality as much as possible in terms of consumer health and food safety, which is important in the food industry. In this context, the water activity of the strawberry would be reduced by the osmotic dehydration process and at the same time, stevia at different concentrations would be used as a solution in osmotic dehydration. At the same time, using stevia in osmotic dehydration will help diabetic patients to consume this food easily.

Materials and methods

Sample preparation

Fresh strawberries, which were purchased from the local markets in İzmir, were used in this study. After they had been washed with vinegar and baking soda, they were sliced almost the same size. Then, they were immersed in solutions having different sucrose and stevia concentrations. Five different sample groups were prepared; the control group was prepared using only water. Solutions that were used for S1, S2, S3 and S4 contain 50% sucrose; 20% sucrose and 0.1% stevia; 5% sucrose and 0.15% stevia and 0.2% stevia, respectively. After that, they were placed in an ultrasonic bath (Transsonic 780/H, Elma Schmidbauer GmbH, Germany) for 30 min. at room temperature and then kept in this solution overnight. Finally, they were placed in the oven at 40 °C for drying.

Analyses

Strawberries were weighed immediately after they were removed from the solutions and after being dried, and the weight loss was determined (Nowacka *et al.*, 2018).

The total phenolic content of strawberries was determined by the Folin-Ciocalteu method (Bozkır *et al.*, 2019). The antioxidant capacity of dried strawberries was measured by the DPPH method (Li *et al.*, 2020). Color measurements were carried out using a Minolta CR-400 reflectance colorimeter (Osaka, Japan). CIE *L**, *a**, *b** color values were measured (*L**: lightness; *a**: redness; *b**: yellowness). A hedonic scale from 1 (worst) to 5 (best) was used for sensory evaluation. 9-membered trained panelists evaluated the color, odor, taste, and overall acceptability of dried strawberries. All experiments were performed in triplicate. Mean values and standard deviations were calculated for all the determined parameters. Analysis of variance (ANOVA) was performed to investigate the differences ($p<0.05$) in characteristics of dried strawberries. Minitab (Minitab, State College, PA) software (version 19.0 for Windows) was used for statistical analyses.

Results and discussion

Among the five different groups that were dried, the weight loss was found to be 84.35% for S3 group (5% sucrose and 0.15% stevia) and 88.74% for S4 group (0.2% stevia) which have the most similar results to the control group (87.72%) which was prepared using only distilled water (without sucrose or stevia). At the first glance, the higher the sucrose content, the lesser the weight loss. When we compare the S1, S2, S3, and S4 solutions, the least weight loss was seen in S1 which was prepared using 50% sucrose. On the other hand, the highest weight loss was observed in the samples prepared by immersing 0.2% stevia solution. The content of stevia significantly ($p<0.05$) affected the weight loss of samples. As the amount of stevia increased, the weight loss increased. In a study osmotic dehydration was performed using an ultrasonic bath and the quality parameters of cranberries in different solutions were evaluated. According to their results, the cranberries in solutions containing sucrose and stevia for cranberries had the highest weight loss which is similar to our weight loss results (Nowacka *et al.*, 2018).

Color measurements of samples were carried out before and after drying (Table 1). Before drying *a** (redness) values of samples were significantly ($p<0.05$) different from each other. S1 samples had the highest *a** value and S4 group had the lowest one. S2 samples containing 20% sucrose and 0.1% stevia had similar results to S1 group. After drying there was no significant difference in *L** and *a** values of samples, whereas the lowest *b** value was observed for S4 and the greatest one was for S3 sample ($p<0.05$). We can conclude that the addition of stevia didn’t make an adverse impact on the color values of dried strawberries. Solanke *et al*., 2018 applied different sweetening agents, including sugar, jaggery, honey and stevia, as osmotic solutions. They indicated the highest lightness (*L**) and lowest redness (*a**) values for samples prepared by using sugar solution. *L** and *a** values of samples prepared by sugar or stevia solutions are very similar to each other.

Table 1. *L**, *a**, *b** values of dried strawberry samples before and after drying

	Samples	*L**	*a**	*b**
Before drying	Control	44.48 ± 7.64^{a}	22.87 ± 2.95^{bc}	22.16 ± 2.13^{b}
	S1	39.33 ± 6.73^{a}	31.89 ± 3.85^{a}	26.99 ± 5.08^{ab}
	S2	42.67 ± 1.50^{a}	27.81 ± 1.98^{ab}	27.47 ± 3.21^{a}
	S3	44.03 ± 4.01^{a}	19.38 ± 3.94^{c}	23.48 ± 3.55^{ab}
	S4	44.20 ± 6.19^{a}	20.90 ± 4.64^{c}	22.64 ± 1.78^{ab}
After drying	Control	40.21 ± 2.74^{a}	22.35 ± 6.54^{a}	20.91 ± 5.10^{ab}
	S1	37.30 ± 8.16^{a}	20.87 ± 6.99^{a}	17.57 ± 5.64^{abc}

	S2	33.73±4.38[a]	20.69±5.03[a]	14.99±4.27[bc]
	S3	38.86±4.62[a]	26.05±5.74[a]	21.89±3.90[a]
	S4	35.22±6.11[a]	19.19±4.82[a]	13.86±2.61[c]

Values are the arithmetic means of measurements taken from three replicate fruit samples, with their corresponding standard errors (SE).
a-c: Means having different letters within each row denotes a significant difference at $p<0.05$

Strawberry has a significant total phenolic content and antioxidant activity (Table 2). The replacement of sucrose with stevia did not make a significant difference in the antioxidant activity and total phenolic contents of the products ($p>0.05$). On the other hand, the highest total phenolic content and antioxidant activity were observed in the samples prepared using 20% sucrose and 0.1% stevia (S2). The increasing amount of stevia caused a decrease in total phenolic content and antioxidant activity.

Table 2. Total phenolic content and antioxidant activity of strawberry samples

Sample groups	Total phenolic content (g/100g)	Antioxidant activity (g/100g)
Control	0.1569±0.073	0.0409±0.018
S1	0.1909±0.099	0.0435±0.0108
S2	0.2776±0.058	0.0493±0.0061
S3	0.2129±0.128	0.0338±0.0149
S4	0.1606±0.127	0.0254±0.0192

Values are the arithmetic means of measurements taken from three replicate fruit samples, with their corresponding standard errors (SE).

Among the dried strawberry samples, S1 sample was the most appreciated in terms of color, taste, and acceptability (Table 3). There was no significant ($p>0.05$) difference in the odor of the sample groups. When all these criteria were evaluated, control and S4 got the least scores. The reason why these two groups are less appreciated than the others is that stevia might not provide the expected sweetness and odor. In the study conducted by Solanke *et al.*, (2018), sensory evaluation was made between orange slices immersed in different solutions having different concentrations. Among the scores given to sensory parameters, including taste, aroma, color and appearance, texture, and overall acceptability of the osmotic agent, samples prepared by honey solution had the highest scores. In addition, samples prepared by stevia had lower scores than the expected one. Our sensory evaluation results are similar to this study. In the osmotic dehydration process performed with the help of stevia in strawberries, stevia received the lowest score when color, odor, taste and acceptability were evaluated (Solanke *et al.*, 2018).

Table 3. Sensory evaluation results of dried strawberry samples

Name	Color	Odor	Taste	Acceptability
Control	2.45±1.13[ab]	1.56±0.73[a]	1.11±0.33[b]	1.44±0.53[b]
S1	3.89±1.54[a]	2.78±1.3[a]	4.44±0.73[a]	3.89±1.05[a]
S2	3.45±1.01[ab]	2.78±1.3[a]	3.44±1.24[a]	3.89±1.05[a]
S3	3.00±1.00[ab]	2.33±0.5[a]	2.22±1.20[b]	2.11±1.17[b]
S4	2.22±1.30[b]	2.22±0.97[a]	1.22±0.44[b]	1.56±0.73[b]

Values are the arithmetic means of measurements taken from three replicate fruit samples, with their corresponding standard errors (SE).
a-c: Means having different letters within each row denotes a significant difference at $p<0.05$

Conclusion

Consumers generally try to prefer natural and healthy food. For them, nutritious and healthy snacks are considered more preferable. Strawberry is among the fruits that consumers love to consume. Based on the obtained results, dried strawberries could be consumed as low-calorie snacks. Addition of stevia didn't make a significant difference in the total phenolic content and antioxidant activity of strawberries. Although the group produced by using only stevia got the lowest score by panelists, S2 and S3 groups had relatively the highest scores. Consequently, by using stevia in combination with sucrose, a healthy snack could be produced.

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UTILIZATION OF GELLED EMULSION BASED ON CHIA FLOUR WITH PEANUT OIL IN BEEF PATTIES

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Abstract

In this study, four different patty formulations were prepared where beef fat was substituted with gelled emulsion (GE) prepared with chia flour and peanut oil; C (0% GE), G50 (50% GE), G75 (75% GE), and G100 (100% GE). The effects of using GE on color parameters, oxidative changes, and sensory properties during 45 days of storage were investigated. The peroxide values of the samples increased until the 30^{th} day, and the highest value (24.08 meqO_2/kg) was reached in the control samples ($P<0.05$). On the 45^{th} day, a decrease was observed in the peroxide values of all treatments. While no differences were observed between the TBARS values on the 15th day of storage, the lowest TBARS values were observed on the 30^{th} day in G75 and G100 treatments and the 45^{th} day in G50 and G100 treatments. It was observed that the addition of GE to the formulation prevented lipid oxidation regardless of the amount ($P<0.05$). In general, while L* values decreased during the storage, b* values increased and a* values were found to fluctuate. Replacing beef fat with GE did not change the sensory properties and samples with GE were preferable to the panelists. In a conclusion, gelled emulsions prepared with chia flour and peanut oil could be considered a healthier fat replacer for meat products.

Keywords: *Chia, Gelled emulsion, Peanut oil, Beef patty, Fat replacer.*

Introduction

Meat and meat products are key components of the human diet since they include a wide range of high-value substances, including essential amino acids, vitamins, and minerals (Pereira and Vicente, 2013). However, their nutritional quality is called into doubt due to their significant levels of fat, saturated fatty acids (SFA), and cholesterol contents, all of which are highly associated with increased risk of diseases like cardiovascular diseases, diabetes, etc. (WHO, 2009). As a result of greater consumer nutritional awareness, the industry is experiencing growth in recent years for meat products with low-fat content and enhanced fatty acid composition (Serdaroğlu et al., 2016). However, decreasing fat content, and incorporation of fat substitutes or vegetable oils impact the quality of the product in terms of technological (cooking loss, emulsion stability, water holding capacity, and texture) and sensory (mouth feel, flavor, juiciness, and overall acceptability) features, minimizing the consumer acceptance and acceptability of reformulated low-fat meat products (dos Santos Alves et al., 2016). Using dietary fibers, hydrocolloids, or vegetable/marine/seed oils rich in polyunsaturated fatty acids has been recommended to decrease the fat content and enhance the fatty acid profile of formulations containing animal fat (Jiménez-Colmenero, 2007). However, reformulating meat products to reduce or replace animal fat with vegetable/seed/marine oils (high contents of polyunsaturated fatty acids) is never an easy effort, as it has been demonstrated that the using liquid oils have a significant negative impact on several sensory and technological attributes (Serdaroğlu et al., 2016). Recently, emulsion gels, hydrogels, and organogels have been used to compensate for the aforementioned obstacles (López-Pedrouso et al., 2021; Öztürk-Kerimoğlu et al., 2021). Emulsion gels can have a fat-like physical phenomenon in the functional qualities of meat products due to their soft-solid texture. Emulsion gels can be made by dispersing emulsified droplets in a continuous gel matrix or aggregating dispersed droplets in a particulate gel. The use of hydrocolloids as well as heating, acidification, or enzymatic treatment has been utilized to create a gel network. Researchers have been focused on the use of emulsion gels in different food products, specifically reformulated meat products (de Souza Paglarini et al., 2019). The application of different emulsion gels has been recently used as successful fat replacers to produce healthier and high-quality meat products (Serdaroğlu et al., 2017; Nacak, et al., 2017; de Souza Pagliarini et al., 2019; Öztürk-Kerimoğlu et al., 2019; Kavuşan et al., 2020; Öztürk-Kerimoğlu et al., 2021). On the other hand, there are few studies in which gel emulsions created using chia flour are used as animal fat substitutes in meat product formulations (Pintado et al., 2016a; Pintado et al., 2016b; de Souza Pagliarini et al., 2019). Besides, in these studies in which gel emulsions produced with chia flour are used as animal fat replacers, it is seen that there is no consensus on the antioxidative effects of chia flour (Pintado et al., 2016a; Pintado et al., 2016b). Moreover, no study has investigated the use of peanut oil and chia flour in gel emulsion formulation. Palmitic (C16:0), oleic (C18:1 ω-9), and linoleic (C18:2 ω-6) fatty acids are the most abundant in peanut oil (Kim et al., 2015). These oils are also rich in antioxidants such as phenolic or flavonoid components tocopherols and phytosterols, which are responsible for the oxidative stability of peanut oil (Ciou et al., 2021). As far as we know the use of peanut oil in meat matrices was mostly limited (Nacak et al., 2021; Wongpattananukul et al., 2022). Taken all together, this study aimed to discover the probable utilization of emulsion gels formulated with chia flour and peanut oil to replace animal fat in the beef patty formulation and to observe oxidative and sensory changes during storage.

Material and Methods

The beef topside round was kindly donated by Migros Ticaret A.Ş., and beef fat was obtained from a local butcher. Peanut oil was purchased from Smart Kimya Tic. and Dan. Ltd Şti. (İzmir), and chia flour was procured from the Tazemiz company (Mersin). Spice mixes were obtained from the local market of İzmir, while PGPR (polyglycerol polyricinolate), and sodium caseinate used in gel emulsion formulation were supplied from Çağdaş Kimya A.Ş (İstanbul). All other reagents used in the present study were analytical grade and purchased from Merck (Darmstadt, Germany). The cold gelation method was used for the preparation of O/W gel emulsion Pintado et al. (2015). The water phase (W) of the emulsion was prepared by homogenizing chia flour and egg white powder (YBT) with water at 5600 rpm for 45 s (Thermomix). 0.7% microbial transglutaminase (MTG) was added to the obtained homogenate and homogenizing process was continued for 15 s more. Peanut oil, as the il phase (O) of emulsion, was added to the water phase and emulsification was carried out using a high-speed mixer (Thermomix) at 5600 rpm for 3 minutes. The prepared emulsions were incubated at +4°C for 12 hours. The meat is grounded through the 3 mm plate. Minced meat is mixed with beef fat /GE (gel emulsion used in different proportions), and other ingredients (2% salt, 2% spice mix: onion powder, black pepper, and cumin) in a dough mixer until a homogeneous mixture was achieved, then the beef patty was shaped using metal molds (1, 5 cm thickness and 100 mm diameter). During the storage period, samples were stored at -18°C. The experimental design is given below (Table 1). For each batch, 3500 g of patty dough was produced.

Table 1. Beef patties formulation

Treatments	Beef (%)	Fat (%)	GE (%)	Salt (%)	Spice mix (%)
C	76	20	-	2	2
G50	76	10	10	2	2
G75	76	5	15	2	2
G100	76	-	20	2	2

Gel emulsion stability was measured according to the methods of Surh et al., 2007). The Color (L*, a*, b*) of the emulsions was measured with a portable colorimeter (Konica Minolta, Japan). Sensory evaluation of samples was performed using a 9-point hedonic scale. Samples were heated before serving. The panelists (10 male, and 10 female, aged between 20 to 26) were asked to evaluate the following parameters: appearance, color intensity, texture, oiliness, juiciness, flavor, and general acceptability (1: dislike extremely and 9: like extremely). Peroxide value was assessed by using AOAC (2012) method for determining oxidation substances. Also, the thiobarbituric acid reactive substances (werebears) were determined according to Witte et al. (1970). Statistical analysis of data SPSS for Windows V 21.0 package program was performed using the 95% confidence interval. The data of each trial were calculated by using the One-Way and Duncan multiple comparison methods.

Results and Discussion

Regarding the thermal stability (1 hour at 70°C), centrifuge stability (3000 rpm, 3 min), and creaming stability (7 days at +4°C) there was no phase separation in the emulsion structures and the stability of the samples was high. L*(brightness-darkness), a* (red-green), and b* (yellow-blueness) values of O/W gel emulsion were recorded as 55.18±0.8, 8.24±0.10 and 12.78±0.01, respectively. The color parameters measured during the storage are presented in Table 2. The effects of using GE on L*, a*, and b* values were significant ($P<0.05$). Although the use of GE did not have a significant effect on had any significant effect on L* values at the beginning of storage, it was observed that L* values were lower compared to the control treatment after 30 days of storage groups ($P<0.05$), G100 treatment had the lowest L* value and the control had the highest ($P<0.05$). The addition of GE had no effect on b* values ($P>0.05$). This may be related to the fact the strong yellow color of the (12.78±0.01). Pintado et al. (2016a) reported a decrease in L* and a* values and an increase in b* values by adding chia flour (25.84%) to frankfurter samples. On the other hand, when chia flour in gel emulsion form was added to Bologna sausages increase L* values and decrease a^* and b^* values were recorded (de Souza Paglarini et al., 2019).

Table 2. Color parameters of beef patties

	Treatments	Storage			
		0	15	30	45
L*	**C**	36.93 ± 0.55^{aby}	36.03 ± 0.71^{by}	44.49 ± 1.36^{ax}	44.16 ± 1.18^{ax}
	G50	37.45 ± 0.47^{ay}	37.62 ± 1.30^{ay}	40.01 ± 0.73^{bx}	39.74 ± 1.31^{bx}
	G75	37.65 ± 0.62^{a}	37.85 ± 0.64^{a}	39.17 ± 1.42^{b}	39.03 ± 1.46^{b}
	G100	36.14 ± 0.76^{by}	36.58 ± 1.00^{aby}	35.77 ± 0.67^{cy}	37.94 ± 1.25^{bx}
a*	**C**	13.28 ± 0.92^{ax}	8.22 ± 0.93^{ay}	5.46 ± 1.73^{bz}	5.85 ± 0.99^{a}
	G50	12.13 ± 0.59^{bx}	6.44 ± 0.56^{by}	6.05 ± 0.65^{aby}	5.79 ± 0.41^{y}
	G75	12.43 ± 0.41^{abx}	6.71 ± 0.32^{by}	6.32 ± 0.36^{abyz}	5.82 ± 0.79^{a}
	G100	10.63 ± 0.57^{cx}	6.85 ± 0.50^{by}	6.87 ± 0.46^{ay}	6.09 ± 0.19^{a}
b*	**C**	8.39 ± 0.64^{bx}	5.91 ± 1.05^{cz}	7.83 ± 0.73^{axy}	7.10 ± 0.94^{cy}
	G50	10.47 ± 0.95^{ax}	8.69 ± 0.50^{by}	10.47 ± 1.13^{bx}	9.19 ± 1.57^{bxy}
	G75	11.43 ± 0.66^{ax}	9.45 ± 0.83^{abyz}	10.36 ± 0.74^{bxy}	8.62 ± 1.35^{cz}
	G100	11.00 ± 0.50^{a}	9.94 ± 0.80^{a}	10.01 ± 0.57^{b}	10.19 ± 1.24^{a}

$^{a-c}$ Different letters in the same column indicate a significant difference (P < 0.05). $^{x-z}$ Different letters in the same row indicate a significant difference (P < 0.05).

The results of the sensory analysis are shown in Fig 1. Overall, the scores ratings given by the panelists were similar in appearance and color. Replacing beef fat with GE did not result in negative sensory properties of the samples, and even the highest general acceptance scores were recorded in samples added with found (P<0.05). The flavor is particularly affected by the presence of beef fat. However, the samples in which beef fat was completely replaced with GE, except for the 30th day, received the highest flavor scores (8.20). It was observed that the oiliness scores were improved by the addition of gel emulsion, regardless of the amount, until the last day of storage (P<0.05). Texture parameters are important to better understand the acceptance of these products. When the texture scores were examined, it could be seen that the addition of GE formulation significantly affected texture scores samples added with GE had higher texture scores (G50-7.40, G75-7.70, G100-8.12) than the control treatment (P<0.05). When previous findings in similar meat products reformulated with chia emulsion as a fat replacer were examined, it was seen that although differences were detected in the sensory properties of chia reformulated sausages, these products were considered acceptable by the panelists (Pintado et al., 2016b). In another study, the negative effects of replacing 100% of animal fat with gel emulsion on sensory properties were reported (Pintado et al., 2016a).

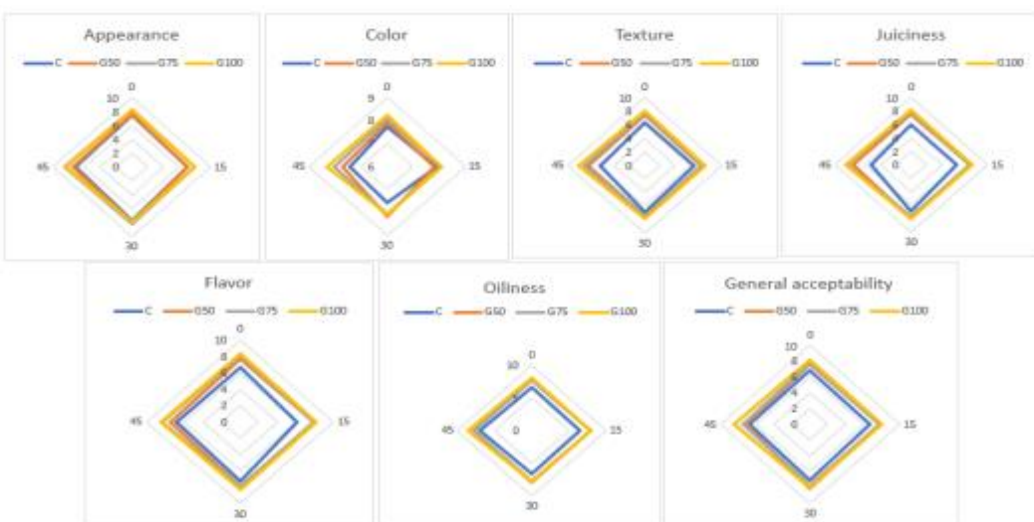

Fig 1. Sensory scores of beef patties

Changes in peroxide values of patty samples during the storage are shown in Fig 2. Initial peroxide values of G75 and (2.68meqO_2/kg) and G100 (2.82 meqO_2/kg) groups were found to be higher than the C and G50 ($P<0.05$). Higher oxidation could be associated with the high unsaturated fatty acid profile of peanut oil. The peroxide values of the samples increased until the 30th day, and the highest value (24.08 meqO_2/kg) was reached in the control group ($P<0.05$). It should be noted that even the highest recorded peroxide value was below the limit value (25 meqO_2/kg oil) stated by Evranuz (1993). There was a significant decrease in peroxide values after the 30th day ($P<0.05$) based on the conversion of lipid peroxides to secondary lipid oxidation products. Similarly, Kavuşan et al. (2020) stated that peroxide values of fresh chicken sausages formulated with GE containing black cumin and linseed oils decreased after the 3rd day of storage. All reformulated patties had lower peroxide values than C treatment, which can be explained by the antioxidant effect of chia or the protective surrounding on peanut oil provided by gelled emulsion. Patties formulated with chia flour were reported to have lower peroxide values than samples that do not have chia flour in their formulation (Erdoğdu, 2019). TBARS values of beef patties during storage are given in Fig 2. The initial TBARS values of the patties were between 0.42-0.60 mg MA/kg. At the beginning of the storage, all samples had similar TBARS values except the G100 treatment which had the lowest TBARS value ($P<0.05$). Similar to our results, it has been reported that replacing 50% of animal fat in pork patties with avocado, sunflower, and olive oils resulted in lower TBARS values than control groups (Rodríguez-Carpena et al. al., 2012). Also, Fernández-López et al., (2019) observed the decrease in TBARS values of Frankfurters with the addition of 3% partially skimmed chia flour. As the storage proceeded (30th day) oxidation in C and G50 was more pronounced ($P<0.05$), however, the lowest TBARS value was registered for the G50 (0.25 mgMA/kg) and G100 (0.28 mgMA/kg) treatments at the end of the storage. Reduced oxidation was also reported using gelled emulsion as a fat replacer in burgers (Barros et al., 2020; Barros et al., 2021). The low lipid oxidation is thought to be caused by antioxidant compounds found in chia seeds or higher oxidative stability of peanut oil due to the oleate/linoleate ratio and iodine value (Andersen and Gorbet, 2002). Also, peanut oil is an important source for natural antioxidants such as resveratrol, tocopherol, p-coumaric acid, squalene and etc. (Akthar et al., 2014). Another factor that will strengthen the antioxidant effect is encapsulated peanut oil droplets in an emulsion gel matrix which acts as a barrier against lipid oxidation. During the whole storage, TBARS values of all-beef patties were found to be lower than the limit value (2 mg MA/kg) (Witte et al., 1970). On the other hand, while peroxide values decreased in all groups on the last day of storage, the decrease in TBARS values may be associated with the onset of protein oxidation.

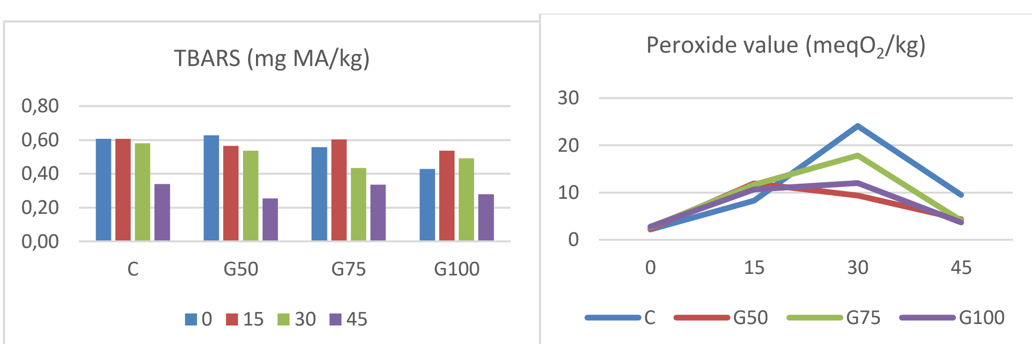

Fig 2. TBARS (a) and peroxide (b) values of beef patties

Conclusions

This research revealed that using a gelled emulsion made with chia flour and peanut oil as a beef fat substitute in beef patties is convenient and can be considered a reasonable alternative for increasing the nutritional value while altering the sensory properties. As a result, the use of gelled emulsions formulated with chia flour and peanut oil can be recommended as fat replacers for minced meat products to develop healthier meat products that have equivalent or better-quality parameters to the standard formulations.

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THE PRESENCE OF PROTISTAN PATHOGENS IN SOME COLEOPTERAN STORED PESTS

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Abstract

Different climatic conditions and insufficient storage areas create very favorable environments for the development of many stored product pests. The protection of stored products is very important both to ensure food safety and to contribute to the country economy. The interest in the use of entomopathogens (EPOs) as an alternative to chemical insecticides in the control strategies against stored pests is increasing day by day. Among the entomopathogens, protistan pathogens play an important role as natural suppressor factor in pest insect populations. The present study includes presence of protistan pathogens in the populations of four important coleopteran stored pests, *Tribolium castaneum* (Hbst.), *Tribolium confusum* (Duv), *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauv.) from five localities in Turkey. During the study, 230 samples of coleopteran stored pests were dissected and searched for protistan entomopathogens. Two pathogens, microsporidium and coccidian were found in the different populations of the pests. While microsporidian pathogen was observed in the populations of *T. confusum, T. castaneum, O. mercator* and *O. surinamensis* (Bursa, Samsun and Gaziantep locations) coccidian pathogen was observed in only one population of *T. confusum* (Bursa location). The infection rates were relatively low, 4.35% for microsporadian and 0.43% for coccidian pathogens. Total infection level was found as 4.78%. However, infection reached to 9.25% in some populations. The present study includes the first records on the prevalence of protistan pathogens in the populations of coleopteran stored pests from five localities in Turkey, however it is needed to increase the number of coleopteran species and samples and populations from different locations to represent the entire Turkey. Furthermore, it is needed to investigate other pathogens and parasites and identify each of them at the species level.

Keywords: *Stored coleopteran pests, Microsporidia, Coccidia, Biological control, Turkey.*

Introduction

Coleopteran stored pests are common storage pest all over the world. Different climatic conditions and insufficient storage areas create very favorable environments for the development of many stored product pests. Thus, serious losses occur in the harvest produced (Reichmuth et al., 2007; Mason and McDonough, 2012; Akci et al., 2016). Damage to stored products is a very serious problem both in Turkey and worldwide due to that stored product pests cause both quantitative and qualitative damage for crops. Both situations are undesirable. The protection of stored products is very important both to ensure food safety and to contribute to the country economy.

Today, stored product pests are generally struggled with chemicals. The very common use of chemical insecticides has led to many negative consequences, resulting in increasing attention given to natural products. Because this insecticides caused insecticide resistance, toxicity to mammals and other non - target animals, environmental pollution (Isman 2006). Alternative control methods are needed for an effective and efficient fight against stored product pests (Freitas et al., 2020). Biological control using entomopathogens is a viable alternative control. In the world and Turkey in stored product insect pests in microbial control are used of some entomopathogenic organisms (Cowan et al. 1986; Knell et al. 1996; Moino et al. 1998; Batta 2016). However, the use of entomopathogenic protozoans for microbial control and biological control of stored product insect pests are not very common (Alfazairy et al., 2019). The interest in the use of entomopathogens (EPOs) as an alternative to chemical insecticides in the control strategies against stored pests is increasing day by day. Among the entomopathogens, protistan pathogens play an important role as natural suppressor factor in pest insect populations. Therefore, studying the presence of protistan entomopathogens in pest populations can provide useful information for decision-making in the biological control against them.

The present study includes presence of protistan pathogens in the populations of four important coleopteran stored pests, *Tribolium castaneum* (Hbst.), *Tribolium confusum* (Duv), *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauv.) from five localities in Turkey.

Material and Methods

Insect Samples

Samples of the different stored products such as hazelnuts, peanuts, walnuts, rice, flour, bean and tarhana soup powder, suspected with infestation by the pests were collected from five localities; Bursa, Muğla, Isparta, Samsun and Gaziantep in Turkey, in 2019 and 2020. In total, 230 samples of four important coleopteran stored pests, *Tribolium castaneum* (Hbst.), *Tribolium confusum* (Duv), *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauv.) were collected from the samples.

Microscopic Examination

After macroscopic examination of insect samples, adult beetles were dissected in Ringer’s solution and wet smears were prepared. Host fat body, Malpighian tubules, gut epithelium, and hemolymph were examined for the presence of the protistan entomopathogens under a light microscope at × 400–1000 magnification (Yaman et al., 2019). When an infection with the spore/oocysts of pathogen was observed, a part of the material was used for the preparation of Giemsa-stained smears. Giemsa-stained slides were examined under a light microscope. Detected spore/oocysts were measured and photographed using a microscope with a digital camera and Soft Imaging System.

Results and Discussion

During the study, 230 samples of coleopteran stored pests were dissected and searched for protistan entomopathogens. Two pathogens, microsporidium and coccidian were found in the different populations of the coleopteran pests examined in the study. Entomopathogenic protistan infections were confirmed by observation of their characteristic spores and oocysts. These protistan entomopathogens were diagnosed as *Nosema* sp. and *Adelina* sp. (Figures 1-4). Fresh spores of the micosporidian pathogen are ellipsoidal and measure ca. 3.73-4.72 µm in length and ca. 2.47-3.06 µm in width. Spherical to ellipsoidal oocysts of the coccidian pathogen measure ca. 32.45–42.75 µm in diameter.

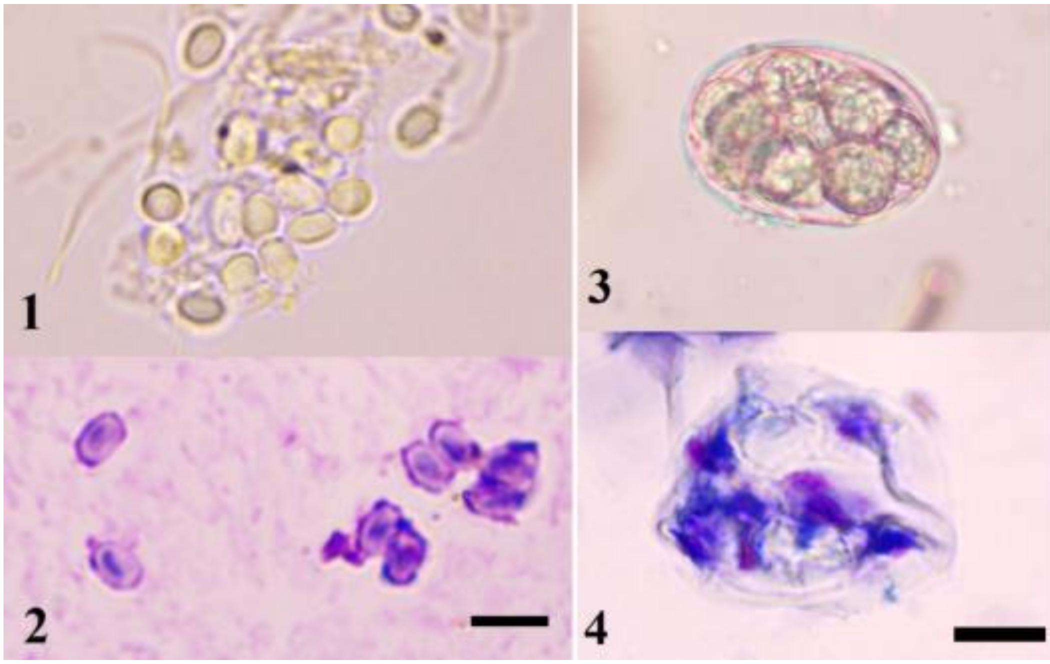

Figure 1-4. Microsporidian (*Nosema* sp.) and coccidian (*Adelina* sp.) pathogens in coleopteran stored pests in Turkey. 1. Fresh microsporidian spores, 2. Giemsa-stained microsporidian spores, 3. Fresh coccidian oocyst, 3. Giemsa-stained coccidian oocyst. Bars: 5 µm for figures 1 and 2, 10 µm for figures 3 and 4.

While microsporidian pathogen, *Nosema* sp. was observed in the populations of *T. confusum, T. castaneum, O. mercator* and *O. surinamensis* (Bursa, Samsun and Gaziantep populations), coccidian pathogen *Adelina* sp. was observed in only one population of *T. confusum* (Bursa location) (Table 1). The infection rates were relatively low, 4.35% for microsporadian and 0.43% for coccidian pathogens. Total infection level was found as 4.78%. However, infection reached to 9.25% in some populations.

Table 1. Occurrence of microsporidian and coccidian entomopathogens in some coleopteran pest populations.

Locality	**Coleopteran pests (adults)**	**Number of the dissected beetles**	**Microsporidium**	**Coccidian**
Bursa	*Tribolium confusum* (dead)	54	5	1
	Tribolium confusum (live)	52	-	-
	Tribolium castaneum (dead)	6	1	-
Muğla	*Tribolium confusum* (live)	10	-	-
	Tribolium confusum (dead)	15	-	-
	Tribolium castaneum (dead)	4	-	-
Isparta	*Oryzaephilus mercator* (live)	19	-	-
	Oryzaephilus mercator(dead)	1	-	-
Samsun	*Oryzaephilus mercator(*dead)	12	2	-

	Oryzaephilus surinamensis (dead)	13	1	-
Gaziantep	*Tribolium confusum* (dead)	44	1	-
Total		230	10	1
Infection			4.35	0.43
Total (%)			4.78	

The present study includes the first records on the prevalence of protistan entomopathogens in the populations of coleopteran stored pests from five localities in Turkey, however it is needed to increase the number of coleopteran species and samples and populations from different locations to represent the entire Turkey. Furthermore, it is needed to investigate other pathogens and parasites and identify each of them at the species level. Detection of these pathogens in natural populations of the coleopteran stored product pests is important in terms of biological control of these important pests.

Conclusions

Entomopathogens can be considered as a more effective and easy method, considering the harmfull of chemicals to the environment in Integrated Pest Management (IPM) (Mantzoukas et al., 2021). Although commercial preparations of entomopathogenic protists for use in biological control are limited, they may play a role as suppressive factors in natural populations of the pests. The present study includes the first records on the prevalence of protistan pathogens in the populations of coleopteran stored pests from five localities in Turkey. Two pathogens, microsporidium and coccidian were found in the different populations of the pests. The presence of two different protistan entomopathogens in natural populations of the coleopteran stored product pests is promising in terms of suppression of the pests under natural conditions. The obtained results encourage to think about the use of protistan entomopathogen in biological control against coleopteran stored product pests. However, it is needed to increase the number of coleopteran species and samples and populations from different locations to represent the entire Turkey. Furthermore, it is needed to investigate other pathogens and parasites and identify each of them at the species level.

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EFFECTS OF OXALIC ACID TREATMENTS ON POSTHARVEST QUALITY AND STORAGE LIFE OF APPLE *CV*. GRANNY SMİTH

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Abstract

Oxalic acid is a natural organic natural compounds are present in fruits and vegetables, and have shown important roles in delaying the ripening process when applied as postharvest treatment. In this study, effects of different concentrations of oxalic acid (OA) on storage and postharvest quality of ‘Granny Smith’ apples were investigated. Apples were dipped in different concentrations OA (2 mM, 4 mM and 6 mM) solutions for ten minutes, while control fruits were immersed in distilled water for the same minutes. Treated fruits were stored at 0 °C and 90% relative humidity for 4 months. Weight loss, firmness, skin color (hue angle), total soluble solid content (SSC), titratable acidity (TA), pH and starch were performed with 30 d intervals during the storage time. According to the results, all oxalic acid treatments maintained firmness, skin hue angle and titratable acid. It was determined that oxalic acid treatments were effective on the preservation of quality characteristics in Granny Smith apple compared the control. During cold storage, the lowest weight loss was determined in 4 mM OA treated fruits. It was concluded that the applied doses gave similar results in general, but 4 mM oxalic acid application could be the appropriate dose for cold storage of Granny Smith apple, especially in reducing weight loss, fruit firmness and maintaining titratable acidity values. For this reason, 4 mM OA treatment was considered to be effective on quality extension of apples *cv*. Granny Smith with decreasing the quality changes of during cold storage.

Keywords: *Apple, Granny Smith, postharvest quality, oxalic acid.*

Introduction

Apple is a temperate, especially cold temperate climate fruit. Apple, which can be grown in almost every region, ranks first among pome fruits in terms of production amount. It is known that, after harvesting, apples can be kept in cold storages for 6-7 months by maintaining their optimum quality characteristics, depending on the variety, and the loss will not exceed 4% under suitable conditions. Apples should be stored at 0 °C and 80-90% relative humidity, depending on the varieties and storage time (Huang et al., 2013). Maintaining the quality features of apples is one of the important issues for market supple year around. The applications that will extend the postharvest life are of great importance. Attractiveness of Granny Smith apples has increased due to its being a good pollinator and its green color. Its production has increased significantly compared to other years. It has gained a special place in the market with its unique sour taste. The leading factor limiting the production increase of Granny Smith apples in the world and in our country is the superficial scald that occurs during the storage of this variety (Yıldırım et al., 2008).

Oxalic acid appears to have some antioxidant activity properties. It is stated that this has an important role in the prevention of oxidation events in plants, systemic resistance to cell death, and antiaging in harvested products (Huang et al., 2013).

It has been stated that exogenous oxalic acid application after harvest is effective in delaying ripening, preventing darkening, reducing susceptibility to chilling damage, reducing respiratory activity and ethylene production, and thus protecting fruit quality and extending shelf life (Sayyari et al., 2010; Valero et al., 2011; Huang et al., 2013).

In this study, the effects of postharvest oxalic acid applications at different doses on the maintenance of quality characteristics and storage time of Granny Smith apple variety were investigated.

Material and Method

Apples c*v*. Granny Smith were harvested from a commercial orchard near in Karaman, Turkey and transferred to the laboratory of the Department of Horticulture, Selcuk University. Apples were selected according to their uniformity in color, size and lack of damages and were randomly divided into four equal lots.

Different doses of oxalic acid (2 mM, 4 mM and 6 mM) were applied to the three lots of fruits for 10 minutes, and the fruits were dried for 2 hours at room temperature (20 °C). The fourth group of fruit was evaluated as a control without any treatment. After the treatments, all fruits were stored in cold storage at 0 °C and 90% relative humidity for 120 days with quality evaluation performed on days 0, 30, 60, 90 and 120.

The weight loss (%) during postharvest storage was determined by periodical weighing, and calculated by dividing the weight change during storage by the initial weight: Weight loss (%) = [(Wi – We)/Wi] × 100, where Wi = initial weight and Me = weight at examined time.

Skin color of apples was performed using a colorimeter (Minolta® CR-400) to obtain the following variables from two equatorial points of fruits: L*, a*, b*. Hue angle (h°) was calculated to determine the color changes (McGuire, 1992).

Fruit firmness was measured with a digital penetrometer (fruit pressure tester, model 53205; TR, Forlì, Italy). In the equatorial region of the fruit, the outer skin was removed as a thin section and the firmness of the fruit flesh was measured, and the results were given as Newton (N).

Soluble solid content (SSC) was measured by hand refractometer in the juice obtained by squeezing the fruits in each treatment and the results were given as %. Titratable acidity (TEA) was determined by titrating fruit juice with 0.1 N NaOH to an endpoint of pH 8.1 and expressed as the percentage of malic acid.

Starch was determined by immersing the half part of the apple fruits into an iodized potassium iodide solution for one minute. At the end of this period, the starch ratio was determined using the 0-6 scale created for the Granny Smith variety.

The experiment was carried out in a randomized design with three replications and each replication contained 15 fruits. The data obtained from the experiment were subjected to analysis of variance using the JMP statistical software. Sources of variation were treatment, storage time and their interaction Means were compared by Student's t-test at $p \leq 0.05$.

Results and discussion

The effects of treatments on weight loss during cold storage were found statistically significant. Weight loss increased during prolonged storage for all oxalic acid (OA) treatments and control. The weight loss value of control fruit was significantly higher than those of oxalic acid treatments. At the end of the storage, the highest weight loss occurred in the control fruits (2.91%), while the lowest weight loss was observed in fruits treated with 4 mM OA (2.40%). 4 mM oxalic acid was more effective than others with a significant differences detected during the storage (Figure 1).

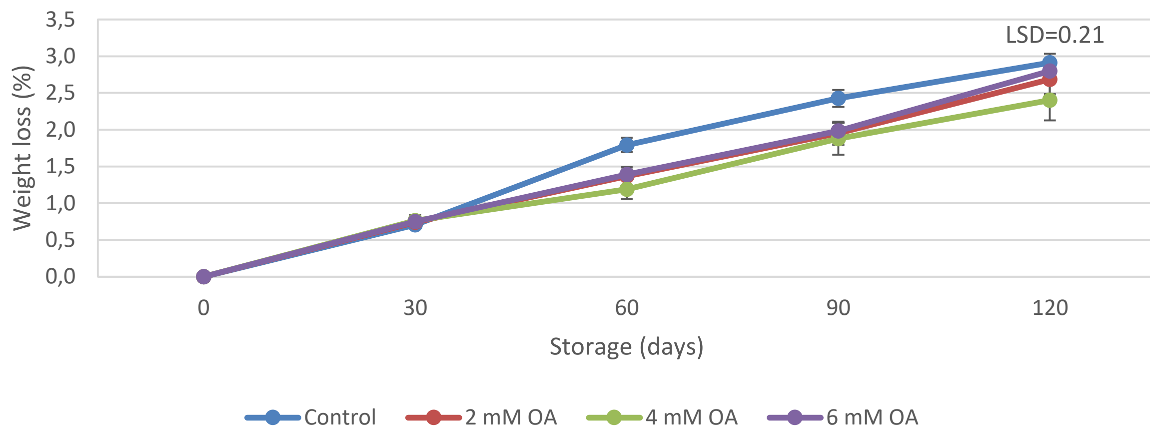

Figure 1. Effects of postharvest oxalic acid treatments on weight loss during storage

The initial skin hue angle value of Granny Smith apples were measured as 114.08°. A slight decrease in the angle value was detected with the prolonged storage. At the end of the storage, hue angle values varied between 109.71° (control) and 110.22° (2 mM OA) (Figure 2).

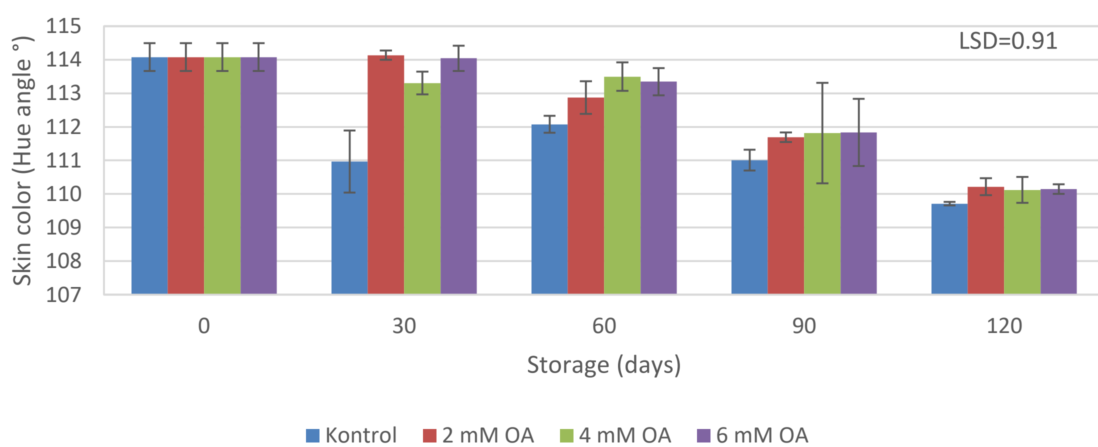

Figure 2. Effects of postharvest oxalic acid treatments on skin color during storage

The effects of postharvest OA treatments on firmness during storage were statistically significant. At harvest, firmness of fruit was 41.58 N and gradually decreased during the prolonged storage (Figure 3). All oxalic acid treatments maintained the firmness compared to control. At the end of the 120 days storage period, the highest firmness value was measured from 6 mM OA (26.67 N), followed by 4 mM OA (26.48 N) and 2 mM OA (25.56 N) treatments, respectively. The lowest firmness value was obtained in control (18.46 N).

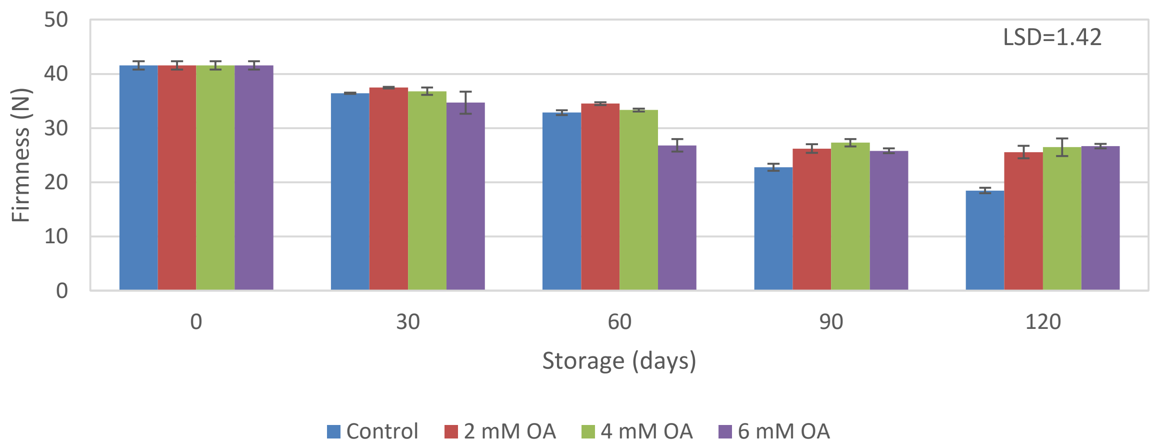

Figure 3. Effects of postharvest oxalic acid treatments on firmness during storage

The effect of the applications on SSC values during storage was significant (Table 1). The SSC value measured as 11.1% at the beginning of storage increased in all applications during storage. At the end of the 4 month storage period, the highest increase in SSC value occurred in fruits that were treated with 6 mM OA (14.2%). The lowest TSS value was obtained from fruits treated with 2 mM OA (13.2%).

Table 1. Effects of postharvest oxalic acid treatments on SSC, TA, pH and starch during storage

Treatments	Storage (days)				
	0	2	4	7	10
SSC					
Control	11.1±0.17	13.1±0.12	13.7±0.61	13.3±0.12	13.3±0.31
2 mM OA		13.1±0.12	13.5±0.50	14.0±0.00	13.1±0.12
4 mM OA		12.9±0.42	13.5±0.31	13.6±0.35	13.2±0.20
6 mM OA		12.9±0.61	13.7±0.42	14.1±0.12	14.2±0.16
TA					
Control	0.95±0.04	0.88±0.02	0.84±0.02	0.81±0.01	0.74±0.01
2 mM OA		0.90±0.01	0.88±0.02	0.88±0.00	0.82±0.08
4 mM OA		0.93±0.02	0.88±0.02	0.83±0.02	0.83±0.02
6 mM OA		0.92±0.02	0.88±0.01	0.88±0.02	0.79±0.02
pH					
Control	3.45±0.04	3.60±0.02	3.05±0.02	3.11±0.01	2.95±0.01
2 mM OA		3.62±0.01	3.06±0.02	3.13±0.00	2.92±0.08
4 mM OA		3.63±0.02	3.06±0.02	3.19±0.02	2.93±0.02

6 mM OA		3.64±0.01	3.07±0.01	3.17±0.02	2.88±0.05
Starch					
Control	1.0±0.00	3.3±0.00	4.4±0.19	5.6±0.19	6.0±0.00
2 mM OA		2.3±0.33	4.8±0.19	5.7±0.33	6.0±0.00
4 mM OA		2.9±0.19	5.0±0.00	5.7±0.00	6.0±0.00
6 mM OA		2.6±0.38	4.8±0.19	5.4±0.19	5.9±0.19

LSD for SSC: 0.51, TA: N.S., pH: 0.04, Starch: 0.29

TA of the tomatoes decreased gradually during the cold storage but the effect of OA treatments on the amount of TA during cold storage was found to be insignificant (Table 1).

TA was 0.95% at harvest while at the end of the 120 days storage period varied between 0.74% (control) and 0.83% (4 mM OA)

During the 4 month cold storage period, the effect of the applications on the pH value of the juice was found to be statistically significant (Table 1). The initial pH value of 3.45 decreased with the progression of the storage time. At the end of the storage period, the highest pH value was measured in fruits treated with 2 mM OA (2.97), followed by control (2.95), 4 mM OA (2.92) applications, respectively. The lowest pH value was measured in fruits treated with 6 mM OA (2.88).

The amount of starch evaluated using the 0-6 scale was determined as 1.0 at the beginning of storage. With the progression of the storage period, an increase in the rate of starch decomposed due to ripening occurred (Table 1). At the end of the storage period, starch was not detected (6.0) in control, 2 mM and 4 mM OA applications, while the starch value was determined as 5.89 in fruits treated with 6 mM OA.

Oxalic acid, which is applied externally after harvest, provides systemic durability by showing antioxidant properties, and also extends the shelf life of products with its maturation and aging retardant effect. In our study, it was determined that oxalic acid applications were effective in preserving the skin color and titratable acid content, decreasing the fruit firmness and delaying the increase in weight loss. The most important effect of postharvest oxalic acid application on fruit physiology is to reduce the change in parameters related to ripening by reducing the amount of respiration and ethylene production (Zheng et al., 2007). In studies conducted on different fruit species such as banana, peach, pomegranate and mango, it has been reported that postharvest oxalic acid applications delay changes in all parameters related to ripening and aging (Sayyari et al., 2010; Huang et al., 2013; Razzaq et al., 2015). These effects of oxalic acid on fruit species are in agreement with the results we obtained.

Conclusion

All three doses of oxalic acid used in the study were found to be effective in delaying the change in quality characteristics when compared with the control. However, it was determined that 4 mM oxalic acid application could be a promising postharvest treatment for Granny Smith apple cultivar, which was kept at 0 °C for 120 days, especially in reducing weight loss, maintaining fruit flesh firmness and titratable acidity values.

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EFFECTIVENESS OF PULSED ELECTRIC FIELD TREATMENT AND PLANT ESSENTIAL OILS ON QUALITY AND SHELF-LIFE EXTENSION OF LICORICE ROOT DRINK

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Abstract

Licorice root drink (LRD) is one of the most popular traditional drinks in Turkey which is usually consumed in summer seasons. It has very short shelf life, and thus, studies are in need to extend its shelf life. Plant essential oils (EO) have been used as natural food additive, but due to their strong flavor and aroma, their use in food is limited. Pulsed electric field (PEF) treatment is defined as the application of high intensity electric field pulses at the magnitude of 20-80 kV/cm for food pasteurization. Although effectiveness of EO and PEF alone or in combination for the preservation of different food are reported, they lack information on preservation and shelf-life extension of LRD. Freshly prepared LRD samples were divided into four group i-control, ii-*Mentha piperita* EO added, iii-PEF-treated and iv-*M. piperita* EO added+PEF-treated samples. Control and *M. piperita* EO added samples were spoiled by the 3rd week; whereas PEF-treated and *M. piperita* EO added+PEF-treated samples had the shelf life of six weeks. Although pH, conductivity, turbidity, color (L*, a* and b*), total antioxidant capacity, and total phenolic substance content of the samples fluctuated during shelf life, numbers of total mesophilic aerobic bacteria and total mold and yeast were significantly lower for PEF-treated and *M. piperita* EO added+PEF-treated samples. These samples also had higher sensory scores for the measured properties. Results revealed that PEF-treatment alone and *M. piperita* EO+PEF treatment were effective to extend shelf-life of LRD.

Keywords: *Pulsed electric fields, Licorice root drink, Shelf-life, Plant essential oils, Microbial inactivation*

Introduction

Recent concerns with extensive use of food additives, consumer demand for more natural products and adverse effects of antimicrobial agents has increased request for more natural products. Plant essential oils (EO), mixtures of volatile and hydrophobic substances from plants, carrying high antioxidant and antimicrobial activities come forward to replace and/or used in combination with commercially available food additives (Baptista *et al*., 2020; Silva *et al*., 2017; Dehghani *et al*., 2018). EOs are organic volatile compounds, with the molecular weight of less than 300 Da. They generally are classified into terpenoids and phenylpropanoid (Zhang *et al*., 2019; Dhifi *et al*., 2016). It has been proposed that EO causes the disruption of the microbial membrane with the leakage of cellular components, loss of ions, and prevention of many cellular

life activities, including energy production and cell membrane transport (Tarıq *et al*., 2019; Nisar *et al*., 2019).
Pulsed electric fields (PEF) involves application of short burst of electric current with high frequency. Applied electric field is transmitted to food by the presence of the ions through the food sample. Electric current at above 1V of the transmembrane potential of the cell membrane can cause electroporation of the membrane by formation of pores resulting in cell death (Álvarez *et al*., 2003; Timmermans *et al*., 2019). PEF processing is usually applied to low viscosity food products for pasteurization with preservation of bioactive compounds, physical and sensory properties (Evrendilek *et al*., 2021).
Being one of the most popular traditional drinks in Turkey and some Middle Eastern countries, licorice root drink (LRD) consumption is limited due to its very short shelf life (Al-Balawnih *et al*., 2017). Due to changes in the physical and sensory properties, thermal pasteurization of licorice drink is not suggested. Thus, several studies were performed by nonthermal technologies such as high-pressure processing and PEF to extend its the shelf-life (Evrendilek *et al*., 2021; Uzuner and Evrendilek, 2017).
Since efforts to extend its shelf life through the heat processing have deteriorated its physical and sensory properties, alternative technologies are being sought to process LRD with the purpose of preserving its physical and sensory properties. Such previous studies involved PEF treatment, combination of mild heat and PEF (Uzuner and Evrendilek, 2017) and ultrasonication (Bakay, 2019); but combination of PEF and plant essential oils. Thus, the objectives of the study were to process LRD by PEF, mixture of plant essential oils (MoEO), and MoEO+PEF and determine the effectiveness of the processes to extend shelf life of LRD.

Materials and Methods

Preparation of licorice root drink (LRD) samples: Dried shredded licorice roots were purchased from a local supplier (Ankara, Turkey). LRD was prepared by immersing 10 g of root in 1- L water at room temperature and leaving at 4°C for 16 h. The drink samples were filtered to remove the particles, and a clear drink with goldish-yellow color was obtained. The samples were kept at refrigeration temperature and processed at the same day of preparation.
Processing of LRD: Prepared LRD samples were divided into four groups coded as below.

1. Control 1-no treatment applied
2. Control 2-only essential oil added (mixture of thymus (33%) and *Mentha pulegium* (67%)
3. Processed 1-only PEF treated (flow rate: 75.00 mL/min, 22.60 kV/cm, total treatment time 262.00 microsec, energy input 33.70 J/sec)
4. Processed 2-Essential oil (mixture of thymus (33%) and *Mentha pulegium* (67%)) added and PEF treated (flow rate: 75.00 mL/min, 22.60 kV/cm, total treatment time 262.00 microsec, energy input 33.70 J/sec)

Pulsed electric field treatment: A bench-scale PEF system (OSU-4A, The Ohio State University, Columbus, OH) was used to treat the control samples. Six treatment chambers of co-field flow type with 0.23 cm diameter and 0.29 cm gap distance were adopted as the PEF system. Square wave bipolar pulses with 3 µs pulse duration, and 20 µs pulse delay time were applied. The used PEF parameters were 500 pps frequency, 22.60 kV/cm electric field strength (EFS), 75.00 mL/min flow rate, and the treatment time of 262.00 µs.

Processing temperature before and after each treatment was monitored using K type thermocouples (Fisher Scientific, Pittsburgh, PA, USA). Frequency, pulse width, and pulse delay time were adjusted using a pulse generator (Model 9310 Pulse Generator, Quantum Composer Inc., Bozeman, MT, USA) and were monitored using an oscilloscope (Model TDS 210 Two Channel Digital Real Time Oscilloscope, Teknotronix Inc., Beaverton, OR, USA). Constant pulseless flow was provided using a gear pump (EW-07002-23 model, Cole-Palmer Inst. Company, Vernon Hills, IL, USA).

Shelf-life studies: 200 ml of the samples were transferred into amber-colored bottles and kept at refrigeration temperature of 7 ± 1 °C. Sampling was made every week during storage. –pH, conductivity, turbidity, *L**, *a**, *b**, TMAB, TMY, total phenolic substance content, total antioxidant activity and sensory analyses were performed during the shelf-life studies.

Measurement of physical properties: pH was measured at room temperature, using a pH meter (pH-2005 model, JP Selecta SA, Barcelona, Spain). Conductivity (mS/cm) was measured using a handheld conductivity meter (Sension 5 model, HACH, CO, ABD). Turbidity (NTU) was analyzed using a turbidimeter MICRO TPI, Model 20008). Color *L**, *a**, and *b** values were measured using a Hunter Color Flex spectrophotometer (Hunter Associates Laboratory Inc., Reston VA, USA).

Determination of total phenolic content: 200 µL of LRD samples were taken and 2.5 mL of Folin-Ciocalteu reagent (diluted 10-fold in deionized water) was added. This mixture was vortexed for 30 seconds and 2 mL of %7.5 Na_2CO_3 was added to the mixture. It was cooled down to room temperature for 5 minutes after keeping it in water bath at 55°C for 5 minutes and the absorbance was measured at 760 nm against the blank containing 200 µL of water (Bakay, 2019).

Determination of antioxidant capacity: Blank sample was prepared with 1 mL of %0.004 DPPH solution added to 2 mL of ethanol. 100 mL LRD samples completed to 2 mL with 1900 mL of ethanol. DPPH solution was added in diluted samples and kept in the dark for 30 minutes. Absorbance of the solution was measured at 517 nm by T80+UV/VIS model spectrophotometer (PG Instruments, Leicestershire, UK) (Atanassova *et al*., 2011).

Microbial count: Inactivations of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) were performed by surface plating of the appropriate dilutions in 0.1 % (w/v) peptone onto PCA for TMAB and PDA acidified with 10 % (w/v) tartaric acid for TMY. PCA plates were incubated at 35 ± 2 °C for 24-48 h, whereas PDA plates were incubated at 22 ± 2 °C for 3-5 days, respectively. Results were reported as log cfu/mL.

Sensory analyses: LRD samples were evaluated for the sensory properties of cloudiness-clarity, dullness-shininess, color intensity, particle distribution, flavor, juice density, licorice taste, bitter taste, sour taste, sweetness and aftertaste. Nine-point hedonic scale test was performed with 30 trained panelists.

Results and Discussion

Both control I and II samples were spoiled by 3rd week of the shelf-life studies whereas processed I and II samples had the shelf life of six weeks. While no significant difference a detected among the samples during shelf life (Figure 1), turbidity of the samples was decreased at the first week and then increased during storage (Figure 2). pH of the sample was not drastically changed during the storage, but there was slight decrease in the pH of the samples (Figure 3). Total antioxidant capacity of the samples (Figure 4) was significantly decreased through the end of the shelf life studies; whereas total phenolic substance content of the samples was significantly decreased at the last week (Figure 5). Both total mesophilic aerobic bacteria and total mold and yeast counts of the samples were significantly increased during 6-week storage (Figures 6-7). Sensory properties of the samples were not significantly changed during shelf life and in fact processed samples had higher sensory scores than that of the control samples.

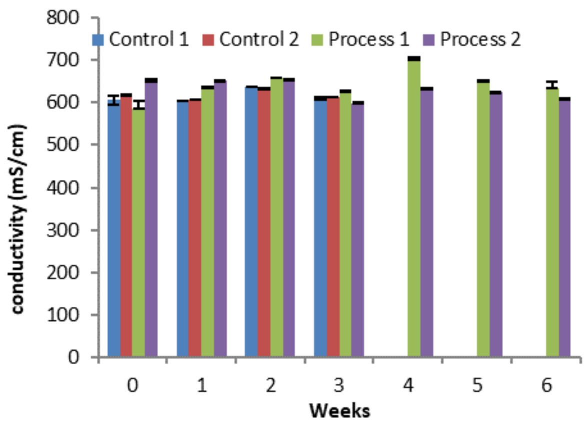

Figure 1. Changes in the conductivity of the samples during storage

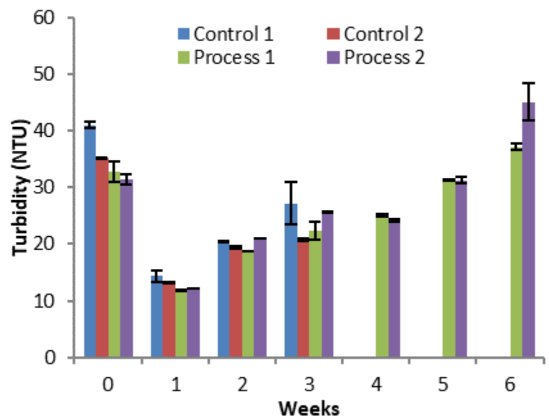

Figure 2. Changes in turbidity of the samples during storage

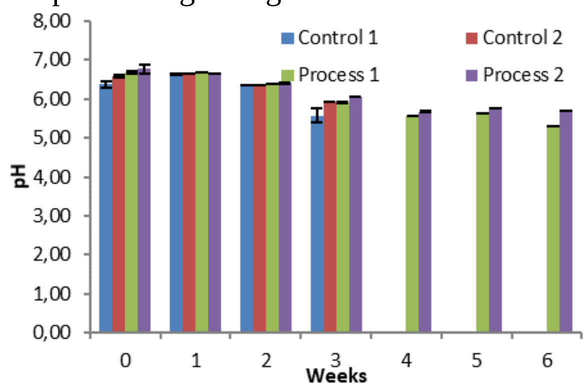

Figure 3. Changes in pH of the samples during storage

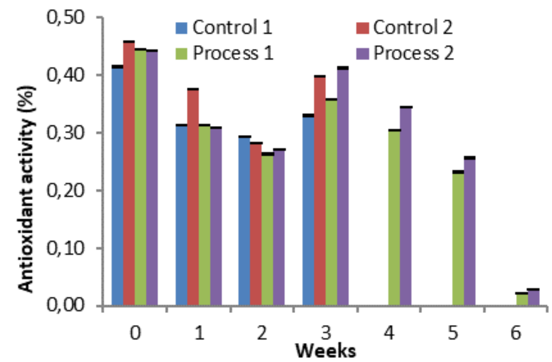

Figure 4. Changes in the antioxidant activity of the samples during storage

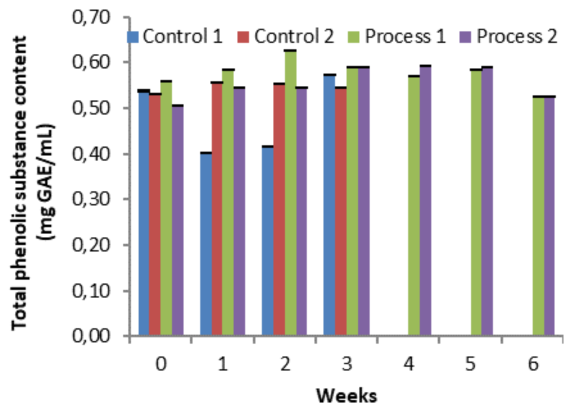

Figure 5. Changes in the total phenolic substance content of the samples during storage

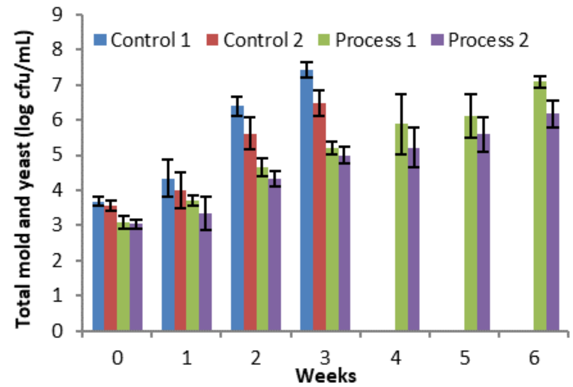

Figure 6. Changes in the total mold and yeast count of the samples during storage

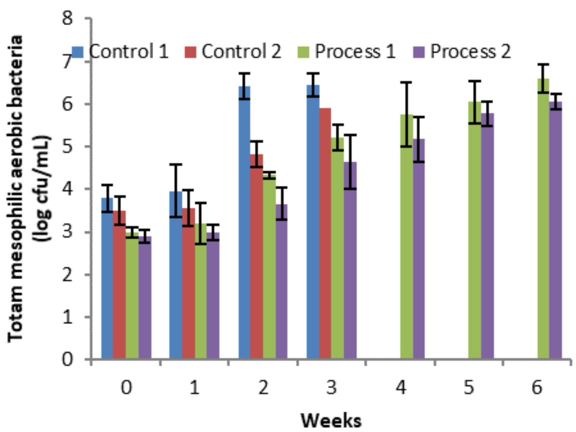

Figure 7. Changes in the total phenolic substance content of the samples during storage

Similar results were reported with PEF and HHP processing of LRD. A study conducted with LRD treatment by PEF (17, 23, and 30 kV/cm) and heat treatments (70°C for 3 min, 80°C for 3 min, and 90°C for 3 min), and combination of mild heat (40°C for 3 min) + PEF (23 kV/cm) revealed that the samples treated by PEF and mild heat + PEF showed no significant difference on pH, TA, conductivity, total soluble solid, color values, total antioxidant capacity, and total phenolic substance content. Heat treatments, however, revealed a significant difference on measured properties compared with control samples. A significant increase on microbial inactivation (TAMB, TMY, *Escherichia coli*, and *Salmonella* Enteritidis) was observed with an increase in electric field strength and temperature. The most effective treatment for microbial inactivation was reported as the combination of mild heat + PEF (Uzuner and Evrendilek, 2017).
HHP processed LRD samples by 200-500 MPa, 3-15 min, and 4-40°C with optimum operational conditions of 500 MPa pressure, 9.90 min, and 18.5°C were stored at both 4 and 22 °C for shelf-life studies. Control samples stored at 4 and 22 °C were deteriorated on days 7 and 2, whereas the HHP-processed samples had an extended shelf-life of 25 days. Initial pH, *L**, *a**, *b**, and C* values in addition to glabridin concentration had a significant decrease, but conductivity and turbidity values, TMAB and TMY counts had a significant increase during storage. HHP-processed samples had no significant change for the initial *h*°, titratable acidity, glycyrrhizin concentration, and sensory properties during shelf-life studies. Except for vanillic, caffeic, and

acetic acids and miristein, no significant change was observed on phenolic compounds and organic acids (Evrendilek *et al*., 2021).

Conclusions

Based on the obtained results it was observed that PEF treatment alone and combination of essential oil and PEF are effective to extend shelf-life of LRD at refrigeration temperature. Consumption of LRD, although very popular, is very low due to its very short shelf-life; thus, this study has a big impact as it reveals a possible approach to extend its shelf life without significantly affecting its physiochemical and sensory properties with inactivation of spoilage microorganisms. Although addition of essential oil and PEF treatment was a viable option for the shelf-life extension of LRD, future studies need to be conducted with addition of different plant essential oils compatible with physical properties the LRD with consumer acceptability tests.

Acknowledgements

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IN-VITRO AND IN-VIVO ANTIFUNGAL ACTIVITY OF FERMENTED COCOA’S RESIDUAL HONEY AGAINST WITCHES’ BROOM DISEASE

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Abstract

Last decades, Brazilian cocoa agribusiness had suffered with the negative impacts caused the witches' broom disease, caused by the fungus *Moniliophthora perniciosa*. Currently, eco–friendly measures are developed to control the spread of this phytopathogen. The main objective of this work is to contribute to the reverse logistics and enhancement of cocoa’s residual honey (CRH) as a natural biofungicide in plants of the *Theobroma cacao* against witches' broom. More specifically, this works explores the potential of the CRH (Tome-Açu/PA, Brazil) fermented during 4 years to inhibit the germination and the growth of *M. perniciosa* (isolated cac-257, CEPLAC/PA, Brazil) through in-vitro and in-vivo essays (using seedlings of the PA-195 genotype of *T. cacao*) compared to negative control. The *in-vitro* essays were performed using 10 diluted solutions of CRH with 3 replications. A gradual reduction of the germination and growth was observed with increasing doses of CRH. In particular, CRH promoted 100% of inhibition in the concentration of 2%. The *in-vivo* essays were carried out using 10 seedlings with 4 replications. Four days after spraying the CRH at a concentration of 5%, the seedlings were subjected to inoculation with the fungus. The results evaluated 45 days after inoculation show a reduction of 5% of the seedlings that present the manifestation of the disease compared to the negative control. The CRH has proved to be a sustainable, low economic and efficient biofungicide against *M. perniciosa* in plants of the *T. cacao*.

Keywords: *Reverse logistics, valorization, biofungicide, Theobroma cacao, Moniliphthora perniciosa.*

Introduction

The fruits of the cocoa tree (*Theobroma cacao*) are of great socioeconomic importance in the world and in particular in Brazil, which is currently the sixth largest producer. The main use is for the production of chocolate and derived products. One of the main biological factor that limits the cocoa production worldwide is invasive and endemic diseases caused by fungal infections, among them witches' broom disease, caused by the pathogenic fungus of the genus *Moniliophthora perniciosa* (Marelli *et al.*, 2019; Lisboa *et al.*, 2020).

Currently, eco–friendly measures have been implemented to control the spread of the phytopathogen, with the advantage to be integrated management controls, more economically and less harmful with human health and environment than the traditional pesticides. One of the most recommended treatments is the combination of physical, biological and chemical agents (Tirado-Gallego *et al.*, 2016). Such strategies include biologic agents control with fungus or bacteria, or natural fungicides, mainly based on essential oils or plant extracts.

Among other pre-processing steps in the transformation chain, cocoa beans are subjected to fermentation, drying and roasting, that generates significant amounts of by-products. In

particular, the residual broth, also called cocoa's residual honey, is an opaque yellow mucilaginous liquid, with a sweet-sour flavor, that is released naturally from the pulp enveloping the cocoa beans during the first days of the fermentation (Silva *et al.*, 2014; Leite *et al.*, 2019).
The main objective of this work is to contribute to the reverse logistics and enhancement of the cocoa's residual honey as a natural biofungicide in plants of the *Theobroma cacao* species against witches' broom. More specifically, this works aims to explore the potential of the cocoa's residual honey to inhibit the germination and the growth of *Moniliphthora perniciosa* fungus through *in-vitro* and *in-vivo* essays.

Materials and methods

The samples of cocoa's residual honey (CRH) were obtained from Michinori Konagano's property, located in Quatro-Bocas, Tome-Açu (02º24'53''S and 48º08'59''W), State of Pará, Brazil. The samples consist of the accumulation of CRH collected directly from the fermentation wooden box during the first four days of the fermentation. After collection, the samples were fermented for 4 years.
The inoculum of the fungus *Moniliphthora perniciosa*, isolated cac- 257, was obtained from infected brooms from the Phytopathology Laboratory of the Executive Committee of the Cacao Plantation Plan (CEPLAC) - José Haroldo Genetic Resources Station, located in Marituba, State of Pará, Brazil. Mature basidiocarps were manually removed from the brooms, washed with distilled water, and dried on absorbent paper.
Seedlings of the PA 195 genotype of *Theobroma cacao*, selected to be susceptible to witches' broom disease, were used. The seeds were collected at the CEPLAC station and after the protrusion of the primary root, the seedlings were transferred to tubes. After 40 days, the seedlings were subjected to the *in-vivo* essays.
The *in-vitro* essays aimed to evaluate the inhibitory effect of the CRH on the germination and growth of *M. perniciosa*. Ten diluted solutions (in sterile distilled water) of CRH were tested, with concentrations of 0.00, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 2.50, 3.00 and 5.00%. The essays were performed using a completely randomized design with three replications.
The germination essays were performed using excavated slides on moistened (with distilled water) filter papers inside Petri dishes. In each slide, a small block of 1.5% agar-water containing *M. perniciosa* spores and 100 µL of the diluted solutions of the CRH were deposited. The negative control was realized using 100 µL of distilled water. The observation of the germination was evaluated after 4 hours of incubation at 25 ± 1 ºC in a BOD-type incubator under the microscope (40x). All spores presenting a germination tube were considered as germinated. The results were expressed as the germination percentage (in %), compared to the negative control.
The growth essays were performed using Petri dishes of 9 centimeters in diameter containing the diluted solutions of the CRH incorporated into the BDA culture medium. For the negative control, no sample was incorporated into the BDA culture medium. Discs of 7 millimeters in diameter containing active *M. perniciosa* fungus were deposited in the middle of the Petri dishes. The Petri dishes were sealed with parafilm and kept in a BOD-type incubator at 25 ± 1 ºC. The evaluation of the growth started after 2 days and was monitored until 12 days. The results were expressed as the fungus colonies diameter (in mm), measured with a caliper, as a function of the time.
The *in-vivo* essays were carried out using a Randomized Block Design, with four replications using the diluted solution of the CRH at a concentration of 5%, resulting in 4 plots with 10

seedlings each. The CRH were applied on the faces of the apical meristem and leaves by hand spray, applying around 20 mL per seedling to the point of drainage. Four days after spraying the diluted solutions of the CRH, the seedlings were subjected to inoculation with a suspension of $2x10^5$ viable basidiospores per mL of *M. perniciosa* fungus by depositing 30 µL in the apical meristem. The inoculated seedlings were transferred to the greenhouse. The symptoms presented by the seedlings individually were evaluated 45 days after inoculation.

Results and Discussion

Figure 1 presents the results of the *in-vitro* essays aiming to evaluate the potential inhibitory effect of ten diluted solutions of the CRH on the germination (Figure 1a) and growth (Figure 1b) of *M. perniciosa*. They show that there is a gradual reduction of the germination and growth with increasing CRH concentration (0 to 5%). The diluted solutions of the CRH with concentrations less than 1% significantly ($p > 0.05$) reduce the germination by approximately 20 – 40% (Figure 1a), but do not significantly affect ($p > 0.05$) the growth kinetics (Figure 1b). The diluted solution of the CRH at a concentration of 1.5% shows to significantly limit the germination with a germination rate of 3.3% after 4 hours (Figure 1a). This same solution allows limiting significantly ($p > 0.05$) the growth kinetics, as it demonstrates a start of mycelial growth only after 5 days (Figure 1b). In particular, the results show that CRH promotes 100% inhibition of germination and growth at a minimum inhibitory concentration (MIC) of 2%.

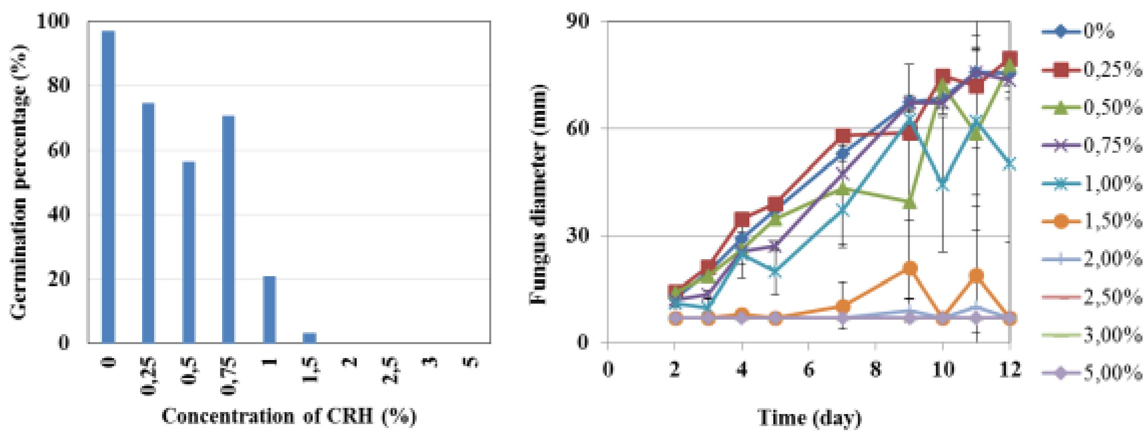

Figure 1. Effect of the concentration of the CRH in the diluted solutions (0-5%) on: a) the germination of spores of *M. perniciosa* (germination percentage - in %), and b) the growth of the fungus *M. perniciosa* (colony diameter - in mm) as a function of time (in days)

This effect may be associated with the content of phenolic compounds, which are proven to be important cacao defense substances. Several studies (Bastos *et al.*, 2007; Silva *et al.*, 2007; Chaussê *et al.*, 2011; *Costa et al.*, 2016) have already been carried out aiming to study the effect of different natural products, mainly essential oils, on the inhibition of germination and/or growth of the *M. perniciosa* fungus through *in-vitro* tests. All these studies obtained MIC far below those obtained in our study. However, it is noteworthy that the CRH has concentrations of essential nutrients much less concentrated than essential oils.

Regarding to the *in-vivo* essays, the results show that 60% of the seedlings that received the diluted solution of the CRH at a concentration of 5% present the symptoms of the disease, while

for the negative control, 65% present the manifestation of the disease. These data indicate that CRH has an inductive protective effect, suggesting a possible resistance mechanism. This fact can be attributed to the physical-chemical characteristics and the potential inhibition of the germination of basidiospores and the growth of the *M. perniciosa* fungus, as presented before. It is worth mentioning that, in this study, the samples of CRH were applied only once to the cocoa seedlings. It is possible that with the use of more applications, before and/or after the inoculation of the fungus, there will be a greater reduction in the symptoms presented.
Several studies have already been carried out in an attempt to control the disease with the use of conventional systemic protective fungicides or natural, such as plant extracts (Resende *et al.*, 2007) or essential oils (Bastos *et al.*, 2007). However, the use of these products is not always economical, and most of time, the concentration and/or the frequency of spraying required is high.

Conclusion

Based on the results obtained in this paper, there are prospects for the experimental use of CRH in the integrated biological control of witches' broom. In addition to being economically attractive and ecological, it presents an excellent source of organic matter, is renewable, and has a low cost. Its bioconversion, from waste to sustainable biofungicide, is a promising alternative contributing to the dynamics of the rural economy, the elimination of the use of pesticides, and the sustainable development.

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STUDY OF THE EFFECTS OF AN INSECTICIDE (CHLORPYRIFOS) AND A FUNGICIDE (TEBUCONAZOLE) ON CHICKEN EMBRYOS

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Abstract

This study aimed to determine the individual and combined toxic effects of Pyrinex 48 EC, a chlorpyrifos-based insecticide (480 g/l) and the Mystic 250 EW, (250 g/l tebuconazole) on the early embryonic development of chicken embryos. Chicken eggs were immersed in the emulsions of the test substances for 30 minutes before the start of the incubation. The insecticide concentration used was 0.4%, and of the fungicide was 0.4%. Subsequently, on the third day of incubation, permanent preparations were made from the embryo to study the early developmental stage. Embryos fixed on slides and stained with osmium tetroxide solution were studied under a light microscope. In addition, embryo mortality and their abnormalities were analyzed by the Fisher test. The single and simultaneous administration of Pyrinex 48 EC increased the mortality up to 20%, but the changes were not significantly different from those in the control group. In addition, the rate of developmental abnormalities did not differ statistically between treated and control groups. Based on the results, there is presumably an addition-type toxic interaction between Pyrinex 48 EC insecticide and Mystic 250 EW fungicide that may reduce the viability of the embryos.

Keywords: *chlorpyrifos, tebuconazole, interaction, ecotoxicology, chicken embryo.*

Introduction

Plant protection must pay close attention to the decrease or complete elimination of the damage to the environment, which affects wildlife because the cultivated plants provide feedstuffs for wild birds and a living and hatching territory. The pesticides sprayed during the plant protection activities might have an influence not only on mature birds but also on the embryos in the eggs. In an environment contaminated by pesticides, these materials change the chemical setting of plants and animals, populations and communities and make it possible for poisoning. The harmful effects of pesticides can be seen in acute damage or destruction of living creatures at a lower or higher level. The destruction of the mature animals can cause the death of the offspring who remain without food and care, even if they are not poisoned. Those who survived the acute poisoning but their resistance decreased can become the victims of different environmental pollution (Várnagy and Budai, 1995). In the toxicological examination of the different xenobiotics and similarly, in the ecotoxicological tests of the pesticides, the chemical agents are generally doneapplied. However, we cannot ignore the fact that the chemical load generally appears in a complex way. Therefore, we can expect the simultaneous, complex toxic effect and interaction of the chemicals present; consequently, the components can modify each other's toxic effects. Therefore, the interest of the researchers turned to the effects of interaction gradually, not only in the field of ecotoxicology but also in any other fields that deal with the question of health care and chemical safety (Oskarsson, 1983; Danielsson *et al*., 1984; Speijers and Speijers, 2004; Youn-Joo *et al*., 2004). Our study aimed to reveal the individual and possible simultaneous embryotoxic effects of chlorpyrifos (Pyrinex 48 EC) insecticide and a tebuconazole-containing fungicide (Mystic 250 EW) which may be dangerous for living organisms.

Materials and Methods

Fertile chicken eggs of a mixed-use hen breed called Farm (*Gallus gallus f. domesticus*) breeds (Goldavis Ltd., Sármellék, Hungary) were used in the study. The eggs were divided into four homologue groups (10 eggs/group) based on their size and weight. They were incubated in a Ragus-type table incubator (Wien, Austria), assuring the required temperature (37-38°C), the relative humidity (65-75%) and the daily twice rotation of them to prevent the adhesion of the embryo to the eggshell (Bogenfürst, 2004).
Before starting incubation, the eggs were immersed for 30 minutes into a solution or emulsion of the test items and their combination at 37ºC (Lehel *et al*., 2021). Then they were placed onto a filter paper after treatment to soak the unnecessary liquid.
The single and simultaneous administration of Pyrinex 48 EC was performed with a concentration of 0.4%, which corresponds to the plant protection practice in the field. This pesticide is an organophosphate insecticide containing 44.4±2m/m% chlorpyrifos as an active ingredient and assigned to marketing category I in Hungary. It is used to control a wide range of insect pests on arable land and in orchards. Mystic 250 EW (0.4%) is a triazole fungicide containing 26.0±1.6 m/m% tebuconazole as an active ingredient and assigned to marketing category I in Hungary. It is used to control fungal diseases on grapes, cherries, almonds, cereals, and rapeseed or canola.
The control group was treated with avian physiological saline solution (NaCl, 0.75%). All eggs and embryos were examined and processed on day 3 of incubation. During the processing rate of embryo mortality and type of developmental anomalies were registered.
In order to study the early phase of development, permanent preparations were made from all embryos on day 3 of incubation. First, the calcic eggshell and the shell membrane were removed above the air chamber, and then the germinal disk was cut around and stained with 0.1% osmium tetroxide solution. Next, the stained germinal disk was placed into avian physiological saline solution (0.75 w/v%) at 38ºC temperature, and it was floated on a slide and fixed with DPX (dibutyl phthalate polystyrene xylene) histological adhesive. Finally, the slide was covered with a coverslip. The permanent preparations were then examined by light microscopy (Sinkovitsné and Benkő, 1993; Kertész, 2001).
In the case of the biometric processing of embryonic mortality and developmental anomalies, an exact test, according to Fisher, was used.

Results and Discussion

Embryonic mortality
Results of the mortality in the control and treated groups are presented in Table 1.
On day three after treatment, there was no embryonic death in the control group (Lehel *et al*., 2014).

As a result of treatment with chlorpyrifos, the rate of embryonic mortality was 20.0%. However, the difference was not significant.
There was no embryonic mortality due to a single administration of the fungicide.
The combined administration of insecticide and fungicide resulted in an embryonic mortality rate of 20.0%. However, the statistical evaluation showed no real change compared to the control and individually treated groups.

Table 1. Embryonic mortality from teratogenicity test of chlorpyrifos and tebuconazole in chicken embryos after single and combined administration.

Treatment	Number of dead embryos/Number of fertile eggs	Rate of embryonic mortality (%)
Control	0/10	0.0
Pyrinex 48 EC	2/10	20.0
Mystic 250 EW	0/10	00.0
Pyrinex 48 EC + Mystic 250 EW	2/10	20.0

Developmental anomalies
Developmental abnormalities and their types are summarized in Table 2 and 3.
During the light-microscopic evaluation of permanent preparations stained with osmium tetroxide, two embryos showing developmental anomalies were found in the control group. Developmental abnormalities consisted of the underdevelopment of one embryo and the poorly developed vasculature of another embryo.
Three embryos (37.5%) showed abnormal development due to the treatment with Pyrinex 48 EC insecticide alone. However, this change was insignificant compared to the control group. The developmental anomaly consisted of retarded development of the embryos and their vascular system.
Four embryos (40.0%) showed abnormal development due to treatment with Mystic 250 EW alone. However, this change was not statistically significant compared to the control group. The developmental anomalies included the retarded development of the vascular system and the body.
Due to the combined treatment, the developmental anomalies rate was 37.5%. However, the change was insignificant compared to the control group and the groups treated with either Pyrinex 48 EC or Mystic 250 EW alone. The developmental anomaly was retarded development of the embryos and their vascular system.

Table 2. Developmental anomalies from teratogenicity test of chlorpyrifos and tebuconazole in chicken embryos after single and combined administration.

Treatment	Number of embryos showing developmental anomalies/Number of alive embryos	Rate of developmental anomalies (%)
Control	2/10	20.0
Pyrinex 48 EC	3/8	37.5
Mystic 250 EW	4/10	40.0
Pyrinex 48 EC + Mystic 250 EW	3/8	37.5

Table 3. Types of developmental anomalies diagnosed in the teratogenicity test of chlorpyrifos and tebuconazole in chicken embryos after single and combined administration.

Treatment	Types of developmental anomalies (incidences of developmental anomalies)
Control	Poorly developed vasculature (1) Poorly developed body (1)
Pyrinex 48 EC	Poorly developed body and vasculature (3)
Mystic 250 EW	Poorly developed vasculature (2) Poorly developed body and vasculature (2)
Pyrinex 48 EC + Mystic 250 EW	Poorly developed body and vasculature (3)

Following immersion treatment of hen eggs with Pyrinex 48 EC insecticide, two embryos died, and three developed developmental abnormalities in an early embryonic study. Based on the results, individual treatment with Pyrinex 48 EC increased the proportion of embryos that died and showed developmental abnormalities compared to the control group. Juhász *et al*. (2005) studied the the toxic effect of a dimethoate containing organophosphate insecticide (BI 58 EC) and the cadmium sulphate on chicken embryos in the early phase of development. It was established by them that individual treatment with dimethoate containing organophosphate insecticide increased the embryonic mortality and the rate of developmental anomalies. In the case of eggs treated with the fungicide Mystic 250 EW, no dead embryos were found during the early embryonic examination; however, developmental abnormalities were found in four cases. In the case of the group treated with Mystic 250 EW alone, there was no statistically significant difference in mortality or developmental abnormality compared to the control group in the developmental phase studied. Major *et al*. (2021) investigated the the embryo toxic effect of a chlorpyrifos containing insecticide (Pyrinex 48 EC) and a tebuconazole containing fungicide (Mystic 250 EW) on chicken embryos. The incidence of malformations resulted in a statistically significant increase compared to the control group in the group treated with Mystic 250 EW.

In the case of co-treatment, two dead and three embryos with developmental abnormalities were recorded on the third day of processing. Six dead embryos and one embryo with a developmental disorder were observed during the late embryonic stage. Based on these results, it can be concluded that in the early embryonic study, there was no significant difference in either embryo mortality or developmental abnormalities in subjects treated with Pyrinex 48 EC and Mystic 250 EW compared to controls. However, there was a kind of increase in treatment for both parameters studied. Mészáros (2021) previously studied the single and combined effects of Pyrinex Supreme and Mystic 250 EW using an injection regimen. Similar result for embryo lethality in early developmental studies was recorded, but statistically significant increase in developmental abnormalities between the control and treated groups was noted.

Based on these results, it can be concluded that immersion treatment induced low embryotoxic effect. This low mortality may be due to the adequate protection of the calcareous shell and the underlying membrane system, suggesting that only small concentrations reach the embryo during treatment. Consistent with the opinion of other authors (Fejes *et al*., 2002; Juhász *et al*., 2005, 2006; Várnagy *et al*., 1996, 2000, 2003), it can be said that interaction avian teratology studies adequately indicate that individual toxic effects change as a result of the combined exposure to foreign chemicals.

Conclusion

The single treatment of Pyrinex 48 EC, a chlorpyrifos-containing insecticide with 1% concentration induced embryotoxic effect in chicken embryo which manifested in not significant increase of mortality and elevated rate of developmental anomalies.

The tebuconazole-containing Mystic 250 EW plant protection product with fungicidal action was also embryotoxic on chicken embryos applied alone and not significantly increased the rate of developmental anomalies. The combined administration of Pyrinex 48 EC insecticide and Mystic 250 EW fungicide induced embryotoxic effect in chicken embryo which represented in not significant increase of mortality and elevated rate of developmental anomalies.

In view of the increased sensitivity of wild fowl species, the studies reported in this paper should be extended to seed-eating birds (pheasants, Japanese quail) and waterfowl (mallards). We also recommend that the interaction studies be complemented with hatchability studies and investigations performed at the postembryonic stage of development, so that the harmful effects of the chemicals under study can be explored more precisely.

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CHANGES IN CHEMICAL AND MICROBIOLOGICAL INDICATORS OF UNSTERILIZED CONCENTRATED CARROT JUICE DURING STORAGE

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Abstract

Carrot is one of the main vegetable crops grown in Belarus on an industrial scale. The processing of carrots into concentrated juices is of particular relevance, since this product is in demand by various manufacturers in the food industry. The purpose of this work is to study the changes in the chemical and microbiological parameters of unsterilized concentrated carrot juice to determine its shelf life. The object of the study were samples of unsterilized concentrated carrot juice obtained under production conditions and stored at T=4±2°C. Sampling for research was carried out monthly, studying the change in the number of bacteria, yeast and mold, the pH, water activity and mass fraction of titratable acids. It was found that the growth of the number of bacteria occurred almost evenly during the four months of storage, reaching tens of thousands of CFU/g. Active reproduction of yeast and mold began after 3 months of storage, reaching a maximum number (several hundred CFU/g) by the 4th month of storage. Further storage of juice samples (up to six months) did not lead to a noticeable development of the microbiota. The value of water activity varied within 0.865–0.900; the mass fraction of titratable acids varied from 0.2 to 0.3%; the pH increased from 4.6 to 5.1 during the first month of storage and then decreased to 5.0 and remained constant. Thus, we can conclude that the shelf life of unsterilized concentrated carrot juice should not exceed three months at T=4±2°C.

Keywords: *Unsterilized concentrated carrot juice, microbiota, chemical indicators, shelf life.*

Introduction

According to statistics, among the semi-finished products of canning products used in recent years by food enterprises in Belarus and other countries, the share of concentrated juices obtained from various types of raw materials is constantly increasing (Statistical report, 2021). The modern technique of juice concentration, which ensures the preservation of almost all biologically active, coloring and nutrient substances, in combination with the capture and return of volatile aromatic substances, makes it possible to obtain products that have a taste and chemical compositions, which differ little from natural juices. In addition, concentrated juices have a number of advantages over natural single-dose juices. For their storage and transportation, they require 5–7 times less consumer packaging, storage facilities and vehicles, they can be stored for a long time without the addition of preservatives and sterilization, they are convenient for consumption, as they easily and completely dissolve in water and store liquid consistency at temperatures down to minus 18°C (Samsonova and Ushev, 1990; Schobinger, 2004*)*. Due to this, recently, juices are exported mainly in concentrated form.
World production of concentrated juices is concentrated mainly in countries where the corresponding types of fruits and vegetables are grown. In Belarus, producers focus on the processing of fruit raw materials, which is due to two main reasons. Firstly, the national standard

STB 1825-2008 "Concentrated fruit juices. Specifications" is valid and applied in the republic, and secondly, some Belarusian canning factories are equipped with technological lines for the production of concentrated fruit juices (usually apple).

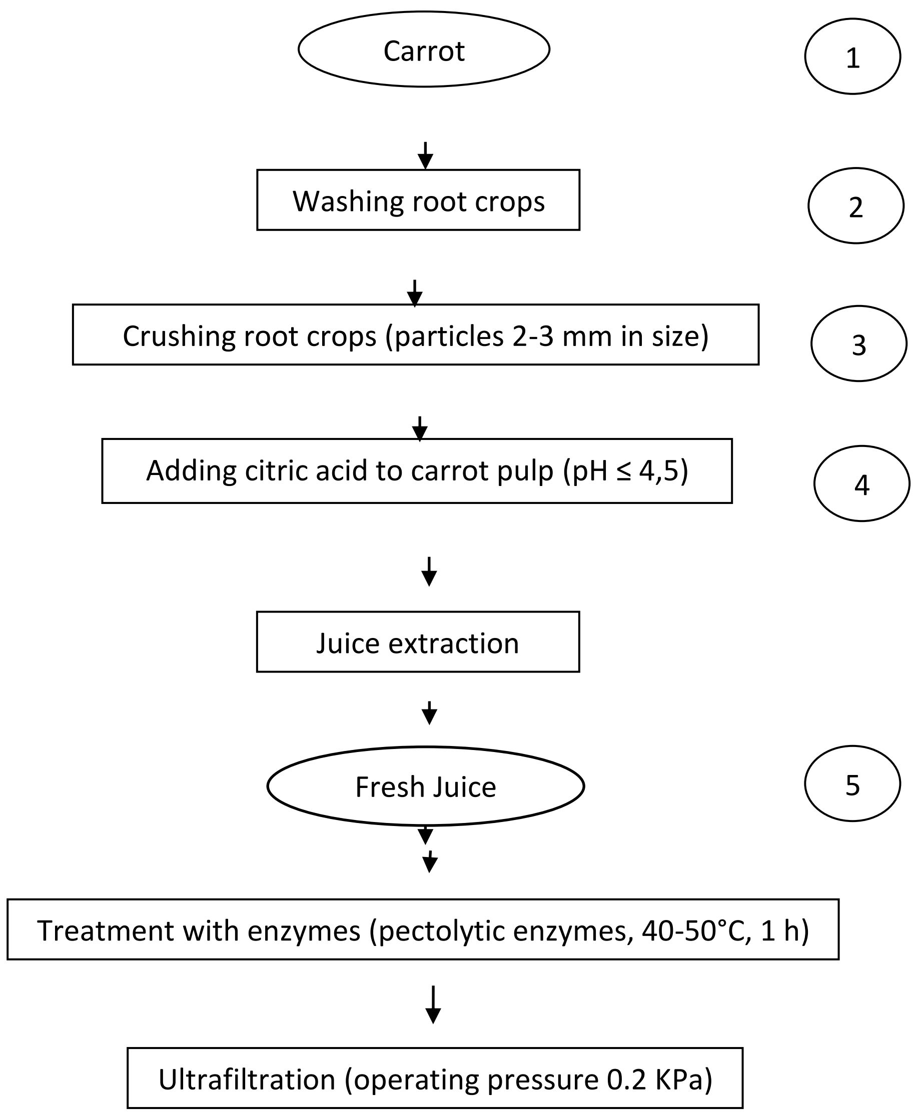
At the same time, carrots (Daucus carota L.) are grown in significant quantities in our country, the chemical com-position of which is determined by the characteristics of the species and variety, agrotechnical and soil-climatic conditions of cultivation, the quality and doses of fertilizers applied, as well as depends of the size of the root crops themselves. Carrot roots contain up to 90% water and about 10% dry matter. Dry matter is represented by almost 50% sugars – glucose and fructose. The content of nitrogen, phosphorus, potassium, calcium, magnesium and other minerals depends on the type of soil, the type of fertilizer used, etc. In total, carrot roots contain about 1% of minerals. Carrots are a source of provitamin A (carotene), the most important medicinal substance, the amount of which depends on varietal characteristics, agricultural practices and climatic conditions of growth. Carrot roots contain fiber, proteins, a small amount of essential and fatty oils, nitrogenous substances, mineral salts of cobalt, iron, copper, phosphorus, iodine, flavonoids, enzymes, vitamins B_1, B_2, B_6, C, E, K, pantothenic acid; a lot in carrots compared to other vegetables of vitamin PP (nicotinic acid). Carrot essential oil consists of such terpenes and terpenoids as pinene, limonene, cineole, geraniol, geranyl acetate, citral and others. Carrot carbohydrates are represented by sugars, starch, fiber, pectin, hemicelluloses. Carrots contain little starch – about 0.2%. It is distributed unevenly in various tissues of the root crop: more often between the core and the pulp and less often closer to the surface of the root crop. Pectin substances are contained both in the pulp and in the core. Carrots also contain bioflavonoids, which have the ability to reduce capillary permeability, thereby strengthening their walls and reducing capillary bleeding. Carrot roots also contain such valuable and useful organic substances as sterols, lecithin, digestive enzymes (amylase, invertase, lipase, catalase, protease, peroxidase). Carrot proteins are represented by the following amino acids: lysine, histidine, cysteine, asparagine, serine, threonine, proline, methionine, tyrosine, leucine. In addition, carrots contain such organic compounds as coumarin, umbeliferon, alkaloids daucine and pyrrolidine (Metlitsky, 1976; Kolpakov, 1998; Wootton-Beard *et al*., 2011; Domagała-Świątkiewicz and Gąstoł, 2012; Sharma *et al.*, 2012; Tretyakov *et al*., 2013; da Silva Dias, 2014; Varshney and Mishra, 2022).

Thus, expanding the range of products of the canning industry through the production of concentrated juice from carrots will satisfy the demand of domestic food enterprises and export products to other countries. To organize the production of concentrated juice, it is necessary to establish its shelf life, which is determined on the basis of an analysis of the results of laboratory tests of products for resistance to microbiological spoilage during storage under specified conditions. Taking this into account, the purpose of our work was to study the changes in the microbiological and chemical parameters of unsterilized concentrated carrot juice during storage at low temperatures.

Material and Methods

The objects of the study were samples of unsterilized concentrated carrot juice (48% of soluble solids), obtained under the production conditions of one of the canning enterprises of Belarus according to the technological scheme shown in Figure 1. Samples of concentrated juice were stored at a temperature of 4 ± 2°C for 6 months in a refrigerated warehouse. Sampling of products was carried out monthly.

The subject of research was mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM), yeast and molds isolated from samples of unsterilized concentrated carrot juice. Microbiological studies were carried out using standardized methods (inoculation on agar nutrient media – nutrient agar GMR and Sabouraud agar with antibiotic). In parallel with microbiological studies, we studied the change in physical and chemical parameters during the storage of concentrated carrot juice. The mass fraction of titratable acids was determined by the potentiometric method (GOST 34127-2017), the pH index was determined by the ionometric method in the measurement range from 2 to 12 pH units (GOST 26188-2016), water activity value (a_w) was measured using a Roremeter RM-10 water activity analyzer, taking into account the requirements of ISO 18787:2017.
Also, the objects of the study were carrot samples taken during the technological process of making concentrated juice (Figure 1 and Table 1), in which the group and numerical composition of the surface microbiota was determined to assess the effectiveness of the applied technological regimes.



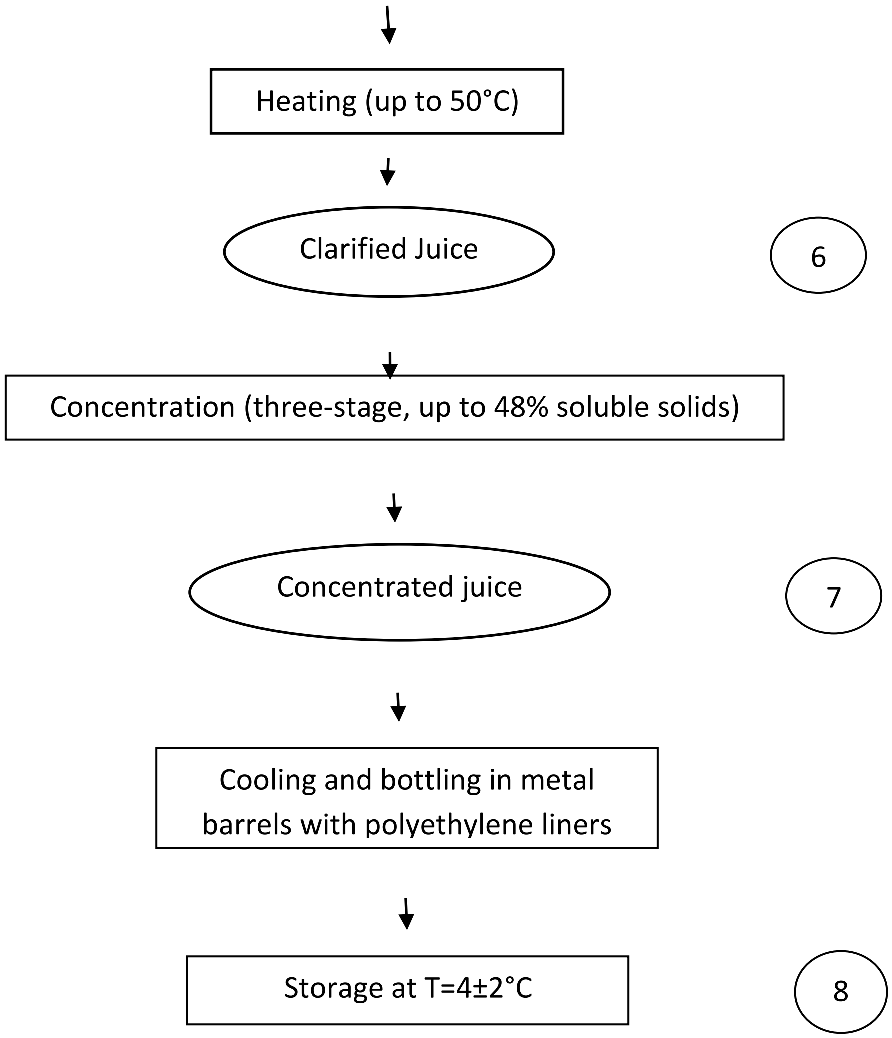


Figure 1. Technological scheme for obtaining concentrated carrot juice under production conditions and sampling points for research

Table 1. Symbols of sampling points in the manufacture of concentrated carrot juice in production conditions

Number of sampling points	Name of the research object
1	Carrots before washing
2	Carrots after washing
3	Crushed carrots
4	Crushed carrots and acidified with citric acid
5	Carrot juice after pressing (fresh juice)
6	Carrot juice after enzyme treatment, ultrafiltration and heating (clarified juice)
7	Concentrated carrot juice
8	Concentrated carrot juice during storage

Result and Discussion

The results of the influence of technological regimes for obtaining concentrated carrot juice on the group and numerical composition of the surface microbiota of carrot roots are shown in Figure 2. From the data presented, it can be seen that technological operations preceding the concentration of carrot juice contributed to a decrease in bacterial and yeast microbiota by two orders of magnitude. Molds found on the surface of carrot roots prior to washing were effectively reduced by the washing operation, enzymatic treatment, ultrafiltration and heating of the juice prior to concentration. These data are generally consistent with the results of similar studies by Jabbar *et al*. (2014) on the bactericidal efficacy of carrot juice blanching at 70°C. At the same time, it was found that such technological methods as crushing carrots, acidifying and pressing the pulp led to an increase in the number of this group of microorganisms almost to the initial value, which is probably due to contamination from elements of the production environment. Juice concentration to a mass fraction of solids equal to 48% led to the death of the vast majority of all microorganisms, and ensured that the end product complies with legislative requirements for microbiological indicators (Technical Regulations of the Customs Union TR CU 21/2011 and TR CU 23/2011).

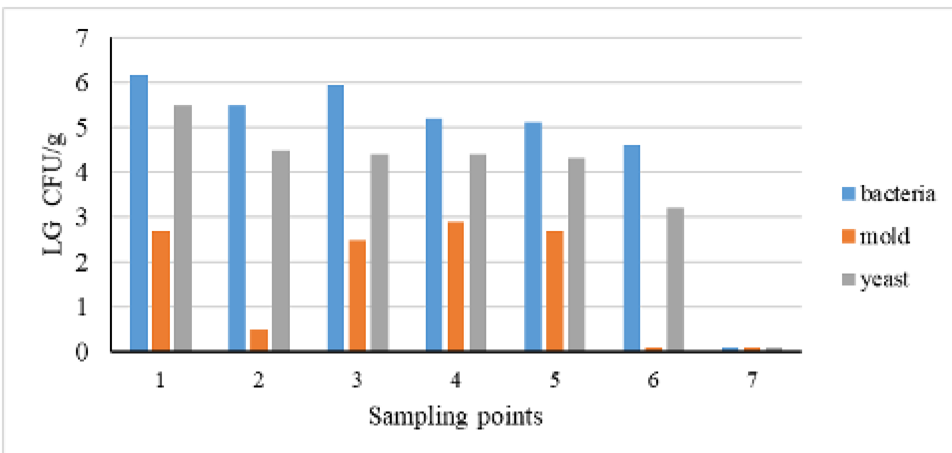


Figure 2. Changes in the quantitative composition of the carrot microbiota in the process of obtaining concentrated carrot juice under production conditions: 1 – carrots before washing, 2 – carrots after washing, 3 – crushed carrots, 4 – crushed carrots and acidified with citric acid, 5 – carrot juice after pressing, 6 – carrot juice after enzyme treatment, ultrafiltration and heating, 7 – concentrated carrot juice

The results of microbiological studies of unsterilized concentrated carrot juice during storage are shown in Figure 3. The data indicate that the development of bacterial microbiota in the final product began already during the first month of storage, molds and yeasts – after the 2nd and 3rd months respectively. Further development of bacteria in the concentrated carrot juice stored in the refrigerator occurred almost evenly up to 4 months of storage (by 1 order of magnitude per month), reaching several thousand CFU/g, and by the end of the experiment the content of the residual bacterial microbiota changed insignificantly. The reproduction of yeasts and molds increased sharply (by ~ 2.5 orders of magnitude) during the 4th month of storage, and their number in 1 g of the product approached the content of mesophilic bacteria. Thus, unsterilized concentrated carrot juice after 3 months of storage did not meet the legislative requirements in terms of microbiological parameters (MAFAnM – no more than $5×10^3$ CFU/g; yeast – no more than $2×10^3$ CFU/g; molds – no more than $5×10^2$ CFU/g).

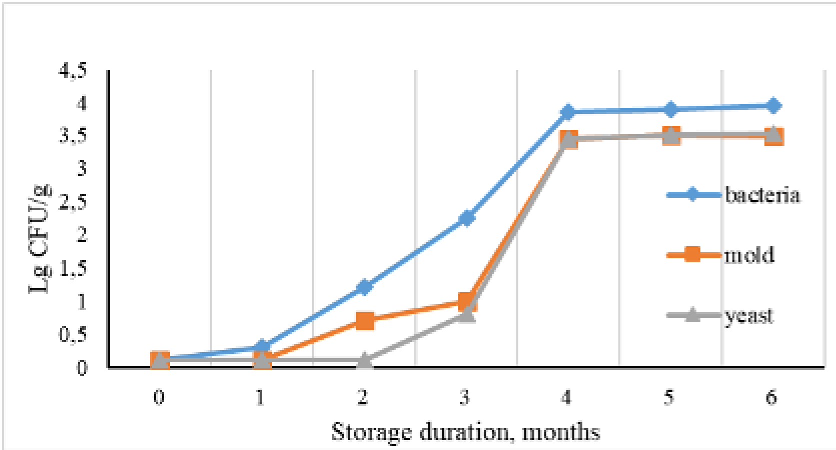


Figure 3. Changes in the quantitative composition of the microbiota of concentrated carrot juice during storage at a temperature of 4±2°C

There are a number of studies in the literature (Hammad *et al*., 2013; Leneveu-Jenvrin *et al*., 2022; Pokhrel *et al*., 2022) on microbial stability during short-term (from 14 to 28 days) storage of carrot juice processed in various ways (blanched, irradiation, after exposure to ultrasound and a pressure of 300-400 MPa). The experimental data of these authors testify to the different rates of development of aerobic mesophilic bacteria, fungi and yeast in the studied samples of carrot juice, leading them to spoilage. Thus, our results confirm the fact that unsterilized carrot juice is not resistant to microbial spoilage for a long time, no matter what pre-decontamination treatment it is subjected to.

Analysis of the dynamic of chemical indicators of unsterilized concentrated carrot juice (table 2) showed the following. The increase value of the water activity of the final product in the first two months of storage may have been due to the presence of air inside the package with the product and the leakage of the package. Then we recorded a gradual decrease in this indicator to the initial value, which coincided with the development of all groups of microorganisms in the product. Thus, water activity was an insignificant barrier factor.

Table 2. Change in the chemical index of unsterilized concentrated carrot juice during storage at a temperature of 4±2°C.

Indicators	Storage duration, months						
	0	1	2	3	4	5	6
Water activity	0.865	0.875	0.900	0.880	0.875	0.870	0.860
pH	4.6	5.1	5.0	5.0	5.0	5.0	5.0
Mass fraction of titratable acids	0.3	0.2	0.2	0.2	0.3	0.3	0.3

A different dynamic was found by us in relation to pH. The decrease in active acidity during the first month of storage by 0.5 pH units may be due to the activity of bacteria, but the subsequent stabilization of this indicator at the level of 5.0 pH units did not reflect any changes in the chemical composition of the product, despite the high content of yeast, molds and bacteria in juice samples. The change in the value of titratable acidity during the experiment ranged from 0.2 to 0.3%, which is within the error of the measurement method used by us. Similar conclusions about the dynamics of acidity and pH value were made by Profir *et al*. (2013) and Pokhrel *et al*. (2022), who studied the change in the composition of carrot juice during short-term storage.

Conclusion

Thus, the conducted studies allowed us to draw the following conclusions. The technology of concentrated carrot juice tested by us in industrial conditions is able to ensure the release of a finished product that is safe in terms of microbiological indicators. At the same time, due to the lack of aseptic conditions for cooling and packaging concentrated carrot juice into consumer packaging, it was contaminated with microorganisms, which led to a limitation of the shelf life of the target product to 3 months. At the same time, the analysis of scientific and technical literature data on the shelf life of unsterilized vegetable juice concentrates showed that such products can be stored at a temperature of 4±2°C for at least a year.

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OCCURRENCE OF RADIONUCLIDES AND TOXIC ELEMENTS IN FEED STUFF FROM NORTH MACEDONIA

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Abstract

Animal feedstuff is one of the most common sources of radionuclides and toxic elements which are responsible for poisonings and dangerous exposures that can lead to adverse health effects and potentially death in animals and humans worldwide. The aim of this study was to determine the content of radionuclides and toxic elements in the samples of animal feed collected from areas with intensive agricultural production on the territory of North Macedonia. The radionuclides (^{226}Ra, ^{137}Cs and ^{40}K) were determined in samples of pig feeds, poultry feeds, lamb feeds and cattle feeds by gamma ray spectrometry, while the toxic elements (Pb, Cd and As) were analyzed by electrothermal atomic absorption spectroscopy (ETAAS), including Hg by using cold vapor atomic absorption spectrometry (CVAAS). The obtained results showed that natural ^{40}K was present in all tested samples. The average 40K activity concentration was 192.85 ± 8.50 for pig feeds, 41.95 ± 3.00 for poultry feeds, 117.0 ± 5.50 for lamb feeds and 61.0 ± 1.50 Bq/kg for cattle feeds. The anthropogenic radionuclide ^{137}Cs was not detected. The trend for average concentrations of toxic elements found in the collected feed samples was as follows: Pb>Cd>As>Hg, except in cattle feeds where Cd was found to be present in lowest average concentration. The Hg content (0.038±0.027 mg/kg) was detected to be higher compared to that of As (0.014±0.018 mg/kg) in lamb feeds. All tested samples contained toxic element concentrations far much below the MLs or within the global average. A conclusion is made that the presence of radionuclides and toxic elements in animal feed and food of animal origin does not pose a risk to the health of animals and humans. Laboratory testing is an important tool as preventive measure for feed safety, which includes monitoring of radioactivity and heavy metals presence in feedstuff from North Macedonia.

Keywords: *radionuclides, heavy metals, pig feeds, poultry feeds, lamb feeds and cattle feeds.*

Introduction

Rapid industrial development and human activities in agricultural practices and livestock production significantly increases the environmental pollution, and thus increases the concentrations of various radioactive substances and toxic substances in it.

The presence of radionuclides and heavy metals is an integral unavoidable part of the environment. Their concentrations depend on the geological and geographical conditions (Tchounwou et al., 2012). The large number of activities in everyday life such as application of soil fertilizers, metal ore processing, and deposition of particles, increase the concentration of radionuclides and heavy metals in the environment, feed and food products (Czarnecki et al., 2015; Mitrović, 2001). Due to the toxic effects and present levels, they are considered major environmental pollutants (Pavlović et al., 2016) The heavy metals lead (Pb), cadmium (Cd) and mercury (Hg) and the metalloid arsenic (As) are of concern because they have toxic properties with no evidence of essentiality. Various toxic effects may occur under conditions of adverse change in biochemical reactions (Lopez-Alonso, 2012). However, the living organism does not have effective homeostatic control mechanisms for heavy metal contaminants and radionuclides. Because of potentially harmful effects on human and animal health (Merck Veterinary Manual, 1973) there has been a significant need for radiological and heavy metal analysis of the presence of the studied toxic elements in the environment, especially analysis of animal feed, considering that it reaches humans through the food chain. In order to enrich the nutritional value, elements that may increase the amount of heavy metals and radionuclide activity in animal feed are increasingly being added, which was an issue of discussion (IAEA, Technical Report Series No. 310, 1990).

Therefore, within the European Commission (EC), the maximum presence of lead, cadmium, arsenic and mercury in feed and feed materials have been regulated in Directive 2002/32/EC, last amended for heavy metals by Regulation (EU) 2015/186 EU.

To comply with these limits, it is necessary to control every stage of the feed supply chain, starting at the primary source of contamination and covering all other appropriate phases of the feed chain. The supply of safe feed products to animals is essential not only to safeguard animal health and welfare but also to reduce human exposure to potentially toxic elements like heavy metals and radionuclides. This study was conducted in order to determine the content of heavy metals and radionuclides in animal feed of plant origin marketed on the territory of North Macedonia. The obtained results included levels of Pb, Cd, As and Hg, as well as levels of ^{40}K, ^{226}Ra and ^{137}C in different types of plant-based animal feed for pigs, lamb, cattle and poultry. The results can be used to define priorities for national monitoring plans.

Material and Methods

Sampling and sample preparation

A total of 121 samples were collected from local wholesale and feed producers. For radiological analysis, after the homogenization, the samples were placed in 0.5 L Marinelli beakers which were fully filled, sealed and stored for some time before the measurements was performed, in order to establish a balance between ^{226}Ra and ^{222}Rn ((IAEA, Technical Report Series No. 295, 1989). For heavy metals testing, the homogenized samples were mineralized in PTFE lined vessels with a mixture of concentrated nitric acid (65%) and hydrogen peroxide (30%), through a wet digestion process in a microwave oven (Ethos up, Milestone Corporation, Italy). The digests were diluted with high-purity water and directly analyzed for heavy metals contents applying ETAAS for Pb, Cd and As and CVAAS for Hg analysis (EN 15550:2007, EN 13806:2002).

Instrument

For radiological assessment the samples were measured on gamma spectrometer instrument (Canberra Packard) with a high purity germanium detector. GENIE 2000 software was used for data acquisition and analysis. The specific activity of ^{226}Ra was calculated from the energy line of 186.1 (keV),^{40}K was determined from its γ-line at 1460 keV, while the activities of ^{137}Cs were determined by means of an estimation of the γ-line at 661.66 (keV). The time interval for calculation (counting) was 65000 seconds.

Lead, cadmium and arsenic analysis was performed using ETAAS (Perkin Elmer AAnalyst 600), and CVAAS by means of flow injection mercury system (Perkin Elmer, FIMS 100) was used for

determining mercury in the selected samples. The measured absorption lines were: 283.3 nm for Pb, 228.8 nm for Cd, 193.7 nm for As, and 253.7 nm for Hg. Palladium was added as chemical modifier to standards and samples for stabilization of Pb, Cd and As at charring temperatures of 1850 °C, while alkaline sodium borohydride was used as the reducing agent for mercury determination in animal feeds (EN 15550:2007, EN 13806:2002).
The internal quality control of the gamma-spectrometry procedure was performed by means of periodical energy and efficiency calibration with standard source MBSS2 (Czech Metrology Institute, Brno, Czech Republic), and weekly control of the background spectrum of the acquisition system. For heavy metals, the quality control check was performed using proficiency test material IMEP 38 (EURL for heavy metals, Geel, Belgium). Recoveries between 90 and 110% were accepted to validate the calibration for all studied toxic elements.
The obtained measurement data were subjected to analysis of arithmetic range, means, standard deviations and medians using MS Excel (MS Office 2013, Redmond, USA).

Results and Discussion

Results of the feedstuff analyses from areas with intensive agricultural production on the territory of North Macedonia are listed by livestock type in Tables 1 and 2.

Table 1. Mean values of specific activities (A) of ^{226}Ra, ^{137}Cs and ^{40}K in pig, poultry, lamb and cattle feeds

Sample type	N*	A ^{40}K ±SD (Bq kg^{-1})	A ^{226}Ra±SD (Bq kg^{-1})	A ^{137}Cs±SD (Bq kg^{-1})
Pig feeds	56	192.85±8.50	3.42±1.22	<1
Poultry feeds	14	41.95±3.00	1.17±0.23	<1
Lamb feeds	28	117.0±5.50	2.57±1.00	<1
Cattle feeds	23	61.0±1.50	1.66±1.50	<1

*N – number of tested samples

The obtained results showed that the highest level of natural ^{40}K is present in all types of tested samples. The mean value for the measured activities in pig feed was 192.85 Bq kg^{-1} for ^{40}K and <1 Bq kg^{-1} for ^{137}Cs. In addition, for poultry feed, the average measured value was 41.95 Bq kg^{-1} for ^{40}K and <1 Bq kg^{-1} for ^{137}Cs. The mean values for lamb feeds varied and the ones for cattle feeds were 117.0 Bq kg^{-1} and 61.0 Bq kg^{-1}. If one considers the activity concentration of the most common ^{40}K found in all samples, it can be concluded that there is a statistically significant difference between the concentration of radioactivity in pig feeds, poultry feeds, lamb feeds and cattle feeds ($p < 0.001$). The highest statistically significant difference in the activity concentration of ^{40}K was found between samples of poultry feeds and the other tested samples. Recently, more attention has been focused on the concept of “farm to fork” adapted into researches as an effort to reduce the contamination of foods by concentrating on source-directed measures.
Preventing contaminated raw materials from entering the food chain is more effective to ensure food safety compared to conventional market control. In a radiation situation, the availability of uncontaminated food and food raw materials to consumers and to the entire production chain is a challenge, especially during the growing season (Rantavaara et al., 2005). It can be seen that the anthropogenic radionuclide ^{137}Cs was not detected in any of the tested samples.

Table 2. Concentration of heavy metals (mg/kg, mean±standard deviation, median) in various animal feeds, in the Republic of North Macedonia

Sample type	N*	Index	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)	Hg (mg/kg)
Pig feeds	56	Range Mean±SD Median	0.012-1.632 0.277±0.376 0.124	0.003-1.962 0.106±0.340 0.022	0.005-0.920 0.105±0.201 0.040	0.002-0.079 0.020±0.024 0.013
Lamb feeds	28	Range Mean±SD Median	0.007-1.124 0.227±0.308 0.110	0.003-1.881 0.194±0.410 0.058	0.002-0.044 0.014±0.018 0.004	0.002-0.068 0.038±0.027 0.041
Cattle feeds	23	Range Mean±SD Median	0.003-0.408 0.107±0.083 0.083	0.004-0.117 0.021±0.027 0.011	0.009±0.064 0.037±0.023 0.037	0.005-0.044 0.023±0.015 0.022
Poultry feeds	14	Range Mean±SD Median	0.016-0.287 0.103-0.092 0.086	0.013-0.066 0.037±0.025 0.031	0.013-0.060 0.036±0.021 0.030	0.011-0.062 0.030±0.028 0.017

*N – number of tested samples

Concentrations found in the accumulated samples in the present study were used to compare them with permitted levels in feedstuffs according to European Commission Directive 2002/32/EC, last amended for heavy metals by Regulation (EU) 2015/186 which are showed in Table 3.

Table 3. EU permitted limits in feedstuffs contaminated with the most common heavy metals and metalloids (Directive 2015/186/EU)

Sample type	Lead EU Limit (mg/kg)	Cadmium EU Limit (mg/kg)	Arsenic EU Limit (mg/kg)	Mercury EU Limit (mg/kg)
Pig feeds	<0.5	<0.5	<2.0	<0.1
Lamb feeds	<0.5	<1.0	<2.0	<0.1
Cattle feeds	<0.5	<1.0	<2.0	<0.1
Poultry feeds	<0.5	<0.5	<2.0	<0.1

The lowest values of the studied elements were below the limit of detection (<0.1 mg/kg) in all feed samples.

Maximal levels of toxic metals were detected to be in pig feeds for lead (1.632 mg/kg), cadmium (1.962 mg/kg), arsenic (0.920 mg/kg) and mercury (0.079 mg/kg), respectively. Among them, the highest contamination was observed in pig (1.962 mg/kg) and lamb samples (1.881 mg/kg) with cadmium concentration above EU allowed limit and the proportion was nearly sixteen times of cattle feeds and thirty times of poultry feeds. The other three heavy metals showed maximal concentrations below the allowed range to the EU legislation

Thus, the median content of pig, lamb, cattle and poultry feeds was below or nearly 0.1 mg/kg.

The determined trend for toxic element levels, according to the average concentrations found in the studied feed samples was as follows: Pb>Cd>As>Hg, except in cattle feeds where Cd was found to be present in lowest average concentration (0,021±0,027 mg/kg).

The mercury mean content (0.038±0.027 mg/kg) was detected to be almost twice higher compared to arsenic mean value (0.014±0.018 mg/kg) in lamb feeds.

Moreover, there was no statistically difference of Hg concentrations between pig, lamb, cattle and poultry feeds.
Almost three times higher average values of Pb were observed in pig (0.277±0.376 mg/kg) and lamb feeds (0,227±0,308 mg/kg) compared to that found in cattle (0,107±0,083 mg/kg) and poultry feeds (0,103-0,092 mg/kg). Finally, cattle and poultry feeds appeared to contain significantly lower mean concentrations of Cd and As corresponded to ones in lamb and poultry feeds. An exception to this was lamb feeds where the mean arsenic value (0,014±0,018 mg/kg) was detected to be the lowest one, while the lowest mercury mean value (0,020±0,024 mg/kg) was showed to be present in pig feeds.
Generally in this survey, the contents of heavy metals in animal feeds was far beyond the range and the mean values reported in other similar studies (Nicholson et al., 1999).
Heavy metals are group of toxic substances that are not metabolized at all, but are persistent and remain in the animal edible products (Kan et al., 2007).
Studies by Vreman et al. (1986) and Kreuzer et al. (1981) demonstrated that certain animal edible products, especially tissues often show a strong relationship to heavy metals in cases when livestock is exposed to them through the diet (Vreman et al., 1986;Kreuzer et al., 1981)
Occurrence of heavy metals in animal feeds as a matter of of importance depends on many factors (Van der Fels-Klerx et al., 2011)
According to Ref (Prankel et al., 2005) three factors proved to be very important in the transfer of heavy metal contaminants from animal feed to animal products, including toxic element concentration in the feed, its chemical form in the feed and duration of feed exposure. Several studies on heavy metal accumulation in grazing animals (Loganathan et al., 1999) demonstrated that carry-over the contaminants into the feed chain strongly hang on soil characteristics and on the degree of pollution from regional metal smelters, industrial, urban and agricultural activities.
The important sources of soil contamination with lead, cadmium and arsenic are combustion of gasoline, and increased use of pesticides and sewage sludge (Alengebawy et al., 2021).
The presence of heavy metals (especially lead, cadmium and arsenic) in soils near highways is related to traffic density and road distance (Trindade et al., 2006).
There is a matter of serious concern for mercury as an environmental pollutant (Hang et al, 2016) showed that there is no clear connection with Hg between plant and soil, indicating that mercury in plant is mostly affected by other factors besides soil mercury, such as external mercury contamination of the animal feed.

Conclusions

Risk management in the field of animal and human nutrition depends on the appropriate information of absorption, metabolism, transfer and toxicological profile of toxic elements as well as on robust sampling and testing schedule performed in practice to further reduce or remove their side effects. Furthermore, to improve risk management of heavy metal contamination, relevant data on management and control of toxic elements in animal production chains are essential. Our research and the obtained results showed that the samples do not pose a risk to animal and human health. The food of plant origin being used in North Macedonia has generally shown safe levels for the analysis of heavy metals and radionuclides. However, as preventive measure continuous monitoring of radioactivity and heavy metals in animal feed should be conducted. Strict safety control will also need to be performed when importing different types of animal feed. Furthermore, a monitoring plan to monitor radiation and heavy metals can prevent the entry of high levels of radionuclides and heavy metals in the food chain. If there are high levels in the analyzed samples, above the permissible limits, they should be diluted with appropriate nutrients, or should be removed from the market and safely destroyed.

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THE INFLUENCE OF THE WAR IN UKRAINE ON THE IMPORT OF WHEAT, CORN AND SUNFLOWER OIL IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

Cereals are the major staple food that feeds the world, and wheat, corn and rice make up more than 40% of all consumed calories. The supply of cereals is continuously decreasing and this year will be the fifth consecutive year with an annual decline in world grain reserves, according to the International Grains Council. Additional problems include higher delivery costs, energy inflation, extreme weather conditions and labor shortages that have made food production even more difficult. The war in Ukraine is additionally raising prices, threatening to bring famine to unprecedented levels. Global food prices are at an all-time high, whereby the UN benchmark index has increased by more than 40 percent in the last two years. Food insecurity has doubled in that period, with an estimated 45 million people on the brink of starvation. This whole situation led the authors to make a more detailed analysis of the conditions. To that end, statistical sources were used, as well as views of prominent experts in the field. All processed data indicate that possible conflicts and wars such as the current one in Ukraine, significantly affect the price of these cereals not only because of the fact that they are mostly produced in Ukraine, but also because at the moment the country has a ban on export of certain cereals in order to replenish its reserves in this crisis situation of the country. At the same time, this significant impact on the price of such cereals is something that is observed today in almost every country in the world, and especially in Europe.

Keywords: *cereals, shortage, Ukraine.*

Introduction

Wheat reached an all-time record on the Chicago Mercantile Exchange in March 2022. Corn and soybeans are traded close to their multi-year maximum values. In order to understand the impact of the current war in Ukraine, one must bear in mind that the country is one of the largest producers of cereals and other crops. Undoubtedly, this conflict causes price shocks and problems with the supply of cereals around the world, and thus an impact on rising stock prices, which in turn brings an increase in trading prices and, ultimately, an increase in retail prices (Focus & Crisis., 2022). Namely, according to the available data, Ukraine is ranked first in Europe in terms of area of agricultural arable land, which means that the country has enough fertile arable land to plant cereals and other types of crops. Thereby, the country is positioned third according to the area of black soil, and this is 25% of the world area of black soil, which makes the soil itself even more fertile and with a higher land capability class. This means that a vast agricultural land is spread on the territory of Ukraine and it has an exceptional level of fertility, which is also observable in the other statistical data that will follow in this part. Namely, Ukraine is on the first place in the world when it comes to sunflower but also sunflower oil. This means that the country has the largest reserves of sunflower and sunflower oil that it exports around the world. Ukraine is responsible for approximately 46% of the world’s sunflower crop and, until the Russian invasion, Ukraine was the world’s main supplier of sunflower oil. The 2021 sunflower has already been harvested and is expected to be processed into oil (Delmy Salin, 2021). Most sunflower oil production plants are completely closed.

Some of the most difficult fights and the worst destruction have taken place in the Ukrainian cities where sunflower processing takes place. The Ukrainian ports on the Black Sea are currently blocked, meaning there are no products leaving or entering Ukraine by sea. Farmers had to abandon their harvest and flee for safety or to stay and fight. Probably there will be no sowing this spring. In addition, the country is one of the largest producers of corn, barley and wheat. In the early 2000s, corn areas in Ukraine were quite small, ranging from about 3 million to slightly more than 4 million hectares (Post-Dispatch., L.2022) That started to change at the end of the first decade of the 21st century and by 2010, Ukrainian corn producers had harvested over 6 million hectares of corn. The corn area continued to rise in recent years, reaching nearly 8 million hectares in 2021, which is more than double in two decades. When it comes to wheat, Ukraine is ranked eighth as a source of wheat globally. Together with the Russian Federation, they account for more than 40% of the world wheat production (Focus S., & Crisis U. 2022). The above mentioned data indicate that possible conflicts and wars such as the current one in Ukraine significantly affect the price of these cereals not only due to the fact that they are mostly produced in Ukraine, but also because the country currently has a ban on the export of certain cereals in order to preserve its reserves in its current crisis situation. Thereby, this significant impact on the price of such cereals is something that is observable today in almost every country in the world, and especially in Europe.

Cereal crops and sunflower oil are staple foods in most human diets, both in developed and developing countries, providing a great portion of dietary energy and nutrients. Cereals such as wheat are composed of approximately 75% carbohydrates, mainly starch and approximately 6-15% protein, and they contribute for more than 50% of the energy supply globally. The importance of cereals and cereal products is also supported by the fact that global food security mostly depends on cereal production, which is approximately 2600 million tons per year (McRae, 2018).

Governments are taking steps to retain food reserves, a move that is likely to continue food inflation. Hungary, Indonesia and Argentina are part of the group of countries that have introduced trade barriers to the export of agricultural products, from wheat to cooking oil, in an attempt to suppress the domestic prices and to protect local food supplies after the Russian invasion led to widespread panic due to the shortage. Russia further supplemented this wave of protectionism when it signaled plans to restrict trade in some raw materials. Ukraine’s main food exporter focused on supplying the Ukrainian army and civilians in the bombed cities.

Trade restrictions could cause further rise in international prices due to the tightening of the global stocks, according to Mr. Steve Matthews, Head of Strategy at Gro Intelligence. Again, observing this from a national security perspective, some countries cannot afford a shortage of grain production or a rise in food prices. Due to the interconnected nature of our food systems, this invasion of Europe’s bread basket certainly poses an associated risk to other countries, some of which are already fighting for food as it is. Everything that is part of food growing is becoming more expensive. Russia, a major supplier of all major crop nutrients, called on domestic fertilizer producers to reduce exports earlier this month, fueling fears of a shortage of inputs for crops that are vital to growers.

Fertilizer prices are rising worldwide due to supply problems and production problems. In Europe, rising natural gas prices - a key contribution to nitrogen fertilizer production - have already forced some facilities to reduce production. The cost of fuel, used by farmers to heat barns and manage equipment which is used for food production is also skyrocketing. The stress is further increased with the sanctions against Russia, the world’s main energy supplier, whereby the United States and the United Kingdom are trying to ban imports of Russian crude oil and other oil products (Shabowski et al., 2011).

Materials and methods

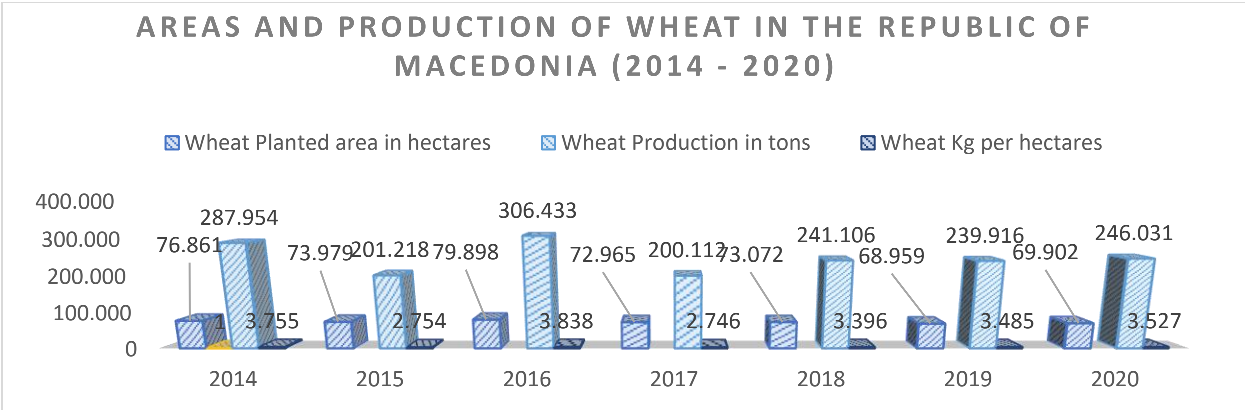
For the needs of this research, a group of data for several years were collected and analyzed, which referred to the production, import and export, as well as the consumption of cereals and other crops as well as oil (sunflower oil) for eating. The analysis of the data will enable correlation between the consumption, stocks and imports of cereals and other crops that are of exceptional importance for North Macedonia and the current war that still continues in Ukraine. The use of the statistical method in the research allows descriptive representations of figures from the past and present, which refer to the production as well as the import and source of these goods and how much these parameters are affected by the state of war in Ukraine.

Results and discussion

This part of the research contains an analysis of the obtained data in relation to the production, import and export and use of cereal crops, that is, corn and wheat as well as edible sunflower oil. After the insight into the statistical data, we can conclude that the sown areas of wheat from 2014 are in constant decline. The reduced sown area of wheat as well as the reduced production, leads to increased import of wheat.

Table 1. Planted areas (wheat) 2014-2020

Crop	**Year**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
Wheat	Planted area in hectares	76.861	73.979	79.898	72.965	73.072	68.959	69.902
	Production in tons	287.954	201.218	306.433	200.112	241.106	239.916	246.031
	Kg per hectare	3.755	2.754	3.838	2.746	3.396	3.485	3.527



Graph 1. Areas and production of wheat in the Republic of North Macedonia (2014-2020)

In accordance with the data presented in the table regarding the areas and production of wheat, we can conclude that since 2014, the sown areas of wheat in hectares are gradually **reducing**, but there is also a **reduction** in wheat production in tons. On the other hand, the import of wheat is increasing and we can notice that in the following table.

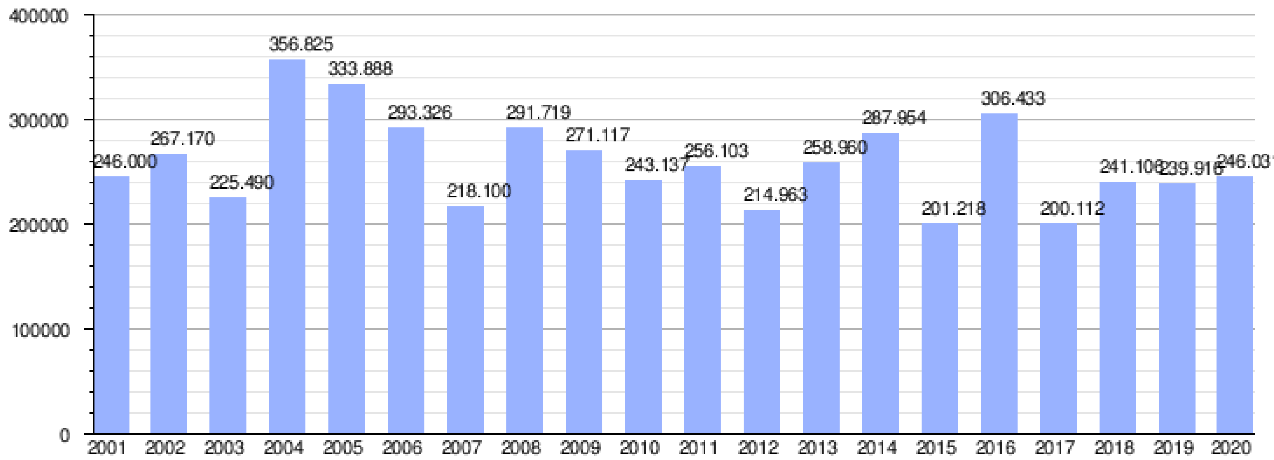
Table 2. Value and quantity of imported wheat in 2017 - 2021 (in euros and denars)

Import according to tariffs	**Wheat/kg/thousand**	**Value in denars**	**Value in euros**
2017	66.265,000	1.858.413,000	30.178,000
2018	80.803,000	2.210.416,000	35.935,000
2019	75.218,000	2.138.089,000	34.763,000
2020	82.778,000	2.301.391,000	37.318,000
2021	80.241,000	2.247.127,000	36.473,000

Table 3. Quantities of items for personal consumption, by type of household, in kg, by years

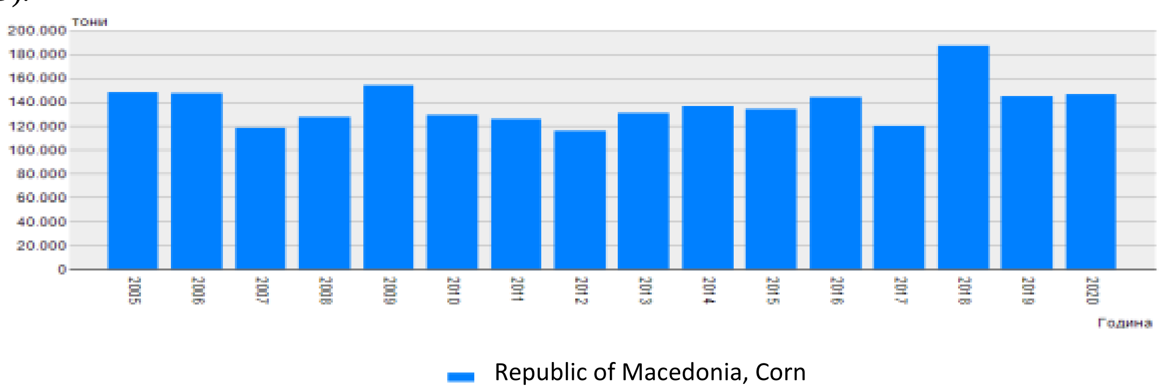
Year	**2016**	**2017**	**2018**	**2019**	**2020**
	Total	**Total**	**Total**	**Total**	**Total**
	average per household	**average per household**	**average per household**	**average per household**	**average per household**
Wheat in grains, packaged	0,6	1,3	1,4	1,4	1,1
Wheat flour type 500	81,2	45,3	54,7	61,3	31
Wheat flour type 400	25,2	44,6	19,5	30,9	50,3

In the Republic of North Macedonia, same as in 40% of the world, wheat is used as one of the basic products for nutrition of the population. Its processing into other products such as bread is crucial to provide enough food for the entire population (Ministry of Finance of the Republic of North Macedonia (2020) *Prices for import-source of cereals*, Skopje: MF.)



Graph 2. Wheat production in the Republic of North Macedonia (in tons)

The graph above shows the production of wheat in tons for individual years. According to the data, the largest quantity of wheat, more than 350.000 tons, was produced in 2004, while the lowest quantity was produced in 2015 or slightly more than 200.000 tons. In 2020, slightly more than 240.000 tons of wheat were produced in North Macedonia. In addition to production, for the country to meet the demand, it additionally imports wheat. The total import of wheat in the period 2008-2011 ranges from 30.190 tons in 2011 to 55.663 tons in 2009 (the average for the period 2008-2011 is approximately 44.000 tons of wheat). The import of wheat from Serbia in the same period 2008-2011 ranges from 26.404 tons or 87.5% of the total import, up to 52.595 tons or 94.5% of the total import. In addition, 71.300 tons of wheat were imported in 2018, and almost 80.000 tons were imported in 2019. In the period 2007-2018, the import of mercantile wheat and flour averaged 110.130 tons, which represents 32% of the total needs of the milling industry (State Statistical Office, Republic of North Macedonia in Figures, 2005 - 2020, Skopje: SSO).



Graph 3. Production of cereal, industrial and vegetable crops in the Republic of North Macedonia (in tons)

According to the presented statistical data, the largest production of corn in the Republic of North Macedonia was in 2018 with more than 185.000 tons while the least produced quantity of

wheat was in 2012, i.e., less than 120.000 tons (State Statistical Office, Republic of North Macedonia in Figures, 2005 - 2020, Skopje: SSO).
North Macedonia is positioned almost at the bottom of the group of ten Balkan countries according to average corn yields, according to the data from the Food and Agriculture Organization of the United Nations. According to the FAO, this situation is due to poor agricultural practices and technologies, which are far below the level of agricultural practices in the countries of the region. Corn is important for the production of domestic animal feed, which will especially impact on the production of milk and on the reduction of the imports of milk and dairy products.
The research of the FAO shows that with the domestic production of animal feed, Macedonia will save approximately 11.3 million euros, money that is spent annually on corn imports. Approximately 50 percent of the total domestic needs for animal feed are covered by imports, of which 35 percent include corn, which accounts for the largest percentage of animal feed. In 2020, weekly corn imports fell to their lowest level since the beginning of the marketing year on 1 July and amounted to 99.150 million tons, of which more than half are imported from Belgium (53.035 million tons). The total imports of that grain have now reached 3.6 million tons, or 17 percent less than last year. Brazil remained the top supplier with 2.36 million tons, followed by Ukraine with 798.991 tons and Serbia with 229.073 tons.
Sunflower is the second most widespread industrial crop after tobacco, with an average production area of 4.300 hectares and an annual production of approximately 6.688 tons that covers only about 7% of the needs for edible oil, while the rest is supplemented by imports. The relatively low purchase price of sunflower seeds, the low yields and the huge competition with cheap raw sunflower oil make the crop unprofitable and unattractive for the producers.
As with other raw materials, this raw material is a basic food product. The factory Brilijant in the Republic of North Macedonia currently covers 60% of the production of sunflower oil in the country. The import of sunflower oil is therefore extremely important if one considers the annual consumption of sunflower oil per capita. Namely, today sunflower oil reaches a huge retail price precisely as a result of the military conflict in Ukraine and the growth of world markets (Ministry of Agriculture, Forestry and Water Economy 2014)
Wheat, corn and sunflower oil are basic food products not only in the Republic of North Macedonia but all over the world. More than 40% of the entire population in the world get their basic food from these products. The military conflict in Ukraine and the ban on the export of basic raw materials from Ukraine, a country that is a major factor in the supply of these raw materials to the world market, has caused multiple increase in prices. In addition, many countries from which North Macedonia has imported some of these raw materials so far, have already banned or are in the process of banning their export. At this moment, in whole Europe, and especially in several separate countries, there is a real possibility of shortage of some of these raw materials, especially sunflower oil (Ministry of Agriculture, Forestry and Water Economy, 2014).
The Republic of Macedonia is a net importer of grain, because the country does not meet domestic needs neither for “bread” grain, nor for other types used for the production of animal feed. Our production is relatively unstable and is a result of the non-standard, old technology that is being used, as well as the weather conditions, and since the yields are below 4 tons per hectare, this is an indicator of poor varieties used for seed materials. In North Macedonia, about 250 thousand tons of wheat are produced annually and an additional 80 thousand tons per year are imported. According to this, about 30% of the needs are imported, including the quantities

for consumption and reserves. Almost all our wheat imports are from Serbia or the total imports of all cereals from Serbia account for 85% of these 80 thousand tons. The remaining part of the imports is insignificant to Russia, and we do not import wheat from Ukraine.

Conclusion

The dedicated approach to agriculture and rural areas in the previous strategic period resulted in terminating or mitigating the negative tendencies of decreasing the physical volume of production as a consequence of the lack of sustainable agricultural policy before 2007, and gradually moving towards positive growth trends, more pronounced in the subsectors with comparative advantages. In this period, with the increase of the support and the investments by the country, the Macedonian agricultural sector shows positive performances of the average growth rate of the agricultural GDP of 3.2%, increased employment in the sector and labor productivity and continuous annual growth of exports which reached half a billion euros a year.

After setting favorable conditions for the functioning of the business entities in the sector through the provided financial support, the beneficial social, tax and credit policies, the complete utilization of the development potential of the agricultural sector, the increase of competitiveness and to a large extent the very development of rural areas in the forth coming period, depend on the change and improvement of the extremely unfavorable structure of the agricultural production. The coping with structural problems will be focused on active treatment of the main cause of such conditions - small and fragmented land plots, insufficient technical and technological equipment resulting from a long period of disinvestment, inadequate variety and species production structure and poor age and gender structure of the rural population.

The crisis and the state of war that currently takes place in Ukraine, have contributed to a significant increase in the prices of cereals and sunflower oil worldwide. In addition to this, a separate problem is the fact that Ukraine is one of the largest producers of cereal crops (especially wheat and corn), as well as sunflower, necessary for the production of cooking oil, and given that the export and production of these goods is almost impossible due to the situation, one can easily conclude that the rise in prices (oil, bread, cereals) will last as long as the state of war in Ukraine lasts.

As for the Republic of North Macedonia, the research shows that the country does not produce enough and it also doesn’t have enough stocks to deal with similar crises in the future and same as most of the Balkan countries, it is largely dependent on other countries, i.e., on the import of cereals. Hence, the country needs an appropriate strategy for the development of agriculture, to a large extent this refers to measures for the possibility of larger plantations for cereals, especially wheat. In addition to this, policies and strategies are required that will use analyses from the past, and which will refer to sufficient replenishment of commodity reserves in order to prevent the possible emergence of another significant shortage of basic food products such as bread or cooking oil on the market.

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RESEARCH ON THE EXTENSION OF THE VALIDITY PERIOD FOR BOILED - SLICED MEATS, MANUFACTURED BY THE NATIONAL MANUFACTURER IN ACCORDANCE WITH THE NORMATIVE ACTS IN FORCE

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Abstract

Currently, the issue of food quality and safety has become a key factor in meeting consumers' requirements. Samples from the category of sliced boiled sausages were inspected: Parizer "Doctorskaia" boiled, of high quality, sliced, non-edible artificial casing, packed in a protective(modified) atmosphere, with three different manufacturing dates (three batches). The investigations were based on complex research in dynamics of physico-chemical and microbiological quality indices, to study the possibility of extending the shelf life up to 30 days at t°C = 0 + 6°C and the relative humidity of the air max.- 75%, of meat products, manufactured according to the company standards and the technological instructions in force of the meat processor. Thus, there were obtained very good results, related to the organoleptic characteristics physicochemical and microbiological indices since they have not changed considerably over time, remaining within the normative requirements even at the end of the shelf life, not affecting the quality of the product.

Keywords: *Parizer „Doctorskaia", normative acts, organoleptic indices, physico-chemical, microbiological.*

Introduction

In the current socio-economic environment, quality has become a strategic tool of global enterprises' management as well as a determining element of their competitiveness. However, by making products for large communities, specialists and producers in the food industry become responsible for the health of the population, participating in one of the most effective ways to health protection and promotion (Nicoleta Stanciuc, 2009; Pop Cecilia, 2011). Food quality and sanitation have direct effects on life, and the issue of food quality is always in the center of attention of the bodies set up to ensure its safety and to protect the interests of consumers and most of all their health. Today, quality has become a key factor in meeting consumers' demands. Of particular interest is the manufacture of products with high organoleptic characteristics which have a long term sales perspective, with long shelf life, without changing the quality of the product (Laslo C. et al., 2008, Oprea A., Vasilica R., 2010). Maintaining the prescribed quality of foodstuffs, including meat products, refers to their ability to retain their original characteristics over time (qualitative and quantitative) and to their resistance to storage, handling and transport. The stability of foodstuffs is limited in time, being determined by the substances in the composition of the products with varying degrees of lability, both in terms of interactions with other constituents and in terms of environmental factors, but which must be ensured throughout the food chain from producer to consumer, (Banu C. et al., 2007; Pop Cecilia, 2004).

Material and Methods

The research on the topic of the bachelor's thesis was conducted in the Agri-Food Testing Laboratory of the Center for Applied Metrology and Certification (CMAC). Samples from the category of sliced boiled sausages were investigated: Parizer *Doctorskaia* packaged in non-edible artificial casing. 3 product samples were taken in part from three different manufacture dates (three batches) - 21.09.21; 22.09.21; 23.09.21. The research was based on the complex study in dynamics of physico-chemical and microbiological quality indices in order to establish the shelf life: 30 days at t ° C = + 0 + 6 ° C and the relative humidity of air of max. = 75%. Thus, quality indexes were determined - organoleptic, physico-chemical, harmlessness and rancidity indices, for all samples taken during the storage period, in accordance with the normative acts in force - GD no. 624/2020 on the approval of quality requirements for meat preparations and products.

The protein was determined by the Kjeldhal Method - the classic method for determining proteins, which is based on the principle of determining total nitrogen and converting it into protein equivalent using the appropriate multiplication factor. The sample to be analyzed is mineralized by heating with concentrated sulfuric acid in the presence of a catalyst.

The fat content was determined by the Soxhlet method - the fat in the research sample is extracted with organic solvents and after removing the extraction solvent, it is weighed and expressed as a percentage. In order to ensure complete extraction, the sample is previously subjected to a heat treatment at a moderate temperature through which the dehydration and destruction of the protein membrane or film of the microstructure in which it is embedded is achieved.

The content of phosphates and nitrites were determined by spectrophotometry, and the content of chlorides, starch by volumetric determinations.

Results and Discussion

The meat product, *boiled Parizer "Doctorskaia*", of high quality, sliced, packaged in modified atmosphere, initially met the requirements of the normative documents in force (GD no. 624 and SF) and presented clean, dry slices of parizer without damage. The appearance in the cut shows a composition of pink color, finely chopped, evenly mixed, without gaps and gray spots. Taste and smell were characteristic of the given type of product, with nuances of spices. Elastic consistency. Pleasant taste, suitably salted, without foreign taste and smell. At the end of the storage period, the organoleptic characteristics of the product did not change. The packaging was airtight, the walls were dry, no broth within the packaging, and the slices of parizer had a clean, dry, damage-free, non-sticky surface. In the cut without color changes, without gaps and gray spots. Pleasant odor, characteristic of the product type, no foreign odor. Pleasant taste, suitably salted, slightly spicy, without foreign taste and smell. Therefore, at the end of the storage period (30 days), the organoleptic properties of *Parizer "Doctorskaia"* boiled, of high quality, sliced, packaged in modified atmosphere, did not change: the appearance, color, taste and smell remained the same. As a result of the quality indices investigations - the mass fraction of protein, fat, starch, chlorides, phosphates and nitrites, in the samples of cooked meat - *Parizer "Doctorskaia*", sliced, of high quality, packaged in modified atmosphere- there was found their compliance with the requirements of the regulations, which indicates that the products can be consumed within the period of validity initially established.

Table 1. Quality indices in Parizer „Doctorskaia”

Examened indices	**Test method**	**Admissible requirements (HG nr.624/SF)** **Obtained results** *Parizer „Doctorskaia”, sliced, h/q* (n=3) **X ± Sx**	**Conformity**
Mass fraction of protein, %, min.	-ISO 937	**(8 / 10)** 11,63 ± 0,088	Conformable
Mass fraction of fat, %, max.	- SM SR ISO 1443:2012	**(35,0 / 35,0)** 33,16 ± 0,10	Conformable
Mass fraction of starch, %, max.	-GOST 10574	**7,0/ Not allowed** Not found	Conformable
Mass fraction of chlorides, %, max.	-GOST 9957-73	(1,0-3,5 / 3,0) 2,0 ± 0,057	Conformable

Thus, it should be noted that the indices related to the mass fraction of phosphates and the mass fraction of nitrites also showed results that fall within the requirements of the regulations in force for this category of products (Table 2).

Table 2. Quality indices in Parizer „Doctorskaia”

Examined indices	**Test method**	**Admissible requirements (HG nr. 229/SF)** **Obtained results** *Parizer „Doctorskaia”, sliced, c/s*, pack.. MAP, (n=3) **X ± Sx**	**Conformity**
Mass fraction of phosphates, mg/kg, max	ISO 13730	**5000/5000** 4298,33 ± 10,928	Conformable
Mass fraction of nitrites, mg/kg, max	GOST 8558.1-78	**150/50** 40,830 ± 0,065	Conformable

Thus, following the initial investigations, it has been established that the products correspond to the requirements of the normative documents: RT “Quality requirements for meat preparations and products” GD No. 624, GD no. 221 of 16.03.2009 "Rules on microbiological criteria for food" and company standards for this category of products. It has been found that the products can be stored for further research. Subsequently, at intervals determined by time, in the samples from three manufacture dates: 21.09.2021; 22.09.2021 and 23.09.2021, the physico-chemical index was analyzed in dynamics - the mass fraction of humidity, %.

Table 3. Dynamics of physico-chemical indices - *humidity,%* in the samples investigated during the storage period (39 days)

Nr.	**Sample**	**Storage period, days**	**Normative requirements HG nr. 624/SF**	**Humidity, % SM SR ISO 1442:2014**		
Manufacture date				21.09.2021	22.09.2021	23.09.2021
***Parizer* sliced, packaged in modified atmosphere (MAP):**						
1	***„Doctorskaia”*, h/q**	Iniţial date	max. 75,0/70,0	**68,2**	**67,4**	**69,2**
		10		**68,2**	**67,4**	**69,0**
		20		**68,1**	**67,3**	**68,9**
		30		**68,0**	**67,3**	**68,9**
		39		**68,0**	**67,0**	**68,8**
Difference between the initial date and 39 days of storage				**0,2**	**0,4**	**0,4**
X ± Sx				68,10±0,082	67,28±0,134	68,96 ±0,124

It can be seen that for *Parizer "Doctorskaia*", sliced, packaged in modified atmosphere, the initial humidity ranged from 67.40% - 69.2% at all reference dates, values that fall within the permissible limits of regulatory requirements in force for samples with different manufacture dates. At the end of the storage period, after 39 days, these values were between 67.0% - 68.8%, these data show an insignificant decrease in the mass fraction of humidity at the end of the storage period. This process is characteristic to meat products during storage period. However, at the end of the storage period the samples had average values of humidity between 67.28 - 68.96%, which correspond to the requirements of the normative document GD no. 624 of 19.09.2020 and the Company Standard for these products. The change in humidity values, with a slight insignificant decrease of 0.2 -0.4% at the end of the storage period, for *Parizer "Doctorskaia*", sliced, h/ q, packaged in modified atmosphere, did not affect the samples' quality. The decrease in moisture did not affect the consistency of the samples or the appearance of the slices until the end of the storage period. Along with the humidity content, which may increase or decrease during storage period, rancidity indices were also appreciated, the determination of which is necessary during the establishment of the shelf life (Table 4-6).

Table 4. Dynamics of physico-chemical indices - *peroxide index*-in the samples investigated during the storage period (39 days)

Nr.	**Sample**	**Storage period, days**	**Peroxid index, mmol/kg $^{1}/_{2}$ O, MI 2586 p.7**		
Manufacture date			21.09.2021	22.09.2021	23.09.2021
***Parizer* sliced, packaged in modified atmosphere (MAP):**					
1	***„Doctorskaia”*, h/q**	Iniţial date	**1,62**	**1,58**	**1,58**
		10	**1,69**	**1,65**	**1,66**
		20	**1,78**	**1,74**	**1,75**
		30	**1,86**	**1,80**	**1,82**
		39	**1,93**	**1,86**	**1,89**
Difference between the initial date and 39 days of storage			**0,31**	**0,28**	**0,31**
X ± Sx			1,776±0,095	1,726±0,092	1,740 ±0,101

The peroxide index evaluated for *Parizer "Doctorskaia*", sliced, h / q and packaged in modified atmosphere, initially shows variations between 1.58 - 1.62 mmol / kg, at all reference data, values that fall within the permissible limits of the regulatory requirements in force for samples with different manufacture dates. At the end of the storage period, after 39 days, these values were between 1.86 - 1.93 mmol / kg, data that show an insignificant increase of the peroxide index at the end of the storage period with average values between 1,726 - 1,776 mmol / kg. This process is characteristic to meat products during the storage period and it did not exceed a maximum of 10 ½ O mmol / kg, according to the admissible requirements of the data in the specialized and scientific literature. The change in the values of the peroxide index, with a slight insignificant increase of 0.28 -0.31 mmol / kg at the end of the storage period for *Parizer "Doctorskaia*", sliced, h /q, packaged in modified atmosphere, did not affect the samples' quality. The appearance, consistency, taste and smell did not change until the end of the storage period.

Table 5. Dynamics of physico-chemical indices - *acidity index*, in the samples investigated during the storage period (39 days)

Nr.	**Sample**	**Storage period, days**	**Acidity index, mg KOH/g MI 2586 p.8**		
Manufacture date			21.09.2021	22.09.2021	23.09.2021
***Parizer* sliced, packaged in modified atmosphere (MAP):**					
1	***„Doctorskaia”*, h/q**	Initial date	**1,28**	**1,18**	**1,26**
		10	**1,35**	**1,26**	**1,32**
		20	**1,42**	**1,33**	**1,40**
		30	**1,47**	**1,38**	**1,49**
		39	**1,52**	**1,42**	**1,56**
Difference between the initial date and 39 days of storage			**0,24**	**0,24**	**0,30**
X ± Sx			1,408±0,077	1,314±0,078	1,406±0,099

The acidity index estimated for *Parizer "Doctorskaia*", sliced, h/q and packaged in modified atmosphere, initially shows variations between 1.18 - 1.28 mg KOH / g, at all reference data, values that fall within the limits permissible by regulatory requirements in force for samples with different manufacture dates. At the end of the storage period, after 39 days, these values were between 1.42 - 1.56 mg KOH / g, data which show an insignificant increase in the acidity index at the end of the storage period with average values between 1,314 - 1,408 mg KOH / g. This process is specific to meat products during storage that didn't exceed a maximum of 4 mg KOH / g, the permissible requirements of the data in specialty and scientific literature. Changing the values of the acidity index, with a slightly insignificant increase of 0.24 -0.30 mg KOH / g. at the end of the storage period, for *Parizer "Doctorskaia*", sliced, h/q, packaged in modified atmosphere, did not affect its quality. Taking into account the fact that the packaging of *Parizer "Doctorskaia*", sliced, h/q, was made in modified atmosphere (MAP), i.e. natural gas (N_2; CO_2) was used, the same as we breathe, but at concentration levels. In order to control the bacteria that cause damage to the products, the carbon dioxide content, %, was also assessed in the samples examined during the storage period (39 days).

Table 6. Dynamics of *carbon dioxide* content,% in the samples investigated during the storage period (39 days)

Nr.	**Sample**	**Storage period, days**	**Normative requirements IT**	***Carbon dioxide, % Instruction nr. 145.277***		
Manufacture date				21.09.2021	22.09.2021	23.09.2021
***Parizer* sliced, packaged in modified atmosphere (MAP):**						
1	***„Doctorskaia”,* h/q**	Iniţial date	max. 30	**30,0**	**30,0**	**29,0**
		10		**30,0**	**30,0**	**29,0**
		20		**30,0**	**29,0**	**28,0**
		30		**29,0**	**28,0**	**27,5**
		39		**29,0**	**28,0**	**27,5**
Difference between the initial date and 39 days of storage				**1,0**	**2,0**	**1,5**
X ± Sx				29,60 ±0,450	29,0 ±0,816	28,20±0,619

Thus, the results presented in the table show us that the carbon dioxide content, %, for *Parizer "Doctorskaia*", sliced, h /q and packaged in modified atmosphere, initially shows variations between 29.0 - 30.0%, at all reference data, values which fall within the permissible limits of the regulatory requirements in force for samples with different manufacture dates. At the end of the storage period, after 39 days, these values were between 27.5 - 29.0%, the data show an insignificant decrease in the carbon dioxide content, %, at the end of the storage period, with average values between 28.2 - 29.60%. The change in carbon dioxide content, %, with a slightly insignificant decrease of 1.0 - 2.0%, at the end of the storage period for *Parizer “Doctorskaia*”, sliced, h/q packaged in modified atmosphere did not affect the samples’ quality.

The microbiological investigations were carried out in accordance with the normative documents in force GD no. 221 of 16.03.2009 "Rules on microbiological criteria for food" and CS for the given product both at the beginning of the shelf life and over 10, 20, 30, and 39 days. During the storage period, Listeria monocytogenes, Bacteria coliforme, E. Coli, yeast and mold were not detected in the examined meat product. At the same time, during the storage period of the meat product under study *Parizer "Doctorskaia"*, sliced, h /q, packaged in modified atmosphere, there is a gradual increase in the amount of MMAFA. In the samples kept at the regulated temperature the growth of mesophilic-aerobic and optionally anaerobic microorganisms practically does not differ from the samples kept at advanced temperature. But in both cases the growth of microorganisms does not exceed the limit of one degree, and the obtained values are below the allowable limit. To a large extent, the minimal changes in the studied samples are due to the fact that the carbon dioxide content in the package has decreased very little. The evaluation of the microbiological indices shows the lack of their negative dynamics during the 39-day storage process for *Parizer "Doctorskaia*", sliced, h /q, packaged in modified atmosphere, which presents a positive criterion in justifying the shelf life of the product under investigation. This fact demonstrates the possibility of setting the 30-day shelf life, as requested by the local manufacturer, for *Parizer "Doctorskaia*", sliced, h /q, packaged in modified atmosphere, provided they are stored in a cold room with a temperature of 0 to 6^0 C and relative air humidity of 75%.

Conclusions

The stability of the organoleptic indices during the storage period (30 days) indicates good quality of the product, as there were no changes in the taste qualities or in the appearance of the slices of the Parizer.

The physico-chemical parameters have not changed considerably over time, remaining within the normative requirements even at the end of the shelf life. The insignificant changes in the peroxide index, the acidity index, the decrease in the amount of moisture did not affect the consistency of the samples or the appearance of the slices. The product quality was not affected.

Throughout the storage period of the product (30 days), the microbiological indices corresponded to the requirements of the normative documents, and the obtained results show us the lack of negative dynamics of the microbiological indices during the 30-day storage process, for *Parizer "Doctorskaia*", sliced, h /q, packaged in modified atmosphere, which gives us a positive criterion in justifying the shelf life of the product under investigation.

The research shows the possibility of establishing the validity period of 30 days, requested by the local producer, for *Parizer "Doctorskaia*", sliced, h/ q, packaged in modified atmosphere, provided it is kept in the cold room at a temperature from 0 to at 6^0 C and a relative humidity of 75%.

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ESTIMATING THE MAGNITUDE OF FOOD LOSSES AND WASTE GENERATED IN PERU

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Abstract

The last decade has witnessed a rapidly growing number of studies on the quantification of food loss and waste (FLW) in various regions around the globe, but little work has been done to have food waste data in Latin American countries. This paper contributes to addressing this knowledge gap by examining the magnitude of FLW along the whole food supply chain (FSC) in Peru. The methodological approach was based on the top-down mass flow analysis at all steps of the FSC for the 2007–2017 period, including different food commodity groups (CGs), such as cereals, roots and tubers, oil seeds and pulses, fruits, vegetables, meat, fish and seafood, and milk. Results show an annual average of FLW of 12.8 million tonnes, which represents 47.76% of the national food supply. Regarding per capita quantities, the average amount of FLW was 426.56 kg per year when considering the entire FSC, and 67.34 kg per year when considering only the consumption step. This study suggests which steps of the FSC and CGs are the most promising targets for FLW reduction strategies in Peru.

Keywords: *Food security, Food policy, Food supply chain, Organic waste, Latin America.*

Introduction

The last decade has witnessed a rapidly growing number of studies on the quantification of food loss and waste (FLW) in various regions around the globe. This trend is due to FLW importance in achieving food and nutrition security (Irani et al., 2018; Parfitt et al., 2010; Abbade, 2020), to help tackle food poverty (Galli et al., 2019), and to improve the environmental footprint of countries (Chen et al., 2020; Read et al., 2020). There is now a broad consensus on two key issues that need to be addressed: the need for research to focus on generating more data on the amount of FLW outside the current focus area (U.S. and Europe) (Koester et al., 2020; Xue et al., 2017), and the need to fully capture estimates of FLW along the complete food supply chain (FSC) (Skoet et al., 2020). Accordingly, this research aims to contribute to the current scientific literature on FLW in Latin America by calculating the distribution of FLW along the FSC in Peru.

Definitions of "food loss" and "food waste" are not universal, which has been extensively discussed by Chaboud and Daviron (Chaboud and Daviron, 2017). The distinction between both terms is because the stakeholders involved in the FSC. Food losses occur at production, post-harvest, and processing steps. This refers to the decrease in the quantity or quality of food resulting from food suppliers' decisions and actions in the chain. Instead, food waste decreases the quantity or quality of food at the end of the food chain, resulting from decisions and actions by retailers, food services, and consumers (Parfitt et al., 2010; FAO, 2020; Gustavsson et al., 2011). For this study, however, the masses of food lost and wasted are analyzed together because

the purpose of this paper is not to identify the causes of FLW definitively; instead, it is to determine the total quantity of FLW along the FSC.
The estimation of FLW was based on the top-down mass flow analysis, following Dal’ Magro and Talamini’s methodological approach (Dal´Magro and Talamini, 2019). They used this methodology to estimate the magnitude of the FLW in the Brazilian FSC, in the same line as Oelofse and Nahman (Oelofse and Nahman, 2013). They calculated the quantity of FLW in the FSC in South Africa. Mass flow analysis is based on the law of the conservation of matter. It has become an important tool in waste management because it allows comparing all inputs, stocks, and outputs of a process (Brunner and Rechberger, 2004). Adelodun and Choi (2020) recently used the same methodological approach to study food waste along the supply chain in Korea.

Materials and methods

The Peruvian-specific data, including domestic food production, food trade (import and export food quantity), and stock variation, were obtained from FAO Food Balance Sheets (FAOSTAT, 2020). Given that Peru’s latest accessible data is dated from 2007 until 2017, the status of FLW over eleven years was examined. FAO Food Balance Sheets have been used to quantify FLW in many investigational studies, among them the most highly cited studies on FLW to date: Gustavsson et al. (2011) and Kummu et al. (2012).
On this basis, the FSC was divided into five steps, including (i) agricultural production, (ii) post-harvest handling and storage, (iii) processing and packaging, (iv) distribution, and (v) consumption at the household level. On the same basis, the following seven food commodity groups (CGs) were considered: (i) cereals, (ii) roots and tubers, (iii) oil seeds and pulses, (iv) fruits and vegetables, (v) meat, (vi) fish and seafood, and (vii) milk.
Figure 1 shows the variables used to calculate the amounts of mass in each step of the FSC, from agricultural production to consumption. The agricultural production step is represented by the quantity of food production, which is summed up to the quantity of food in stock at the post-harvest handling and storage step.

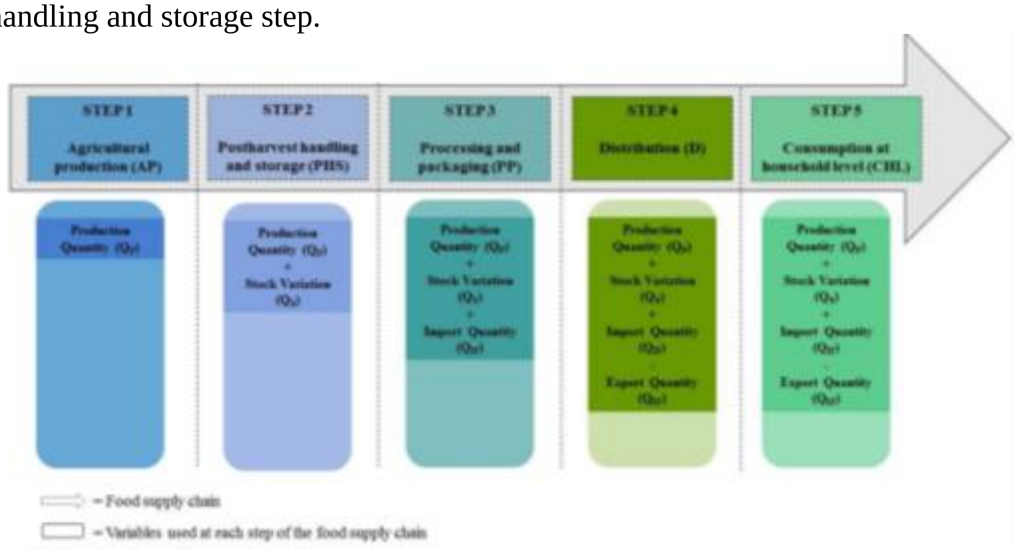

Figure 1. Variables at each step of the food supply chain (FSC). Source: own work based on Dal’ Magro and Talamini (2019).

The import quantity is inserted at the processing and packaging step because part of the imported foods enters the processing step. The domestic supply of food in Peru is represented by the distribution and consumption at household level steps. For this reason, the export quantity variable was excluded from the count. With these variables, the FLW was quantified at each step of the FSC, as shown in Figure 2.

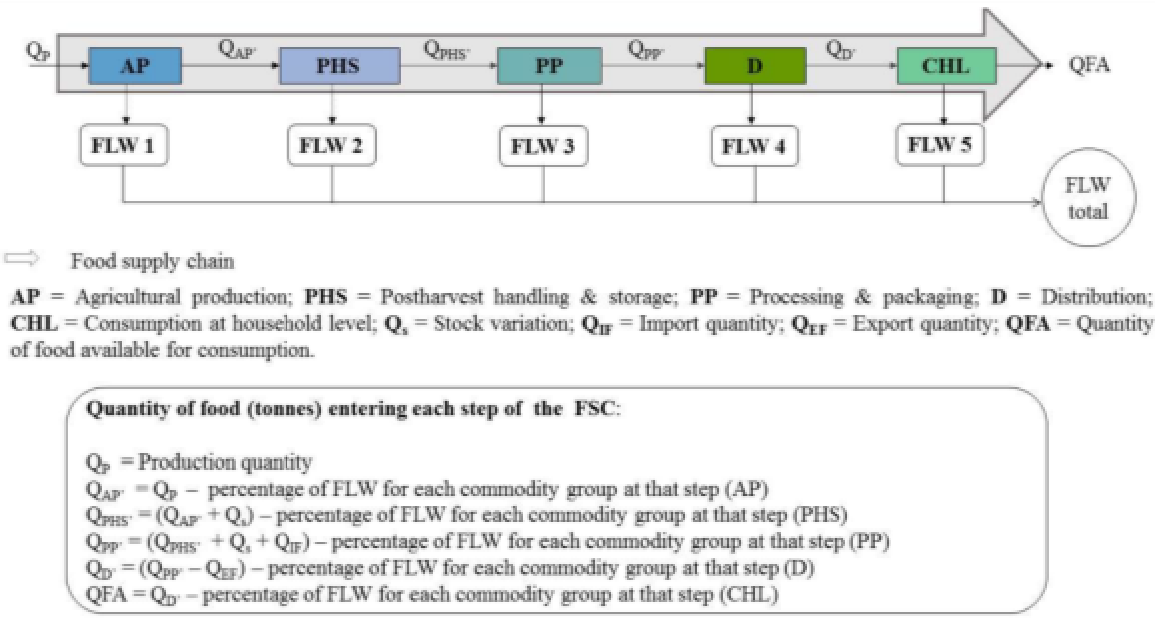

Figure 2. Food loss and waste (FLW) in each step of the food supply chain (FSC). Source: own work based on Dal' Magro and Talamini (2019).

The percentages of FLW per mass of food that enter each step of the FSC, by CG, correspond to the coefficients of FLW for Latin American countries, following Gustavsson et al. estimates (Gustavsson et al., 2011). The same percentages were considered for all analyzed years. Thus, the FLW calculated at each step of the FSC was subtracted from the previous step's accumulated food quantity. After quantifying FLW for each step of the FSC, the conversion factors were applied to determine the edible mass. The waste percentages and the conversion factors calculated by Gustavsson et al. (2013) have also been used in other studies on FLW (see Oelofse and Nahman (2013); Adelodun and Choi (2020); Reutter et al. (2017)).

Results and discussion

The total amount of FLW across the entire FSC was 10.7 million tonnes during 2007 and 14.4 million tonnes during 2017. Similarly, the average amount of FLW in the 2007–2017 period was 12.8 million tonnes per year, and the largest was generated during the pre-consumption steps (10.8 million tonnes per year or 84.21%). This quantity accounted for 47.76% of the average value of the domestic food supply (26,755,000 tonnes).
The Food Bank of Peru (Banco de Alimentos Perú) considers that 9 million tonnes of food are wasted per year (BAP, 2018). However, it is unknown whether food losses are considered in the count and the accounting methodology applied. Similarly, results are hard to compare since other scientific studies in Latin America focus only on specific steps of the FSC. Only the Dal' Magro and Talamini (2019) study reported that the quantity of FLW in the entire Brazilian FSC reached

82.12 million tonnes per year during 2007–2013. This equates to 42% of its domestic food supply’s average value. During the period 2007–2017, the total amount of FLW generated in Peru was 3.3 million tonnes in agricultural production, 2.3 million tonnes in post-harvest handling and storage, 3.5 million tonnes in processing and packaging, 1.7 million tonnes in distribution, and 2 million tonnes in consumption. Figure 3a,b illustrates that in 2007 and 2007–2017, approximately 53% of total FLW corresponds to the agricultural production (25%) and processing and packaging (28%) steps.

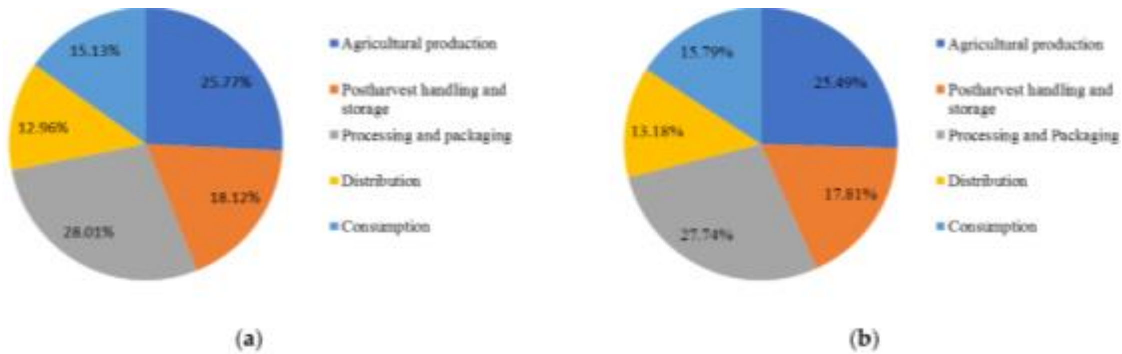

Figure 3. Percentages of food loss and waste (FLW) by step of the food supply chain (FSC), during (a) the year 2007 and (b) the 2007–2017 period.

These findings are similar to those reported in previous studies from upper-middle-income countries, such as South Africa (Oelofse and Nahman, 2013) and Brazil (Dal´Magro and Talamini, 2019). In both countries, the largest FLW estimate also occurred in the agricultural production and processing and packaging steps. It was estimated at 26% and 27% for South Africa, respectively (during 2007). The Brazilian case was estimated at 26% and 24%, respectively (during the 2007–2013 period).

According to Gustavsson et al. (2011), FLW in the so-called developing countries are associated with financial, managerial, and technical limitations in harvesting techniques, storage, and cooling facilities in difficult climatic conditions, infrastructure, packaging, and marketing systems. Various studies conducted in Peru can help to analyze the determinants for food waste in Peru during agricultural production and processing and packaging steps.

They are related to the difficulties faced by farmers during production because of unfavorable climatic conditions, pests, and diseases (Delgado et al., 2017; Velasco et al., 2019); the market inconsistency and product rejections by agri-food corporations due to quality requirements (Tobin and Glenna, 2019); the immense diversity of supply channels in formal and informal food chains (Navarro et al., 2015; Mercado, 2018); the lack of cold chain logistics in the agri-food and fisheries sectors (Freon et al., 2014). Another determinant is that roads are asymmetrically distributed across regions in Peru, which decreases market access for producers and processors (for a complete discussion, please see Aguirre et al. (2018) and Martincus et al. (2013)).

Figure 4a illustrates the contribution of each CG to total FLW in Peru for the year 2007. The group of fruits and vegetables, with roots and tubers, contributed 65.13% of the total FLW flow. The groups of cereals and fish totalled 28.07%, and the group of pulses, meat, and milk represented 6.8% of FLW in the FSC. The same analysis for the period 2007–2017 is illustrated in Figure 4b. It is also highlighted that the groups of fruits and vegetables, together with roots and tubers, accounted for 66.03% of the FLW flow. In comparison, the groups of cereals and fish accounted for 26.41%, and the groups of pulses, meat, and milk accounted for 7.56%.

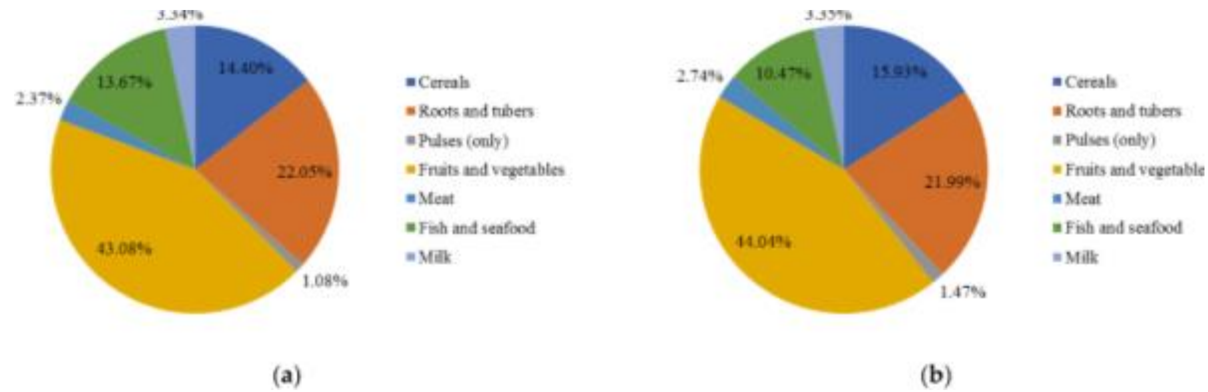

Figure 4. Percentages of food loss and waste (FLW) by commodity group (CG), during (a) the year 2007 and (b) the 2007–2017 period.

These results highlight the most wasted food groups; at the same time, Peru faces nutritional problems. In Peru, only 1 out of 20 individuals consumes the recommended amount of fruits and vegetables per day (Arribas-Harten et al., 2015). Kovalskys et al. (2019) also reported that the mean consumption of fruits, vegetables, grains, and fish in Peru is 117.8 g/day, 88.3 g/day, 14.7 g/day, and 28.9 g/day, respectively. However, considering the literature, these amounts are below the minimum requirement evidenced to decrease chronic diseases’ relative risk (200 g/day of fruits and fish and seafood, 300 g/day of vegetables, 100 g/day of grains and beans).

The per capita amount of food waste by consumers in Peru was 57.41 kg in 2007 and 85.49 kg in 2017. On average, 67.34 kg of food was generated annually during the period analyzed. Gustavsson et al. (2011) estimated for the year 2007 that the per capita food waste by consumers in Latin America reaches a value of 25 kg/year, while this rises to 95–115 kg/year in Europe and North America. The authors also reported that the sum of food loss and food waste in Latin America rises to 225 kg/inhabitant. Much more FLW was reported in the Peruvian case, which indicates an amount equal to 379.41 kg/inhabitant during 2007, and 426.56 kg per inhabitant/year during the period analyzed (that being 326.08 kg corresponding to the edible portion of food). Therefore, it is suggested that Peru is probably among the Latin American countries with the highest level of FLW. This may be explained by the fact that Peru is the fifth-largest country by population in Latin America and is considered an upper-middle-income country, where the food industry accounts for almost 22% of the industrial GDP (USDA, 2019). Thus, trading standards, aesthetic requirements and regulations on food safety in the production, handling, and distribution of foods can cause significant amounts of FLW, as Koester et al. (2020) discussed.

Finally, Figure 5 summarizes the quantities of FLW concerning the domestic food supply in Peru from 2007 to 2017.

Figure 5. Representation of food loss and waste (FLW) concerning the domestic supply quantity (2007–2017).

Conclusion

The current study shows how much food is lost and wasted along the FSC in Peru. Data from 2007 to 2017 were analyzed, and the results show that, on average, Peru generates 12.8 million tonnes of FLW per year. This amount represents 47.76% of the national food supply. On a per-capita basis, when considering the entire FSC, the quantity of FLW is 426.56 kg per inhabitant/year (326.08 kg corresponding to the edible portion of food). Nevertheless, when considering only the consumption step, the quantity of FLW is 67.34 Kg per inhabitant/year. Accordingly, the results suggest that in Latin America, Peru is probably among the countries with the highest level of FLW because Gustavsson et al. (2011) have estimated that the per capita food waste by consumers in Latin America reaches a value of 25 kg/year, and the sum of food loss and food waste rises to 225 kg/inhabitant.

This study highlights which steps of the FSC and CGs are the most promising targets for FLW reduction strategies in Peru. Thus, the agricultural production and processing and packaging steps represent 53% of the average amount of FLW in the FSC. At the same time, fruits and vegetables and roots and tubers groups are responsible for the highest amount of FLW. These food groups account for 44% and 22% of the total, respectively. At the consumption step, the food groups presenting the highest amount of FLW are cereals (43%), fruits and vegetables (35%) and roots and tubers (12%).

This research has limitations that have been pointed out. Therefore, this paper provides a preliminary evaluation of the food waste generation in Peru and guidance to researchers seeking to collect primary data on FLW or to ensure robust support for decision making about FLW reduction. This paper also contributes to promoting knowledge of FLW in Latin America.

Acknowledgement

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ANTIFUNGAL ACTIVITY OF PLANT ESSENTIAL OILS TO THE FUSARIUM VERTICILLIOIDES ORIGINATED FROM GARLIC

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Abstract

Garlic (*Allium sativum* L.) is the second most cultivated *Allium* species worldwide. Many viral, fungal and bacterial diseases attack garlic plants and can be a limiting factor to garlic production. Fungal diseases control is mainly conducted by fungicides, which have detrimental impact on the environment. In this work, we explore the possibility of utilizing essential oils (EOs) as antifungal agents against a fungal isolate originated from symptomatic garlic cloves from Kraljevci locality, Srem District, Serbia, morphologically and molecularly identified as *Fusarium verticillioides.* EOs used in this study originated from several medicinal plants: Turkish pickling herb (*Echinophora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*) and myrtle (*Myrtus communis*). Minimum inhibitory concentrations (MIC) were determined by microdilution method in 96 well microtiter plates. Microtiter plates were incubated for five days at 28°C. The experiment was repeated four times with trifloxystrobin as a positive control. The lowest concentrations without visible growth were defined as the minimal concentrations inhibiting fungal growth. Fungal spores were washed from the surface of potato dextrose agar (PDA) and spore suspension was adjusted to a concentration of approximately 5.0×10^4 in a final volume of 100µl per well. The values of minimal inhibitory concentration (MIC) were carried out by Duncan’s multiple range tests. An analysis of variance was performed on MIC data for four EOs applied on *F. verticillioides*. A significance was evaluated at $p<0.05$. STATISTICA v.7 (StatSoft, Inc.) was used for statistical analyses. The results of the antimicrobial activity of EOs using microdilution method showed a wide range of antifungal activity against *F. verticillioides*. The basil EO proved to be the most potent one (MIC-0,325±5,10µg/mL), followed by oregano (MIC-0,775±0,05µg/mL), myrtle (MIC-5,5±0,05µg/mL) and Turkish pickling herb (MIC-55±5,10µg/mL). The data obtained here suggest that the selected EOs can be applied as inhibitors to prevent growth of the phytopathogenic fungus *F. verticillioides*.

Keywords: *Garlic, Essential oil, Antagonistic, Minimum inhibitory concentration.*

Introduction

Garlic (*Allium sativum* L.) has been cultivated for more than five thousand years as people recognized garlic's physiological, nutritional and health benefits. Nowadays, garlic is the second most cultivated *Allium* species worldwide, as is in Serbia. Many phytopathogenic viruses, fungi and bacteria pose a threat to garlic cultivation, and can be a limiting factor to garlic production. Management of fungal diseases is mainly conducted by fungicides, which have detrimental impact on the environment.

Dry rot disease of garlic is a postharvest problem that can result in a severe attack of up to 30% of the garlic bulbs (Mondani et al., 2021). The disease symptoms are visible during the storage as necrotic spots on garlic bulbs, often with a developed mycelium.

Garlic protection from phytopathogenic fungi is difficult because there is a small selection of pesticides that are safe for human health. Garlic dry rot control is not fully established and only a few studies on the effectiveness of chemical treatments against *Fusarium* spp. in garlic are available (Mondani et al., 2021). Elshahawy et al. (2017) demonstrated *in vitro* assays, that Carbendazim had an inhibitory effect on fungal growth when followed by Metalaxyl + Mancozeb (8% + 64%) and thiophanate-methyl. Gálvez et al. (2017) investigated the ability of three commercial fungicides to control *F. proliferatum* in vitro with promising results, but field treatments failed to control garlic rot during the commercial stage. In our country, there are no registered chemical preparations for application in garlic against Fusarium Basal Rot (Collective of authors, 2020). Furthermore, a frequent use of pesticides have resulted in a development of fungal resistance and destruction of the environment. That is why an eco-friendly pesticide alternative is desirable to protect the quality and the yield of garlic crop.

Bacillus isolates and *Trichoderma* spp. are reported in literature as potential biocontrol agents against garlic dry rot. Over the years, essential oils have received renewed attention due to a wide spectrum of biological activities against several pests, especially microorganisms (Gayoso et al. 2005; Vitoratos et al., 2013; Mahilrajan et al., 2014). The antimicrobial activity of some selected species has already been demonstrated (Maksimović et al., 2005; Stević et al., 2014; Starović et al., 2016; 2017).

In this work we explore the possibility of utilizing plant essential oils as antifungal agents against *Fusarium verticillioides*.

Materials and methods

Isolation and identification of fungal species

During 2021, garlic plants showing symptoms of yellowing and rotting at early and intermediate stages of crop development, were observed. Rotted areas of the bulb progressed from the basal plate towards the neck of the bulb (Figure 1). Affected roots became dark brown to dark pink, while fungal growth was visible at the base of infected bulbs. The isolate used in this study originated from symptomatic garlic cloves from Kraljevci locality, Srem District, Serbia, and was morphologically and molecularly identified. Morphological identification of the isolate was made using the criteria of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006). PCR was carried out for the purpose of identification of *Fusarium* species by amplifying the translation elongation factor-1a gene (tef-la) using the primer pairs EF1 and EF2 as described in O'Donnell et al. (1998).

Antifungal activity of EOs

Essential oils (EOs) extracted by hydro-distillation from several medicinal plants: Turkish pickling herb (*Echinopora tenuifolia*), oregano (*Origanum vulgare*), basil (*Ocimum basilicum*) and myrtle (*Myrtus communis)* - obtained by the kindness of the Professor M.M. Özcan from the Department of Food Engineering, Faculty of Agriculture, Selçuk University, 42079 Konya, Turkey, were used in antagonistic assays with a *Fusarium verticillioides* isolate originated from symptomatic garlic cloves from the locality Kraljevci (Serbia). A minimum inhibitory concentration (MIC) was determined by modified micro-dilution method in 96 well micro-sterile

0.75% saline containing 0.1% Tween 80 (vol/vol) (Balouiri et al., 2016). The fungal spore suspension was filtered and adjusted with sterile saline to a concentration of approximately $4{,}0x10^5$ spores per ml using a hemocytometer. In each well with 90 µL potato dextrose medium with appropriate dilutions of the EO, 10 µL of fungal inoculum was added. The experiment was repeated four times. Trifloxystrobin was used as a positive control. The MIC was defined as the lowest concentration of essential oils (EO) which completely inhibited the visible fungal growth.

Results and discussion

The morphological characteristics (Figure 1) of the pathogen correspond to the records for *F. verticillioides* described by Leslie and Summerell (2006). The amplification and sequencing of tef-la gene produced a sequence identifying garlic isolate used in this study as *F. verticillioides*.

FIGURE 4. Symptoms of *F. verticillioides* on garlic cloves (a, b); Colony morphology of *F. verticillioides* (c)

Garlic dry rot is a postharvest disease, but garlic infection starts in the field (Mondani et al., 2021, Stanković et al., 2007).
All tested oils showed some antifungal activity against the tested fungal pathogen. The results of the antimicrobial activity tests using microdilution method are summarized in Fig.2 and 3. The EOs showed a wide range of antifungal activity against *F. verticillioides*. The antifungal potential of oil tested can be presented as: *Ocimum basilicum* > *Origanum vulgare*> *Myrtus communis*> *Echinopora tenuifolia*. The oregano EOs proved to be the best inhibitor of the tested fungal isolate (MIC 0.033±51.96 µg/mL), followed by basil (MIC 0.075 ±0.05 µg/mL), then myrtle (MIC 5.500±0.50 µg/mL) and Turkish pickling herb MIC 55.000±5.19 µg/mL).

Echinophora tenuifolia		
Origanum vulgare		
Ocimum basilicum		
Myrtus communis		

Essential oils MIC

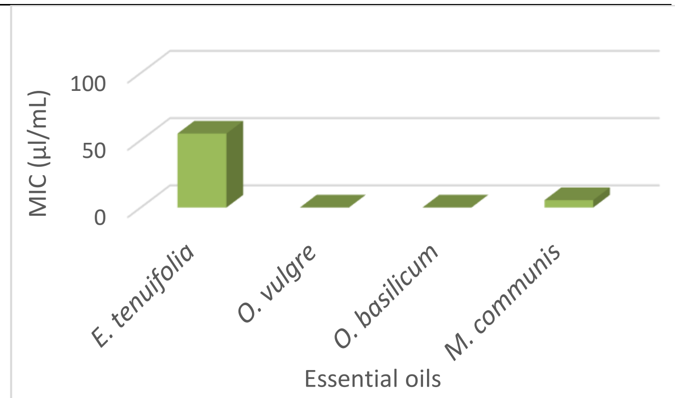

FIGURE. 2. Antifungal activity of essential oils expressed as minimal inhibitory concentrations (µg/mL)

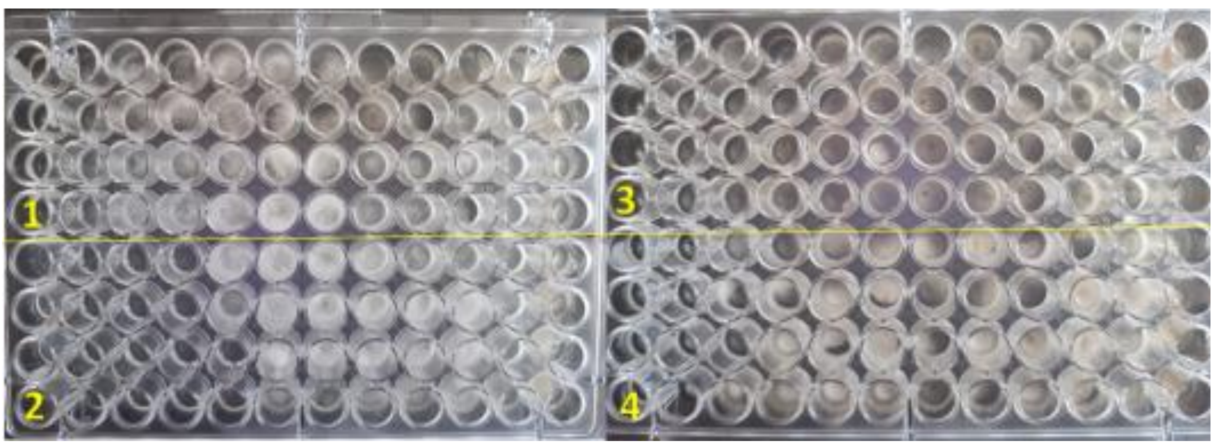

FIGURE 3. Micro-dilution method: antifungal effect of investigated essential oils (1) *Echinopora tenuifolia*; (2) *Origanum vulgare*; (3) *Ocimum basilicum*; (4) *Myrtus communis*

According to Stanković et al. (2007), garlic dry root causal agent in Serbia has been identified as *Fusarium proliferatum*. Here we provided the first report of the presence *F. verticillioides* on diseased bulbs with symptoms described as garlic dry root.

The resulted from our previous study (Starović et al., 2016; 2017) showed that that the oregano EO and basil from Turkey, manifest the high inhibitory effect against *Phomopsis theicola* (MIC5.5 µg/mL and 75.0 µg/mL respectively), while myrtle EOs showed very strong effect against *Fusarium* sp. especially *F. verticillioides* (MIC 3.25 µg/mL). Stević et al. (2014) demonstrated that oregano oil originating from Serbia could inhibit the growth of *Phomopsis* species at concentration of 70 µg/mL, while basil oil demonstrated a smaller effect (MIC 5950 µg/mL). Kocić-Tanackov et al. (2012) showed that the concentration of 25 µg/mL of oregano EOs inhibited the growth of *F. verticillioides* by 88%. The obtained results from this study indicate a very similar effect of selected EOs originating from Turkey and Serbia.

Conclusion

Based on our study, oregano and basil showed promising results in *F. verticilioides* control. Further studies could focus on the selection of essential oils and their application *in vitro*. These data can be a starting point for further experiments and development of biofungicides.

Acknowledgement

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EFFECTS OF ANTI-BROWNING SUBTANCES WITH HONEY ON QUALITY OF APPLE SLICES DURING STORAGE

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Abstract

Fresh-cut or minimally processed fruits have gained particular attention recently and their consumption has been increasing. However, physiological disorders such as browning on the cut surface along with loses in water and texture quality in these produces proceed more rapidly compared to intact products and thus their shelf life is shorter. In the current study, effects of anti browning substances such as citric acid and ascorbic acid employed with honey on slice quality and browning of apples *cv.* Granny Smith were investigated during cold storage. Apple fruits harvested at commercial maturity were divided into lots and the sliced commodity were immersed into pure water (control) or solutions of 2% ascorbic acid, 1% citric acid, 10% honey, 2% ascorbic acid plus 10% honey or 1% citric acid plus 10% honey. Treated slices were packed and stored at 4°C for 15 d. Honey combined with ascorbic acid treatment after slicing were effective on decreasing the weight loss, maintaining the firmness, titratable acid and visual quality and delaying the browning incidence of slices. Considering the overall investigations, treatments of ascorbic acid combined with honey after slicing were effective on protecting the postharvest quality of apples *cv.* Granny Smith during 15 days storage.

Keywords: *Fresh cut, Granny Smith, browning, honey.*

Introduction

Apple, which constitutes approximately 12% of the total fruit production in the world, is an important species that ranks second after banana. Apple is produced on an area of approximately 1.7 million decares in Turkey. Isparta, Karaman and Niğde are the provinces with the highest apple area. (Anonymous, 2019). Granny Smith apple variety is one of the globally popular horticultural produces with its medium large, juicy, sour, partially thick skinned crispy fruits. Granny Smith fruits can be stored in cold stores for up to 9 months under suitable conditions (Valizadeh, 2011). Fresh cut (minimally processed) products are one of the areas whose importance and usage has increased rapidly in recent years. These products are mostly preferred by consumers because they are close to fresh. However, due to processes such as peeling, cutting and slicing, respiration and ethylene activity increase rapidly in these products and the products can deteriorate quickly. This has caused color changes, softening, microbiological deterioration and changes in taste in the products. Color changes are the most important changes that cause a decrease in visual quality in freshly cut products. Physical and chemical methods have been used to reduce these changes and prevent browning in the products.

The chemical methods used are divided into different groups according to their shape of effect. Ascorbic acid are some of the chemicals used such as citric acid. Ascorbic acid is a very good antioxidant; citric acid, on the other hand, helps to preserve a certain quality in the product by inactivating unwanted enzymes as well as being a great antioxidant (Martinez et al., 2013). Citric

acid is a weak organic acid found naturally in many fruits and vegetables. Ascorbic acid is a weak water soluble acid and a natural antioxidant that can be used for food preservation, nutritional supplementation, and also treatment (De'Nobili et al., 2016). Ascorbic acid is widely used as an antioxidant food additive. In order to increase the stability and quality of the product, ascorbic acid is provided to contact the foodstuff directly or in solution. In this way, it helps to preserve the characteristic features of the foodstuff (Sarkar and Sinha, 2016). As a result of the increase in reactions to chemicals that will adversely affect human health in societies, alternative protective measures have been started to be investigated. It has been stated that the use of some types of honey gives positive results in order to preserve the browning and quality of freshly cut fruits and vegetables. However, it was thought that the use of honey alone may have a limited effect on preventing browning.
In the study, it was investigated whether honey alone or in combination with other chemicals has an effect on preventing browning and maintaining slice quality in apple *cv.* Granny Smith.

Material and Method

In this study, Granny Smith apple variety which are the most preferred commercially in fresh cut products were used. Fruits harvested at commercial maturity were brought to the Selcuk University Faculty of Agriculture Horticultural Laboratory under appropriate conditions. Here, the damaged fruits were separated and a sample fruit was selected in terms of size and color and divided into 6 equal parts to be sliced. Before slicing, the fruits were washed with tap water, immersed in a solution containing 1% hypochlorite for 5 minutes, washed with distilled water again, and left to dry for a while at room conditions. The fruits were divided into 8 equal parts with an apple slicer so that they would not be damaged. Sliced apples were immersed in the solutions prepared for application for 5 minutes. After cutting, control (distilled water), 2% ascorbic acid (AA), 1% citric acid (CiA), honey solution (the percentage ratio was adjusted not to exceed the average SSC value of the fruit), honey + 2% ascorbic acid (AA+honey), honey + 1% citric acid (CiA+honey) were applied to the slices. After the applications, the apple slices were kept on blotting paper for a while in order to remove the excess water on them, and then they were placed on polystyrene plates and covered with a film. Control and treated slices were stored at 4 °C in a cold storage with 90% relative humidity for 15 days. Visiual quality were performed with some physical and chemical analyzes in sliced apples at the beginning of storage and at 5 days intervals (on the 5th, 10th and 15th days).
The plates on which the weight loss slices were placed were numbered at the beginning of the enclosure and weighed on a precision scale. These plates were weighed on the analysis days during storage and the differences at the end of the study were calculated as % weight loss. Soluble solid content (SSC) was determined by extracting the juice from the slices in each application and measuring it with a hand refractometer. Titratable acidity (TA) was determined by the titration method of water obtained from apple slices with 0.1 N NaOH until the pH reached 8.1 and expressed as % malic acid. Slice color was determined by reading L* a* and b* values on the opposite surfaces of 6 slices from each plate during storage using a CR 400 model Minolta brand color instrument. Hue angle (h°) values were calculated to determine color changes (McGuire, 1992). The browning index, which is used as an indicator of browning in sugar containing products, is expressed as the browning index. The browning of the slices during storage was calculated using the following equation (Perez-Gago et al., 2006; Putnik et al., 2017). During the storage, the flesh firmness of the apples was measured with a digital

penetrometer from 2 different parts of each slice of fruit, and the results were given as Newton (N). Visiual quality was evaluated on a scale of 1-9 by panelists at analysis periods (9 = excellent, freshly cut; 7 = very good; 5 = good, marketable margin; 3 = adequate, limit of availability; and 1 = bad, unconsumable) (Perez-Gago et al., 2006).
The study was established in a randomized plot design with 3 replications and 10 slices in each replication. The data obtained from the experiment were subjected to analysis of variance using the JMP package program, and the differences between their means were grouped according to Student's t-test multiple comparison test (P<0.05).

Results and Discussion

The treatments on the weight loss of apple slices during storage was found to be statistically significant. The weight loss increased with the progression of storage time. At the end of the 15 day storage period, the highest weight loss in apple slices occurred in the control group (2.04%). It was observed that the treatments of AA slowed down the increase in weight loss and at the end of the storage period, the lowest loss occurred in fruits treated AA + honey (1.51%) (Figure 1).

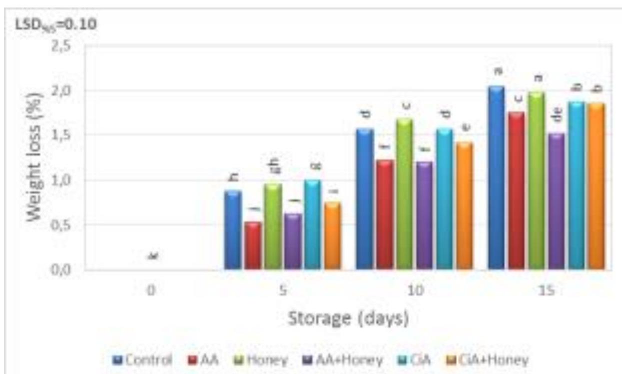

Figure 1. The effects of antibrowning treatments on weight loss of fresh cut apple slices during cold storage.

One of the most important factors effecting the quality of the apples the slice firmness. The effect of treatments on fruit firmness was found to be statistically significant during storage. As illustrated in figure 2, firmness of the slices gradually decreased during the prolonged storage. However honey treatments when used alone or combination with AA and CiA significantly maintained the slice firmness in comparison to control. Initial firmness value of slices were 25.5 N. At the end of the storage period, the highest firmness value was obtained from AA+honey treatment (23.8 N), followed by honey, CiA+honey, CiA and AA treatments (23.0, 22.9, 21.8 and 21.5 N, respectively). On the other hand, fruits of control treatment showed the lowest firmness value (20.9 N). It is thought that honey treatment creates a thin film on the surface of the slice and slow down the loss of water and thus the decrease in hardness.

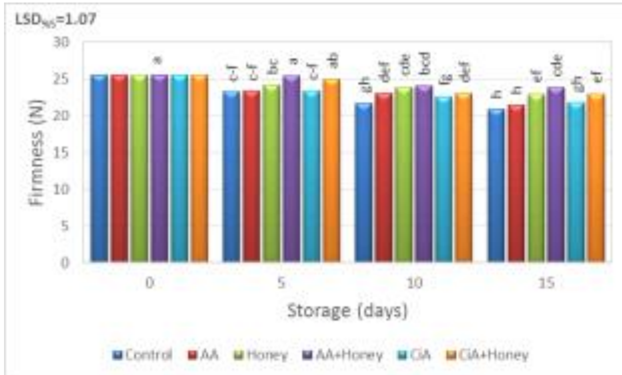

Figure 2. The effects of antibrowning treatments on firmness of fresh cut apple slices during cold storage.

The effect of the treatments on SSC of apple slices was found to be statistically significant. The value which was 13.0% at the beginning of storage, was measured in fruits with the highest honey treatments at the end of the storage period (12.3%), followed by AA + honey (11.9%), control (11.8%), AA and CiA + honey (11.7%) and CiA (11.53%) (Figure 3A).

The effect of postharvest treatments on TA during storage was found to be statistically significant. The TA value which was initially 0.617% in apple slices, showed a rapid decrease from the 5th day of storage (Figure 3B). While this decrease occurred the fastest in the control group fruits, it was observed that the decrease in TA was delayed in the treatments of AA + honey and CiA. At the end of the storage period, the lowest TA value was measured in the control group (0.525%) and the highest CiA treatments (0.572%).

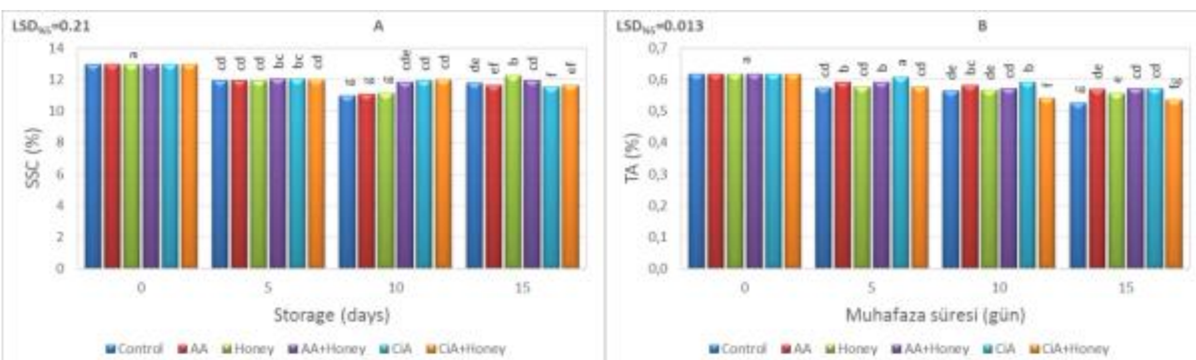

Figure 3. The effects of antibrowning treatments on SSC (A) and TA (B) of fresh cut apple slices during cold storage.

The effects of antibrowning treatments on the slice color were found to be statistically significant. With the prolonged of the storage time, a decrease in the hue angle value was recorded due to the browning of the slices. From the 5th day of storage, AA + honey treatment was found to be effective in slowing down the change of hue angle value (Figure 4A). At the end of the storage period, the highest hue angle value was measured in AA+Bal treatment (103.42°) followed by AA (102.79°), honey (97.78°), CiA + honey (97.29°) and CiA (95.71°) respectively. The lowest hue angle value was determined in the control (94.55).

Changes in browning index of slices during the storage were presented in Figure 4B. Initial index value of slices was 22.7 and underwent a remarkable increase due to browning. AA and AA+honey treatments significantly delayed browning index incerase. At the end of the storage, the highest browning index was deteced in the control group (42.25) also CiA (41.99) and honey (41.84) treatments were also statistically in the same group. While the lowest browning index value was determined in the AA treatment (27.33) was followed by AA + honey treatment (30.96).

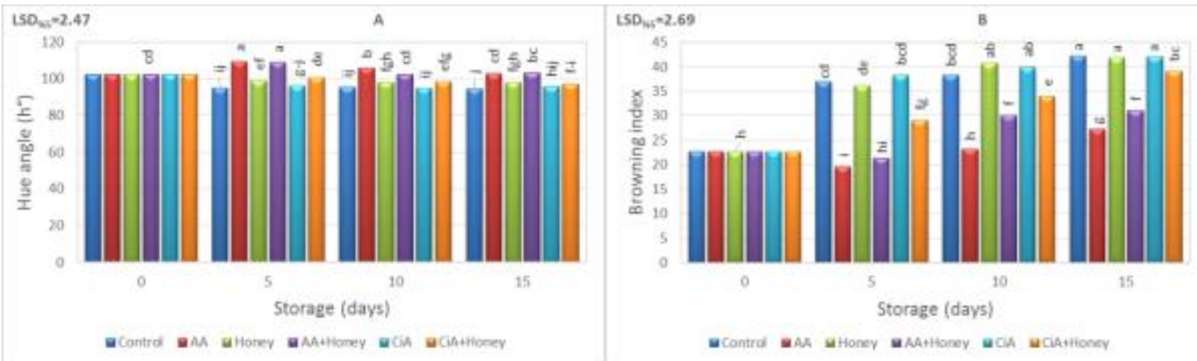

Figure 4. The effects of antibrowning treatments on hue angle (A) and browning index (B) of fresh cut apple slices during cold storage.

Visiual quality evaluatian performed by the panelists using quality feature such as color, browning and firmness during cold storage, it was determined that treatments significantly affected the visual quality of the slices (Figure 5). A significant decrease was determened in the control slices from the 5th day while it was observed that especially AA and AA + honey treatment preserved the quality significantly during the storage period. At the end of the storage, only AA + honey (6.0) and AA (5.16) treatments were able to score above the marketable limit value of 5.0. The lowest score (1.33) was determined in the control slices.

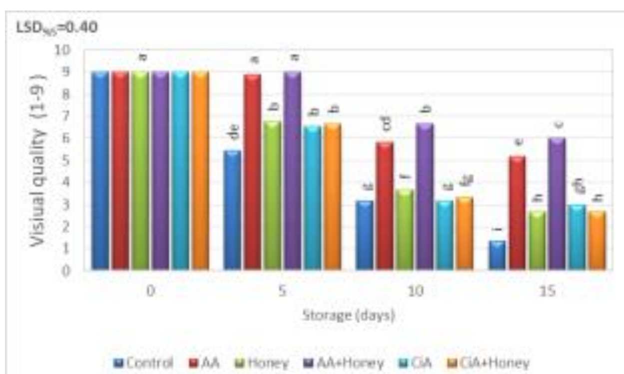

Figure 5. The effects of antibrowning treatments on visual quality of fresh cut apple slices during cold storage.

Browning inhibitors are chemicals that are effective in preventing or slowing the browning reactions of many freshly chopped fruits and vegetables. These chemicals are divided into 6 groups according to their mode of action; acid regulators, reducing agents, chelates, complexing agents, enzyme inhibitors and enzyme applications (Suttirak and Manurakchinakorn, 2010; Sabır, 2017). The use of sulfites, which was the first chemical group used to prevent browning, has been banned by the FDA due to its negative effects on human health (Garcia and Barrett, 2002). Among the reducing agents, ascorbic acid is the most widely used antibrowning agent.

Ascorbic acid and its derivatives are used effectively in delaying enzymatic browning in many fresh cut fruit and vegetable types at concentrations ranging from 0.5% to 4.0% (Sabir, 2017). Chen et al. (2000) stated that the polyphenol oxidase activity and browning index value of honey applied to fresh cut fruit and vegetables decreased by 2-45 and 2.5-12 units, respectively, and this decrease may vary according to the source of the honey. Erbay (2007) successfully maintained the quality of Golden Apple slices by dipping them in solutions of ascorbic acid, citric acid, sodium metabisulfite, honey, sugar and 4-hexyresorcinol for 15 days at 4 °C. As a result of their study, it was stated that the sugar and ascorbic acid solution preserved its quality properties and prevented color browning in the best way. While the browning inhibitory effect of honey may not be effective alone, its effect increases when used together with other chemical (ascorbic acid or metabisulphites) or physical applications (Chen et al., 2000).

Conclusions

The effects of antibrowning treatments (such as ascorbic acid, citric acid, honey) on apple slices of Granny Smith variety on fruit quality during cold storage were investigated. According to the results, it was determined that the treatment of ascorbic acid + honey was effective in reducing weight loss also delaying the decrease in slices firmness, titratable acidity and the increase in the browning index compared to the control. It has been determined that the treatment of ascorbic acid gives more effective results than the application of citric acid in maintaining the important quality properties of apples under many storage conditions. As a result of the study, it was concluded that the application of ascorbic acid + honey can be an effective treatment in extending the storage life of the fresh cut Granny Smith apple slices by preserving its quality properties during 15 days of cold storage.

Acknowledgement

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SPECIES OF THE GENUS *PYRENOPHORA* - BARLEY PATHOGENS IN THE REPUBLIC OF SRPSKA AND THE WORLD

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Abstract

Pathogenic species of the genus *Pyrenophora* are significant causes of diseases on barley and other small grains. There are almost no literary references related to the species of the genus *Pyrenophora* in our country. In this paper we will describe the most important species of the mentioned genus with their most important characteristics. Recently, research on diseases of the genus *Pyrenophora* in the Republic of Srpska has been initiated. The aim of the paper is to provide an overview of the most important species with an aspect of distribution, economic importance, symptomatology, life cycle and control.

Key words: *barley, Pyrenophora* spp.*, disease, control of disease.*

Introduction

Numerous pathogens with different systematic characteristics have been described on barley and other small grains in the world. These fungi appear in almost all barleys' production areas in the world where they cause economically significant diseases. However, fungi of the genus *Pyrenophora* are among the least described and studied pathogens of barley and other small grains in the Republic of Srpska, which served as a reason for us to start extensive research as part of my doctoral dissertation on these barley pathogens in the Republic of Srpska. To date, we have established the presence of two species: *Pyrenophora teres* Drechsler i *P. graminea* Ito & Kuribayashi. Further research is on-going and in this paper we are presenting an overview of pathogenic species of the genus *Pyrenophora* that can appear on barley and other small grains. In the continuation of this work, the seven most important pathogenic species of the genus *Pyrenophora* that appear as pathogens of barley in the world will be described as follows:

Pyrenophora teres Drechsler (anamorph: *Drechslera teres* (Sacc.) Shoemaker) – cause of barley net blotch

Distribution and economic importance. Net blotch is widely present in all major barley-growing agricultural regions worldwide. The pathogen *P. teres* was first described a little over 120 years ago (Webster, 1951), and the sexual stage of this fungus was first described by Drechsler in 1923. Economically, it is a very important pathogen that can significantly reduce the yield, as well as the quality of production, which directly affects the economic profit and justification of production. Yield losses can vary depending on the sensitivity of the variety, climatic conditions, the presence and amount of inoculum, the time of infection, the stage of crop growth, the method of testing, and ranged from 10-40% average, and even up to an incredible 100% in conditions where sensitive varieties are grown and where the crop is heavily affected by disease attack

(Oğuz *et al*., 2019). In addition to yield reduction, the disease can affect root size, percentage of dry matter, size of healthy leaves, number of spikes, number of grains per spike, etc.
Hosts. In addition to cultivated barley, the causer of net blotch can also infect its wild relatives (*Hordeum vulgare* ssp. *spontaneum* (K. Koch) Thell. and *H. murinum* ssp. *glaucum* (Steud.) Tzvelev), but also other plants from different genera such as *H. leporinum* Link, *Triticum aestivum* L., *Avena sativa* L., *Agropyron* sp., *Bromus* sp., *Elymus* sp., *Hordelymus* sp., *Stipa* sp. and other species of the genus *Hordeum* (McLean *et al*., 2009).
Symptoms. Based on the symptoms of the disease, Smedegård-Petersen (1971) divided the pathogen into two forms, the spot form or form *maculata* and the net form or form *teres*. The most common symptoms of the disease can appear on barley leaves, which can be infected at all stages of growth, although the pathogen can also cause symptoms on other plant organs, such as the leaf sheath, stem, but also the flower and grains. Symptoms of the net form initially appear as small spots or lines that gradually increase in size and form narrow, dark-brown longitudinal transverse stripes in the form of a reticulated pattern. The affected parts of the leaf become brownish-brown, and the adjacent tissues become chlorotic. In the later development of the disease, the lesions are surrounded by yellow chlorotic margins that can spread over the entire leaf, causing wilting from the tip to the base of the leaf. Symptoms of spot form appear on the tops of the leaves and on the branches. It can cause different types of dark-brown elliptical lesions, 3 x 6 mm in size, surrounded by a chlorotic zone, of different widths, which can spread over the entire leaf and lead to drying. Severe infection can lead to complete senescence and drying of the leaves.
Life cycle and epidemiology. The sources of pathogen infection for the next growing season are infected plant residues, infected seeds, wild barley plants, but also other pathogen host plants (McLean *et al*., 2009). Infected seeds are also the main source of pathogen introduction into new areas. Very often, infected seeds do not germinate or develop plants with disease symptoms, which represent a significant loss of yield, but also a source of secondary infections. On plant remains, i.e. on infected stubble, perithecia are formed, which play an important role because asci with ascospores mature in them during the winter in cold and constant moisture conditions at ground level, and conidia can also develop from infected stubble (McLean *et al*.,2009). The role of perithecia is twofold, both for the production of asci with ascospores and for the formation of conidia on the ascostoromata setae (Smedegård-Petersen, 1971). Harvest residues are a very important source of potential inoculum, which indicates the mandatory application of crop rotation, deep tillage and other measures to destroy infected residues. *P. teres* is unable to survive in soil for more than 2 weeks after being separated from its host (McLean *et al*., 2009).
Control. The application of preventive measures, first of all, the production and sowing of healthy seeds, the creation of resistant varieties of barley, conventional tillage (ploughing), the application of crop rotation, knowing that the pathogen can be maintained in plant residues as well as wild barley. The amount of inoculum can be reduced by plowing or burning crop residues (Brien, 2005). Considering that the pathogen is maintained on plant residues, a break of two years in the barley plots would be desirable for good control against this disease, as well as sowing in plots far from the barley plots to limit the spread of the inoculum by wind. Barley cultivars show differences in susceptibility/resistance to *P. teres*. Cultivar like Rojo, Coast, CIho 9819, CIho 5791, CIho 7584, CIho 5822, ND B112 i FR 926-77 were resistant to all tested isolates of net and spot forms, which also originate from different parts of the world (Wu *et al*., 2003). The use of fungicides gives good results. Among the chemical groups of fungicides in disease control that are used for seed disinfection and foliar formulations of fungicides are

stolburins, triazoles, benzimidazoles, chlorothalonil, morpholine, chlorophenyl, anilinpyridine, guanidine, carboxamide and dithiocarbamates, and a mixture of stolburin and triazole is recommended in order to reduce emergence of resistance (Brien, 2005). In addition to fungicides, the application of biological measures, that is, biological organisms in the fight against various pathogens, is being tested. Bacteria that showed inhibitory efficiency in vitro are *Trichoderma viride* Pers., *Trichoderma koningii* Oudem., *Trichoderma pseudokoningii* Rifai, and acotinomycetes *Micromonospora* sp. (Ali-Haimound *et al*., 1993). The inhibitory efficiency of *Trichoderma* spp. on the radial growth of *P. teres* isolates was determined by (Tančić-Živanov *et al*., 2017). *Pseudomonas fluorescens* bacteria strains (MKB 100 and MKB 156), *Pseudomonas* sp. (IKB 158), *Pseudomonas* sp. (IKB 194) were effective in suppressing pathogens in a study by Brien (2005).

Pyrenophora graminea **Ito & Kuribayashi (anamorph: *Drechsera graminea* (Rabenh. ex. Schlecht) Ito) – cause of barley leaf stripe**

Distribution and economic importance. Barley leaf stripe the causer *P. graminea* is one of the most widespread seed-borne diseases, which was first described by Carne and Campbell back in 1924 almost a century ago in Western Australia (Khan and Loughman, 1988). The disease is distributed worldwide wherever barley is grown Canada, UK, USA, Europe (Tekauz and Chiko, 1980) and Asia (Karakaya *et al*., 2017). The pathogen can cause significant economic losses. Susceptible plants may stop growing, wilting, which leads to a drastic reduction in yield (Tekauz and Chiko, 1980). Attack plants by *P. graminea* significantly reduce the weight of 1000 grains, the number of spikes, the number of tillers, the total biomass, and the yield in sensitive cultivars by 44-92% (Arabi *et al*., 2004).
Hosts. The causal agent of barley leaf streaking is a pathogen that does not have such a wide host range, more precisely; the pathogen infects plants of the *Horedum* genus, with the fact that it can infect wheat and oats (Stojanović, 2004).
Symptoms. The disease affects the whole plant, but more typical symptoms appear on the leaves. Symptoms appear from the tillering stage until ripening. The streaks initially sometimes consist of partially necrotic tissue, while in other cases only chlorosis is initially visible. In severe forms of infection, the symptoms were in the form of deformation and curling of the first leaf, often associated with a dark-green watery area, along the leaf nerve, followed by rapid necrosis. Young seedlings emerging from infected seeds may stop growing and wilt. The yellow streaks quickly become brown as the necrosis progresses, leading to typical leaf streak symptoms. The leaves are further split under the influence of rain and wind, and the ear is partially released from the stem and becomes sterile (Alasić, 2009). In infected plants, the lower leaf is usually completely necrotic, and infected plants are usually shorter than healthy ones, with small and thin spikes that do not come out of the leaf sheath. The spikes rarely become visible or protrude abnormally from the leaf sheath. The disease is very easily recognized in the field.
Life cycle and epidemiology. *P. grminea* is a pathogen that is maintained only in the infected seed as the only source of inoculum in the form of mycelia and can maintain vitality for several years. The pathogen is monocyclic and strictly seed borne and survives as mycelium in the hull, pericarp and seed coat (Khan and Loughtman, 1988). The pathogen in stored grains can survive for several months, until the next sowing and cause infection during germination. The conditions of the external environment also have a decisive influence on the development of the disease, so cool and dry weather at the beginning of the season favours the development of the disease. The

severity of the disease increases when conditions of high humidity and rain prevail at the time of heading. During the growing season, the fungus produce a mass of conidia on the leaves of diseased plants, which are further carried by the wind to the grains in the spikes of healthy plants that are susceptible to infection from the flowering to the dough stage, and the achieved seed infection gives the possibility of the infection multiplying from one season to another (Cockerell *et al*., 1995)
Control. Special attention should be paid to the production of exclusively healthy seeds, but also to the creation and production of resistant varieties of barley. In pathogenicity studies, different cultivars of barley show different resistance to isolates of *P. graminea* (Arabi *et al*., 2004). Seed disinfection with fungicides is one of the most effective measures. In the beginning, seed disinfection was performed using organo-mercury compounds, formalyl, thiram (Cockerell *et al*., 1995). Satisfactory efficiency of foliar treatments was achieved using preparations based on triadimenol, triadimenol + imazalil, flutriafol and fenfuram, almost 20 years ago (Loughman and Khan, 1993). Disinfections with biological preparations based on *Bacillus subtilis* (Serenade) showed good effectiveness against *P. graminea*, as well as the Tillecur preparation based on white mustard flour (Koch *et al*., 2006). The efficacy of seed coating with strains of *Pseudomonas putida* and *Bacillus subtilis* against *P. gaminea* was confirmed by Adam *et al*. (2017).

***Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker; syn. *Helminthosporium tritici-repentis* Died.) – cause of yellow leaf spot**

Distribution and economic importance. The disease has been identified worldwide as economically important. The pathogen in the USA and Australia causes yield reductions of 3-15%, and often up to 50% (Hosford *et al*., 1982; Rees and Platz, 1983). In Serbia, symptoms of the disease were first recorded on wheat in 1993, and later in other parts of the country, where in 2006 it was identified in several locations (Jevtić *et al*., 2006). The same authors proposed, based on the observed symptoms, that the name of the disease in the Serbian language should be yellow-brown spotting or chlorotic necrotic spotting.
Hosts. The pathogen has a very wide host range. It can cause symptoms on wheat, barley, rye, millet, but also on numerous other grass species (Bakonyi *et al*., 1997).
Symptoms. The pathogen usually forms oval-shaped lesions, often with a darker center, surrounded by a chlorotic halo. Under favourable conditions, spread, cover the entire leaf and lead to its wilting. Resistant varieties are characterized by the absence or presence of a small amount of necrosis and/or chlorosis. By testing the virulence of a large number of isolates on different varieties of wheat by artificial inoculation, it was established that the pathogen forms different types of lesions (necrosis, chlorosis), on the basis of which the pathogen is divided into pathotypes (races). Lamari and Bernier (1989; 1991) established 4 different pathotypes: Pathotype 1 (nec+chl+); Pathotype 2 (nec+chl-); Pathotype 3 (nec-chl+); Pathotype 4 - avirulent (nec-chl-). Pathotypes were later renamed races. A new race 5 (nec-chl+), a race very similar to pathotype 3 (nec-chl+), was soon identified, with race 5 causing symptoms on cultivars previously resistant to race 3 (Lamari *et al*., 1995). Strelkov *et al*. (2002) identified a new race 6, and two more new races were subsequently identified (Lamari *et al*., 2003).
Life cycle and epidemiology. Infected seeds can be one of the main ways of transmission and introduction of pathogens to new areas. Plants with fewer spikes, lower plants and yield developed from infected grains (Fernandez *et al*., 1998). *P. tritici-repentis* produces perithecia on

infected plant debris, with asci and ascospores that mature during the winter, and conidia, which are the main source of inoculum for the following season. Pseudothecia can retain infectivity and survive on plant debris for at least two years (Fernandez *et al*., 1998). Pseudothecia mature during autumn and winter to forcibly erupt ascospores during the wet spring period. Ascospores are usually used to spread pathogens over shorter distances, while conidia are used to spread over longer distances by wind. Ascospores are considered the primary inoculum, present throughout the growing season and infect the leaves of young plants located in the immediate vicinity of plant debris, and conidia, unlike ascospores, are considered a secondary inoculum, which are produced several times during the growing season (Ciuffetti *et al*., 2014).
Control. Mandatory application of the previously mentioned preventive measures. Proper nutrition can reduce the occurrence of disease in the crop. Huber *et al*. (1987) state that increasing the amount of nitrogen, even in sensitive varieties, drastically reduces the severity of the disease and the development of lesions, in contrast to varieties where the nitrogen dose was lower. Great attention should be paid to the destruction of wild cultural and wild (weed) plants in plots and around plots with cereals where the pathogen can survive and overwinter. Harvey *et al*. (2015) published testing the effectiveness of triazole (propiconazole and prothioconazole) and SHDI (isopyrazam, bixafen + prothioconazole and fluxapiroxad + epoxyconazo) fungicides. Propiconazole, prothioconazole, isopyrazam, bixafen + prothioconazole and fluxapiroxad + epoxiconazole gave similar levels of control either alone or in combination, while epoxiconazole and defenoconazole were the least effective.

Pyrenophora semeniperda (Brittleb. & D.B. Adam) Shoemaker – cause of black fingers of death

Wallwork *et al*. (1992) states that this pathogen is present on species of the genus *Hordeum*. It does not have a great economic importance for our country and there is no data on its presence in our country so far. Wallace (1959) was among the first to point out that *P. semeniperda* occurs on the genera *Triticum*, *Avena* and *Bromu*s. It was later found to be a pathogen causing leaf spot and seed infection in plants from over 36 genera of annual and perennial grasses including winter cereals and some dicotyledonous plants. This pathogen is present in grasslands worldwide in Argentina, Australia, Canada, Egypt, New Zealand, South Africa and the USA, while there is no information on its presence in Europe and Asia (Medd *et al*., 2003). Later, in Greece and Turkey, the first presence of this pathogen was confirmed on *Bromus tectorum* seeds (Stewart *et al*., 2009). The symptoms of this disease are in the form of characteristic ring-shaped lesions 1−4 mm in size, lighter in the middle and darker around the edges. Infected seeds are symptomless until germination, when stroma appears on which conidiophores and conidia develop, as well as perithecia with asci and ascospores (Medd *et al*., 2003).

Pyrenophora erythrospila A.R. Paul (anamorph: *Drechslera erythrospila* (Drechsler) Shoemaker; syn. *Helminthosporium erythrospilum* Drechsler) – cause of red leaf spot

The name of this fungus comes from the Latin word '*erythrospilum*' meaning red spot (Paul, 1972). Very little is known about this fungus, which is indicated by the low availability of literary references. This pathogen hasn't been established in our country. The disease is present mainly in the central and northern Japan, and causes diseases on plants of the genus *Agrostis* (Paul, 1972). Wallwork *et al*. (1992) state that the pathogen has been recorded on the genus

Hordeum, as well as on plants from the genus *Triticum*, and is present worldwide in Europe, Australia, New Zealand, and North America (Sivanesan, 1990). During the growing season, conidia are spread by the wind. Characteristic symptoms of the pathogen are formed near the edges of the leaves or on the tips of mature leaves in the form of dull reddish-brown spots, which are followed by wilting and death (Paul, 1972).

***Pyrenophora trichostoma* (Fr.) Fuckel – cause of yellow leaf spot**

Pyrenophora trichostoma is a widespread pathogen worldwide, especially in Europe and the USA, and is known as a serious pathogen on cereals, especially wheat (Goonasekara *et al*., 2020). The disease appears on grasses from several different genera such as *Bromus, Hordeum, Poa, Secale* i *Triticum* and it was introduced in 1961 as a telemorph species of *Heminthosporium*. Previously, the species *P. trichostoma* and *P. tritici-repentis* were synonymized based on morphological characteristics. In the research of Kodsueb (2006) the phylogenetic analysis confirmed that these species are very similar but differ in several base pairs and should be treated separately. *P. trichostoma* as a special species was also shown by phylogenetic analysis in the research of Goonasekar *et al*. (2020). The pathogen was identified in the USA in 1968 on wheat, and it caused symptoms in the form of light-brown lesions with a pronounced yellow halo on the leaves (Robert and Hasford, 1971). Symptoms appear during spring and early summer, and yellow spots spread to cover the entire leaf, which dries up and becomes dormant (Ghazanfari-Hesamabadi, 1983).

***Pyrenophora wirreganensis* (Wallwork, Lichon & Sivan.) Y. Marín & Crous (anamorph: *Drechslera wirreganensis* Wallwork, Lichon & Sivan.) – cause of wirrega leaf spot**

The pathogen *Drechslera wirreganensis* has no significance for our areas. There are few references in the literature about this pathogen. It was identified in Australia on a barley crop thirty years ago, when the fungus was isolated and studied (Wallwork *et al*., 1992). This fungus has also been found on wheat, barley grass, *Bromus grasses*, ryegrass in parts of South and Western Australia (Wallwork *et al*., 1995). Yield loss in the susceptible cultivar Skiff was estimated to range from 13 - 36% (Wallwork *et al*., 1995). Wallwork *et al*. (1992) described two symptoms that this pathogen can cause. More serious symptoms are in the form of large medium brown spots, surrounded by narrow chlorotic tissue, which was much wider at the ends of the lesion. In the middle of the lesion, the tissue was necrotic, and in some cases holes appear on the leaf. The lesions spread to the entire leaf, while the stems are not attacked. Another symptom is in the form of small dark-brown spots 2−5 mm with a light centre, with little or no chlorosis, which are called rings. In this type of lesion, holes also appeared in the middle. The symptoms of this disease are recognizable both by the presence of a hole and a teardrop in the middle of the lesion (Capio *et al*., 2004).

Conclusion

Barley is one of the most economically important cereals grown in our country, and it is used as a basis for making nutrients for domestic animals, as well as for the production of beer malt. It is of particular importance to know the spectrum of the most significant barleys' diseases that can significantly disturb the quality and quantity of barley yield. Based on visits to plots under barley on a large number of production plots, the presence of symptoms manifested in the form of various types of spotting, necrosis and drying of the diseased tissue of barley leaves, which indicate the potential presence of species of the genus *Pyrenophora*, was determined. The significance of this occurrence is all the greater because the symptoms caused by certain species of the genus *Pyrenophor*a on diseased barley leaves are difficult to distinguish visually in most cases, so correct diagnosis is the basis for their successful control. Through the laboratory analysis of the collected samples, we determined that the mentioned changes on the diseased barley leaves were caused by two species from the genus *Pyrenophora*: *P. teres* and *P. graminea*. In addition to these species, in the paper we have given an overview of potential species whose occurrence could be expected, among which the most significant are P. tritici-repentis, P. semeniperda, P. erythrospila, P. trichostoma and *P. wirreganensis*. In the future, continuous surveillance of barley plots across the state is needed to adequately respond to future challenges with the emergence of new species of barley pathogens.

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ANTIMICROBIAL RESISTANCE OF *Salmonella Enteritidis* & *Salmonella Typhimurium* ISOLATED FROM FOODS OF ANIMAL ORIGIN

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Abstract

The problem of resistance to antimicrobial drugs was mostly associated with the spread and mechanism of resistance in human pathogenic bacteria. However, the appearance of resistance in zoonotic bacteria began to affect therapeutic interventions in humans as well. The use of antibiotics in animals whose meat is used for human consumption has opened the question of developing resistance in the animal's body, as well as the possible transmission of resistant bacteria through the food chain to humans and the development of foodborne illnesses that are difficult to treat. Salmonella is one of the leading foodborne pathogens. They are a common cause of alimentary infections in humans. Secondary contamination of salmonella is also possible along the entire food chain. Salmonellosis is a self-imitating infection, so antimicrobial therapy is used only in children, older people and in cases of systemic infections. Serotypes *S. Enteritidis* and *S. Typhimurium* lead to most gastrointestinal infections of humans, pathogenic for animals as well and the outcome of infection ranges widely, from severe systemic diseases to asymptomatic conditions. The aim of the study is to examine the sensitivity of *Salmonella Enteritidis* and *Salmonella Typhimurium* serotypes (isolated from food) on antimicrobial drugs. To investigate the susceptibility of salmonella to antimicrobial drugs a disc diffusion method was used. To perform the disk diffusion method, disks of the manufacturer "Conda" and Oxoid were used. All tested isolates were 100% resistant to imipenem (IMI), linkomycin (MY), bacitracin (LS), canamycin (K), vancomycin (VA) and cefuroxime (CXM), 80% on nalidic acid (NA) and 40% amiac acid isolates (AK) and gentamicin (CN). Moderate susceptibility to amoxicillin (AML) and tetracycline (TE) showed 60% of isolates 20% according to amicacin (AK) and gentamicin (CN).

Keywords: *antimicrobial drugs, antimicrobial resistance, multi-residue strains.*

Introduction

A leading problem in public health is bacterial resistance to antimicrobial drugs. With the introduction of penicillin in the treatment of bacterial infections in the early 1940s, the number of fatal diseases decreased. However, due to the excessive use of antibiotics, bacteria have acquired many mechanisms of resistance to all groups of antibiotics which are used in the treatment of human and animal infections. The use of antibiotics as growth promoters as well as their use for other non-therapeutic purposes contributed to the increase in resistance to antimicrobial substances. Excessive use of antimicrobial drugs has led to the spread of resistance in opportunistic bacterial pathogens. Also, the increase in the number of multi-resistant bacteria associated with the lack of new antimicrobial drugs poses a threat to global health. The use of antibiotic drugs in animals used for food can also have a major impact on the appearance of resistant species in humans. Salmonellosis is the main public health and economic problem as well as a problem related to veterinary and human medicine. The best and most effective way to prevent the spread of food infection is to reduce salmonellosis in the animal population. In most countries of the European Union, the most common strains finding in human gastroenteritis is *S. Enteritidis* and *S. Typhimurium* (Karabasil et al. 2014).

The causative agent of human salmonellosis in most cases is *Salmonella Enteritidis* of poultry origin. Since the mentioned Salmonella serotype does not usually cause clinical symptoms in infected birds, therapeutic treatment with antibiotics is not carried out, so it is to be expected that resistance to antibiotics rarely occurs in *S. Enteritidis*. However, during an examination of antimicrobial resistance and virulence factors in *Salmonella enterica* isolates collected on turkey farms where antibiotics are not applied either through food or water, 36% of the isolates were resistant to two to five antimicrobial agents isolated from turkeys and different samples of the animal environment despite the absence of antibiotics in the facility (Sanad YM et al., 2016).

In their research on antimicrobial resistance in isolated salmonella serovars Brazilian scientists found that 51.9% of the isolates were resistant to at least one antimicrobial drug. Multiresistance was recorded in 10.5% of isolates. The highest rate of resistance was determined to streptomycin (35.9%) and nalidixic acid (16.9%). There were no strains resistant to cefoxitin, cephalothin, cefotaxime, amikacin, ciprofloxacin, and imipenem (Rowlands RE et al., 2014).

Antimicrobial resistance studies of *Salmonella spp.* isolates in New South Wales confirmed the resistance of the investigated strains to amoxicillin and ampicillin (5.51%), tetracycline (4.13%), cephalothin (2.06%) and trimethoprim (0.68%) and sensitivity to cefotaxime, ceftiofur, ciprofloxacin, chloramphenicol, gentamicin, neomycin, or streptomycin (Pande VV et al., 2015).

In a study in which the resistance of *Salmonella* isolated from fresh goat and poultry meat from retail stores in India was examined and determined 100% sensitivity to ciprofloxacin, while 96.87% of the isolates were sensitive to gentamicin, imipenem, 93.75% to ceftazidime. Resistance to erythromycin was recorded in 93.75% and oxytetracycline in 59.37% of isolates (Naik VK et al., 2015).

S. Typhimurium stands out from the bacteria that cause diseases in a large number of animal species as well as humans. *Salmonella enterica* serovar *Typhimurium* with phagotypes DT104 and U302 is often resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline and represents the main zoonotic pathogens (Yu CY et al., 2008)

Salmonella serovars *S. Typhi* and *S. Paratyphi* A, B and C are primarily pathogenic for humans who are their only reservoir, and cause typhus and paratyphus in humans. Other Salmonella can cause septicemia, gastroenteritis, various focal infections, and carrier state in humans. Such infections caused by any serotype are known as zoonoses, which, in addition to the oral route, can be transmitted through food and water and from person to person (interhuman).

The aim of this research was to give an overview of the antimicrobial resistance of *Salmonella Enteritidis* (3 isolates) and *Salmonella Typhimurium* (2 isolates) isolated from food of animal origin, because so far, no research of this type has been done in the territory of Republic of Srpska.

The results will show which antimicrobial drugs are used in animals intended for human consumption and how widespread resistance to multiple antimicrobial drugs is.

Material and methods

The isolates of 5 bacterial strains (*Salmonella Enteritidis*- three isolates and *Salmonella Typhimurium*- two isolates) from the culture collection of the Laboratory for Microbiology of food, animal feed and water were used as material in this research. Antimicrobial susceptibility testing was performed according to EUCAST (European Committee on Antimicrobial Susceptibility Testing).

S. Typhymurium and *S. Enteritidis* cultures were seeded in nutrient broth and incubated for 18 hours at 37ºC. After the incubation the test microorganisms were transferred under aseptic conditions using a inoculation loop into a test tube with sterile peptone water and suspended by intense shaking on a vortex (IKA vortex). The density of the suspension was adjusted using a densitometer (DENSILA METER - "Erba") so that it corresponds to the value of 0.5 McFarland standard. Cultures prepared in this way were sown on a suitable substrate (Müeller - Hinton agar, Cinda, Spain). The sensitivity of the strains to antimicrobial drugs was carried out by the disc-diffusion method by placing reference antimicrobial discs on the surface of the prepared petri plates under sterile conditions. After that, the plates were left in the refrigerator for 30 minutes to allow the sample to diffuse into the substrate, and then they were incubated for 24 hours at a temperature of 37ºC.

To perform the disc diffusion method, discs manufactured by "Condo" Spain were used: amikacin (AK30 µg), amoxicillin (AML30 µg), ceftriaxone (CRO30 µg), cephalexin (CL30 µg), cefuroxime (CXM30 µg), nalidixic acid (NA30 µg), gentamicin (CN30 µg), imipenem (IPM10 µg), tetracycline (TE30 µg), kanamycin (K30 µg), chloramphenicol (C10 µg), bacitracin (BA10 IJ), streptomycin (S10 µg), vancomycin (VA30 µg) and "Oxoid" (Great Britain) neomycin (N30 µg) lincomycin (LY15 µg). Routine quality control was performed in accordance with the recommendations given by EUCAST (EUCAST, 2018b).

Results and discussion

The problem of bacterial resistance to antimicrobial drugs is present all over the world and is getting bigger with time. Despite the increased awareness of the possibility of developing resistance in different types of bacteria antimicrobial drugs are still used excessively and often unnecessarily. The percentage of sensitive and resistant strains according to individual antimicrobial drugs in this study is shown in graph 1.

From the presented results it is evident that all tested strains are 100% sensitive to streptomycin (S), while the sensitivity to ceftriaxone (CRO), cephalexin (CL), chloramphenicol (C) is 80%, to neomycin (N) 60%, and 40 % to amoxicillin (AML), tetracycline (TE), amikacin (AK) and gentamicin (CN). 20% of isolates are sensitive to nalidixic acid (NA).

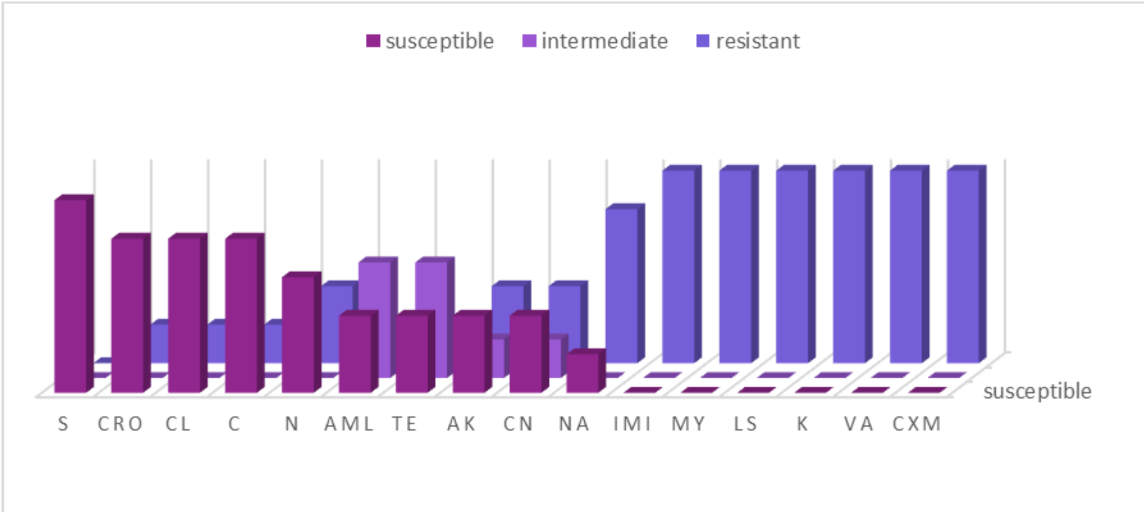

Chart 1. Relationship of susceptible and resistant isolates to antimicrobial preparations

All tested isolates were 100% resistant to imipenem (IMI), lincomycin (MY), bacitracin (LS), kanamycin (K), vancomycin (VA) and cefuroxime (CXM), 80% to nalidixic acid (NA) and 40% isolates to amikacin (AK) and gentamicin (CN). Moderate sensitivity to amoxicillin (AML) and tetracycline (TE) was shown by 60% of the isolates and 20% by amikacin (AK) and gentamicin (CN). Resistance to several antimicrobial drugs was found in all isolates, and the results are shown in graph 2.

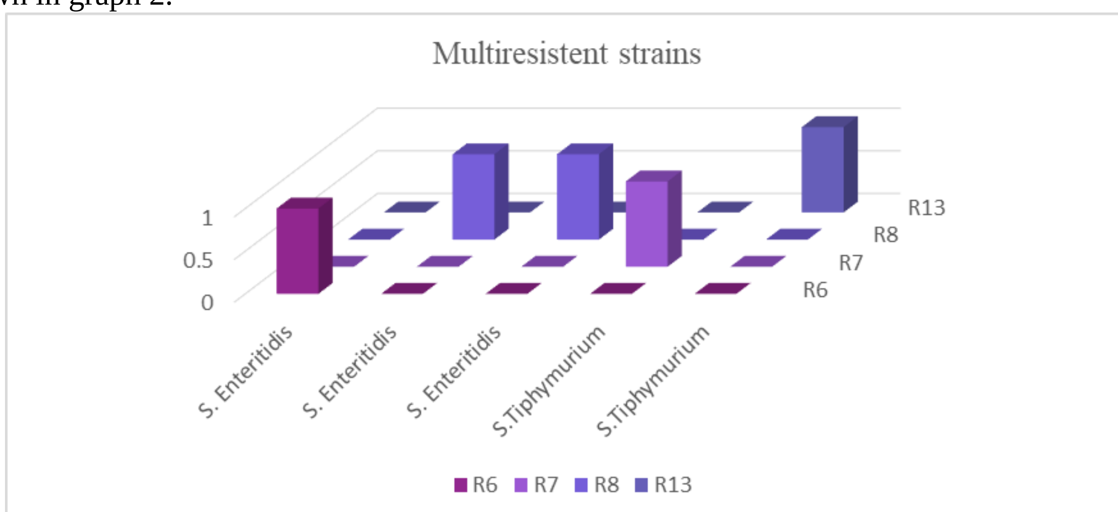

Chart 2. Resistance of *S. Enterica* and *S. Typhimurium* isolates to several antimicrobial drugs

All five isolates were resistant to six of the same antimicrobial drugs (CMX, VA, LS, K, IMI and MY). One *S. enteritidis* isolate was resistant to these six and the other two isolates, which are resistant to a total of eight antimicrobial drugs, were also resistant to AK and NA, respectively to N and NA. One *S. Typhymurium* isolate was resistant to 13, sensitive to 1 (S) and conditionally sensitive to 2 (TE and N) out of a total of 16 tested antimicrobial drugs. The second isolate of *S. Typhymurium* is resistant to 7 antimicrobial drugs, sensitive to 6 (CRO, CL, CN, TE, C, S) and conditionally sensitive to 3 (N, AML, and AK).

Although human salmonella infections are rarely treated with antimicrobial drugs, in some cases adequate antibiotic therapy is required (Darby et al., 2008). In severe infections caused by salmonella the drugs of choice were ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol. The increase in resistance to these antimicrobial drugs significantly reduced their effectiveness, so fluoroquinolones and extended spectrum cephalosporins began to be used in the therapy of invasive salmonella infections (Winokur, 2000). However, resistance to these antimicrobials has also developed.

In this study, the examined isolates showed resistance to some of the tested antimicrobial drugs from the group of penicillin’s, cephalosporins, aminoglycosides, tetracyclines, macrolides, polypeptides, which agrees with other researchers who were involved in testing the resistance of bacteria *S. Enteritidis* and *S. Typhimurium*. A major problem of antimicrobial resistance lies in the fact that most antimicrobial drugs used in animal therapy belong to the same groups as antimicrobial drugs used in human medicine.

According to data published by EFSA in the countries of the European Union during 2016, *Salmonella spp*. isolates originating from sick people were resistant to sulfonamides (34.6%), ampicillin (29.5%) and tetracyclines (29.2%). A high percentage of *S. Typhimurium* isolates showed resistance to ampicillin (60.6%), sulfonamides (50.0%) and tetracyclines (51.3%), while S. Enteritidis isolates were resistant to nalidixic acid (18.4 %), colistin (17.5%) and ciprofloxacin / pefloxacin (12.3%). *Salmonella spp*. isolates originating from poultry meat showed resistance to ciprofloxacin (64.7%), nalidixic acid (61.5%), sulfamethoxazole (55.6%) and tetracycline (46.1%), while resistance for ampicillin was 19.7%, and for trimethoprim 14.8% (EFSA and ECDC, 2018).

Antimicrobial sensitivity tests of salmonella isolates isolated from poultry meat in Egypt revealed 100% resistance to erythromycin, penicillin, and amoxicillin, while 98.8% of the isolates were resistant to nalidixic acid, 96.4% to sulfamethoxazole, 95.2% to oxytetracycline and 91.6% to ampicillin. Multiresistance was recorded in 92.8% of isolates (Abd-Elghany et al., 2015).

Studying the epidemiology of *Salmonella Enterica* serovar *Enteritidis* in Greece by comparing isolates from food and animal feed with clinical isolates in a period of three years high resistance to nalidixic acid was detected in isolates of human origin and isolates from poultry (~25%), indicating unsuccessful treatment with fluoroquinolone preparations (Papadopoulos et al., 2016).

Examination of the resistance of *S. Enteritidis* isolated from human food and feces showed that genotypically highly related salmonellae can be found in humans and foodstuffs as well as in poultry. Out of 60 randomly selected isolates only 3 isolates had a multiresistant phenotype. Two isolates isolated from the feces of sick people were resistant to tetracycline (TE), ampicillin (AMP) and the combination of trimethoprim-sulfamethoxazole (SXT), and one isolate from chicken meat showed resistance to ampicillin (AMP), cephalothin (CFT), tetracycline (TET) and nalidixic acid (NAL) (Kozoderović et al., 2011).

The genes responsible for Salmonella antimicrobial resistance are transferred by different ways, including conjugation, transformation, and transduction (Foley and Lynne, 2008). Transfer of resistance genes crosses the species barrier, so transfer can occur between Salmonella (Ferguson et al., 2002), but gene transfer is also possible between *Salmonella spp.* and other bacterial species (Walsh et al., 2008).

Conclusion

Based on the results of the research within this work the following conclusions were performed:
- All tested isolates were 100% resistant to imipenem (IMI), lincomycin (MY), bacitracin (LS), kanamycin (K), vancomycin (VA) and cefuroxime (CXM), 80% to nalidixic acid (NA) and 40 % isolates to amikacin (AK) and gentamicin (CN).
- Moderate sensitivity to amoxicillin (AML) and tetracycline (TE) was shown by 60% of isolates and 20% to amikacin (AK) and gentamicin (CN).
- Resistance to six or more antimicrobial drugs was found in all isolates.

The problem of resistance to antimicrobial drugs was mostly associated with the spread and mechanism of resistance in human pathogenic bacteria. However, the emergence of resistance in zoonotic bacteria has begun to affect therapeutic interventions in humans.

Bearing in mind the fact that the sources, routes, and ways of spreading salmonella in the food chain are inextricably linked to the risks of infecting and getting sick in humans, epidemiological research must include the monitoring and typing of these pathogens both in humans and in animals intended for human consumption, in all stages of the process production and distribution of food.

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ALLIUM URSINUM: MICROBIOLOGICAL AND ANTIBACTERIAL PROPERTIES

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Abstract

Allium ursinum is so far under-tasted an eatable plant species that is used both in culinary and traditional medicine. The aim of this work is to investigates the microbiological property and antibacterial activity of *Allium ursinum* plants from the territory of the Entity of Republic of Srpska in Bosnia and Herzegovina. Microbiological activity was determined by standard ISO BAS methods for *E. coli* and total number of bacteria. Antibacterial activity was determined by disk diffusion method. *Alium ursinum* showed antibacterial activity on *S. Enteritidis* and *S. Typimurium* isolated from food. Based on the results, it can be concluded that *Allium ursinum* is useful as a dietary supplement because it can participate in the control of certain pathogens that can be found in food and the environment.

Keywords: *Allium ursinum, microbiological safety, antibacterial activity.*

Introduction

Allium ursinum popularly known as ‘sremuš’, wild onion, bear's onion, ‘crijemuš’, ‘srijemuš’, ‘skremboš’, ‘cremoš’ is a wild species from the onion family (Alliaceae), that is according to recent systematics it belongs to the Amaryllidaceae family (Govaerts, 2011). The name *Allium ursinum* is of latin origin from the name for onion (Allium) and bear (Ursus) (Konjević et al., 2006.) ‘Sremuš’ has been used since ancient times in traditional medicine as an agent that cleans blood vessels, lowers blood pressure, and helps with gastrointestinal diseases. It has a strong effect on intestinal parasites, prevents inflammation of the intestinal mucosa and is excellent as protection against flu and fever (strengthens immunity) (Igić et al., 2010). ‘Sremuš’ has a beneficial effect on the cardiovascular system, prevents atherosclerosis and helps with dizziness and insomnia (Grlić, 1986), also helps with indigestion (Randjelović, 2011), diabetes and obesity (Kalaiarasan et al., 2009). The eatable parts of the *Allium ursinum* plant are used in nutrition and in the treatment and prevention of a large number of diseases (Sabha et al., 2012), and even cancer (Sengupta et al., 2004). In recent years, the phytochemical and pharmacological properties of *Allium ursinum* (Sobolewska et al., 2015) as well as the antimicrobial properties (Lupoae et al., 2013) have been investigated. It is considered that *Allium ursinum* is a natural preservative in food, so it is recommended as an additional means to control the development of pathogens in food (Roller, 2000).
The goals of this research were to test the microbiological examination and antibacterial activity of *Allium ursinum* on the growth of pathogenic bacteria isolated from food and to determine the type of action.

Material and methods

The plant material used in this research is a fresh leaf of *Allium ursinum* harvested on the mountain slopes of Kruševo Brdo, municipality of Kotor Varoš (Republic of Srpska). 'Sremuš' leaves (150 leaves) are chopped in a blender and preserved with sunflower oil (35 ml) with the addition of salt (5 g). The prepared *Allium ursinum* dressing was placed in a sterile jar and kept in the Laboratory for Microbiology of food, feed, and water until the beginning of the test. For the purposes of the experiment, the 'sremuša' product/dressing was further diluted with sterile distilled water in a 'sremuš': water ratio of 80:20%.

Test method

Microbiological analysis of *Allium ursinum* was performed using standard BAS ISO methods: BAS EN ISO 4833-1 and BAS ISO 16649-2.

To test the effect of *Allium ursinum* on the growth of bacterial strains *Salmonella Enteritidis* (3 isolates) and *Salmonella Typhimurium* (2 isolates), the agar diffusion method was used on a solid sterile nutrient medium, Müeller-Hinton-agar ("CONDA" Spain).

Cultures of microorganisms were transferred under aseptic conditions using a microbiological loop into a test tube with nutrient broth and suspended by intense shaking on a vortex (IKA vortex). The density of the suspension was determined using a densitometer (Densila Meter - "ERBA") and adjusted to correspond to the value of 0.5 McFarland standard. Metal cylinders with a diameter of 9 mm were placed on the surface of a solid nutrient medium on which a certain pure bacterial culture had previously been sown. 100 µl of *Allium ursinum* was dripped into the cylinders with a micropipette. As a control, 100 µl of 96% distilled water was dripped into the cylinder. To accelerate the diffusion of 'sremuš' into the agar, the plates were left for 30 minutes at 4°C and then incubated for 18 hours in aerobic conditions at 37°C±1°C.

The results for the examined parameters were obtained by measuring in three repetitions and expressed as mean value ± standard deviation.

In addition to the above, the type of action of *Allium ursinum* was also determined. To see if *Allium ursinum* has bactericidal or bacteriostatic activity a small piece of agar was taken from the zone of inhibition and added to the nutrient broth. Incubation was carried out for 24 hours at 37ºC. If the broth became cloudy after incubation it is considered that *Allium ursinum* had a bacteriostatic effect or if the broth remained clear after incubation, the effect of *Allium ursinum* is bactericidal.

Results and discussion

The microbiological examination of *Allium ursinum* dressing for the presence of *E. coli* and the total number of bacteria was tested by standard BAS ISO methods, and the results are shown in the table (Table 1).

Table 1. Microbiological analysis

Microbiology parameter	Meausuring unit	Allowed value	Found value	Method
Escherichia coli	cfu/g	$<10^2$	<10	BAS EN ISO16649-2
Total number of bacteria	cfu/g	$<5.0x10^6$	<10	BAS EN ISO4833-1

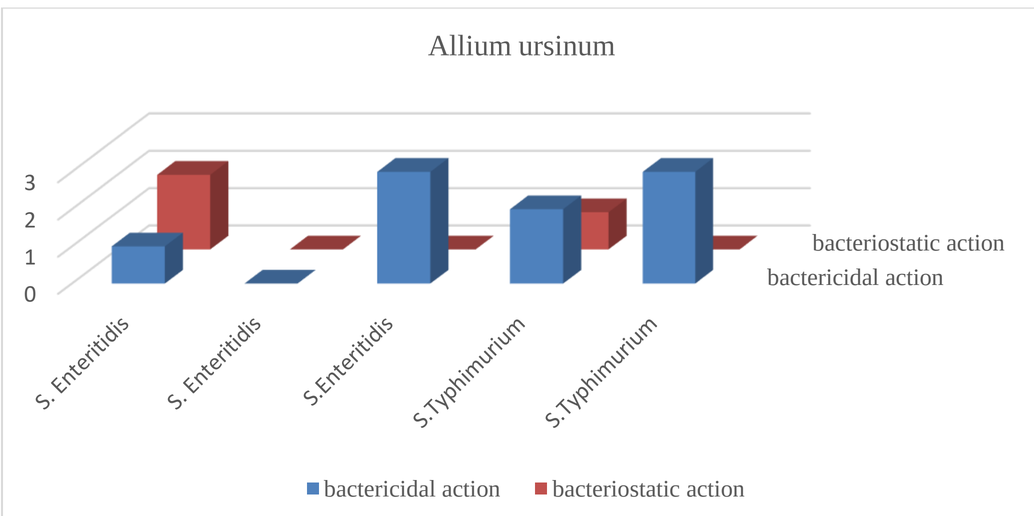
Microbiological analysis of *Allium ursinum* dressing showed that this domestic product does not contain unwanted microorganisms.

Microorganisms	Zones of inhibition were measured in mm
	'Sremuš': water 80:20 %
S. Enteritidis	17,33±2,05
S. Enteritidis	0,00
S. Enteritidis	13,33±1.24
S. Typhimurium	16,33±0.81
S. Typhimurium	16,00±0.46

Allium ursinum dressing as an original domestic product, was tested using the disc diffusion method and showed an antibacterial effect on four out of five tested pathogenic bacteria isolated from food samples. The range of action from 13.33 mm to 17.33 mm. On both isolates of S. Typimurium, Allium ursinum acted with a circumference of 16.00 mm and 16.33 mm, while on one isolate S. Enteritidis acted with a range of 17.33 mm, on the other with a range of 13.33 mm. There was no antibacterial effect on one S. Enteritidis isolate.

The results of this research showed that Allium ursinum has a certain antibacterial effect on the examined bacterial pathogens (members of the Enterobacteriaceae family).

Allium ursinum had a 100% bactericidal effect on one isolate of S. Typhimurium in all three repetitions, while on another isolate of S. Typimurium it had a bactericidal effect in two repetitions and a bacteriostatic effect in one repetition. Also, bactericidal activity was confirmed in all three repetitions on one S. Enteritidis isolate, while bacteriostatic activity was confirmed in two repetitions on another S. Enteritidis isolate. The graph (Graph 1) shows the test results of the type of action of Allium ursinum on the tested pathogens.



Graph 1. Bactericidal and bacteriostatic action of *Allium ursinum*

The results of this research agree with the results of Synowiec et al., (2010) who conducted comparative tests of aqueous and methanol extracts of *Allium ursinum* and showed greater antimicrobial activity of the methanol extract on: *S. aureus*, *B. subtilis*, *E. coli*, *P. mirabilis*, *S. enteritidis*. In the literature, there are data on the testing of different extracts from *Alium ursinum*. Ivanova et al., (2009) investigated the antibacterial activity of acetone, chloroform, ethyl acetate, n-butanol and water extracts of fresh flowers and leaves of the Bulgarian species *Allium ursinum*. The results of their examination showed that none of the extracts showed antimicrobial activity against *E. coli*.

In this research, *Allium ursinum* was used without the addition of stabilizers and diluents, so the result of antibacterial action on selected pathogenic bacteria is the more interesting. According to the available data, *Allium ursinum* is best used at its source, i.e., immediately after picking due to its instability, because the moment the leaf is picked it begins to lose its active compound (allicin) very quickly which begins to oxidize in contact with air, decomposes and lost medicinal properties very quickly. It is believed that *Allium ursinum* loses half of its medicinal properties within 24 hours and in 48 hours they disappear completely leaving only a tasty herb. According to available data, the antimicrobial activity of *Allium ursinum* bulb juice correlated with storage temperature and pH levels. Its activity against selected bacteria and fungi decreases with storage at a temperature above 4°C and with an increase in pH value (Tinecka et al. 1993).

The domestic product/dressing from *Alium ursinum* used in this research is 48 hours old (from the time of picking to the time of processing in the laboratory) and as such has shown satisfactory results.

One of the reasons for the different antimicrobial effect of our product/dressing from *Alium ursinum* can be explained by the different solubility of the active components. Hydrophobicity is an important characteristic of essential oils and plant extracts because it increases the permeability of the bacterial cell membrane and enables easier passage of components into its lipid layer. The change in the permeability of the cell membrane is usually accompanied by the loss of osmotic control of the cell which is considered the basic principle of antimicrobial action of plant extracts and essential oils (Bajpai et al., 2012 Hyldgaard et al., 2012). Due to the different solubility of antibacterial components in water different methods of testing antibacterial activity are used. The disc diffusion method was used in this work, which depends entirely on the hydrophobicity of the active components and the speed of their diffusion through the agar (Kalaba et al. 2019, Bubonja et al., 2008). The results and interpretation of the results of the disk diffusion method can be influenced by numerous factors, including the bacterial species and strain, growth conditions (time, temperature), pH of the substrate, etc. (Kalaba et al., 2019).

The antibacterial activity of *Allium ursinum* probably originates from sulfur-containing compounds (Sobolewska et al., 2015). Allin is a natural ingredient and component of fresh *Allium ursinum* and it is a derivative of the amino acid cysteine which is converted into allicin under the action of the enzyme alliinase. Allicin is a precursor of sulfur compounds responsible for the smell and some pharmacological properties of ‘sremuš’. When exposed to atmospheric air allicin is transformed into another compound (dialydissulphinate) and have an antimicrobial effect. Its advantage compared to antibiotics is that it contains essential oil with allicin and sulfate compounds where viruses and bacteria cannot gain resistance (Wang et al., 2011; Venâncio et al., 2017).

The tested *Allium ursinum* product/dressing prepared in the way it is used in the household has good and promising potential for use in the food, pharmaceutical and cosmetic industries. Testing the antibacterial activity of the plant *Allium ursinum* in this way was done for the first

time in the Republic of Srpska. Also, the obtained results can provide some explanations about the use of ‘sremuša’ in traditional medicine and be the basis for a series of further research that would be directed towards new ways of using this plant as biologically active, natural supplements.
Given that it is not available throughout the year and that it can only be found fresh for a very short time, the ideal way to use it throughout the year is to prepare it in the same way as this examined sample.

Conclusion

Based on the achieved and presented results the following conclusions can be drawn:
- The results of the microbiological analysis showed that *Allium ursinum* is a microbiologically safe product.
- *Allium ursinum* had an antibacterial effect on two of the three tested isolates of *S. Enteritidis* and two isolates of *S. Typhimurium* with a range of action from 13.33 mm to 17.33 mm.
- *Allium ursinum* did not show antimicrobial activity against one tested isolate of *S. Enteritidis*.
- The bactericidal activity of *Alium ursinum* was confirmed in one isolate of *S. Enteritidis* and one A: Typhimurium in all three repetitions, and bacteriostatic in one repetition in *S. Enteritidis* and in two repetitions in one isolate of *S. Typhimurium*.

The results of the research confirmed that *Allium ursinum*, in any form, as fresh or as a dressing prepared from fresh leaves can be used as an additional means to prevent the appearance of pathogenic bacteria in food and have a beneficial effect on the body and human health. The dressing used in this research is an original product, made from sremuš from Kruševo Brdo mountain, Čelinac municipality, Republic of Srpska.

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THE TOMATO LEAFMINER, *TUTA ABSOLUTA* MEYRICK (LEPIDOPTERA: GELECHIIDAE) IN TURKEY: CURRENT STATUS

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Abstract

Tomato (*Solanum lycopersicon* L.) is a major open-field and greenhouse vegetable crop grown throughout the world. Turkey is in the third rank among the top three tomato-growing countries in the world, after China and India with a production amount of 13 million tons. The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most devastating pests of tomatoes in Turkey since it was first recorded in 2009. In the last 13 years, the pest has continued its spread and invaded all of the main tomato-growing provinces in Turkey. *Tuta absoluta* has led to severe damage to tomato production both in open-fields and greenhouses. Chemical control has still the most preferred control method by the farmers in Turkey in order to prevent crop loss derived from *T. absoluta*. However, due to resistance development, failure in chemical control has often been reported. Other control methods such as mating disruption and mass trapping have also been implemented in the control of the pest and the results showed that they were promising alternatives to chemical control. This paper presents the biology of the tomato leafminer according to the regions, damage and economic importance, control methods, updated distribution map, new host plants, and the current status of *T. absoluta* in Turkey. The available literature was briefly reviewed in order to provide an overview of the current knowledge on this pest.

Keywords: *Tomato, Tomato leafminer, Tuta absoluta, Turkey.*

Introduction

Tomato (*Solanum lycopersicon* L.) is a major open field and greenhouse vegetable crop grown throughout the world (Wako and Mulet, 2022). In addition to being consumed fresh, tomatoes are also used as raw materials in the food industry. According to 2020 data, a total of 187 million tons of tomatoes were produced in 168 countries around the world and 52.80% of these tomatoes were produced by the top three tomato-growing countries. Turkey is in the third rank among the top three tomato-growing countries in the world, after China and India with a production amount of 13 million tons (Anonymous, 2022a; 2022b).
The tomato leafminer, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the most harmful pests, that has led to severe damage to tomato production both in open-fields and greenhouses all over the world (Han et al., 2019). This pest originated from South America, and was first described in Peru in 1917 by Meyrick (Meyrick, 1917). In Europe, it was observed for the first time in eastern Spain, in 2006 (Urbaneja et al., 2007) and then subsequently spread to other Mediterranean countries. Currently, *T. absoluta* has invaded most of Europe, Africa, the Middle East, and parts of Asia (Han et al., 2019).
Tuta absoluta was reported for the first time in Turkey, in İzmir (Aegean region) and Çanakkale (Marmara region) provinces in 2009 (Kılıç,, 2010). The pest spread to almost all main tomato-

growing areas in Turkey in a short time due to the high mobility and reproductive potential. About 5 months later, the pest was detected in Antalya where the city with the highest tomato production in Turkey (Erler et al., 2010). In the same year, in April, the pest was detected in Mersin (Karut et al., 2010). In the last 13 years, the pest has continued its invasion and become a key pest of tomato plants.
In this paper, the information about the tomato leafminer, including its biology according to the regions, damage and economic importance, control methods, updated distribution map, new host plants, and the current status of *T. absoluta* in Turkey was summarized and updated. The available literature was briefly reviewed in order to provide an overview of the current knowledge on this pest.

Biology

Tuta absoluta complete their life cycle in four biological stages: egg, larva (four stages), pupa and adult. It is a multivoltine species and has a high reproductive potential that allows the pest population to increase very quickly. The adults are nocturnal and usually hide between leaves during the day. Females lay eggs usually under the leaves, buds and sepals of immature green tomato fruits. During its lifetime a female can lay 120-260 eggs. Depending on the environmental conditions, pupation takes place in a silky cocoon; in the soil, on the tomato plant or in the galleries that the last stage larva open on the tomato plant. Turkey consists of seven geographical regions and tomatoes are grown in almost every region more or less. The biology of the pest is generally the same, with some variations from region to region.
Studies conducted in Mediterranean region revealed that the pest is active throughout the year in Antalya, the adult population generally increases in spring and autumn, and decreases considerably in summer and winter (Tatlı and Göçmen, 2011). As long as food is available, the pest does not enter diapause under suitable conditions, and there may be 10–12 generations per year. The biological cycle is completed in 29–38 days depending on environmental conditions. In open field tomatoes cultivation in Adana, the first adults were captured in traps in April and reached its highest level in June (Portakaldalı et al., 2013).
In Aegean region (Aydın province), the pest gives 7-8 generations in both spring and fall tomato-growing seasons in the glasshouse. In fall season, the first adult was captured on 16 October 2010 in trap, in spring season, the first adult was captured on 29 January 2011 (Demiroz and Gencsoylu, 2016). Aksoy and Karaca (2015) determined that the first adults were caught in pheromone traps on 5 June 2014 and reached the zero point in the last week of October in greenhouse. In the open field, the first adults were caught in traps on 19 June 2014 in Uşak province.
The first adults were captured in traps on the first week of May in open field, the pest could complete up to 4-5 generations per year and could not overwinter in Central Anatolia region (Erdoğan, 2016; Ünlü et al., 2021). In greenhouses with a heating system, the first adults were caught in pheromone traps on 25 October 2011 in Konya province, and the pest continued to damage throughout the production season by forming a peak about every four weeks (Özkan et al., 2017). In greenhouse with no heating system, the first adults were captured in traps in the last week of April-first week of May, and the population reached its highest level towards the end of the growing season (August) and the pest gave 2-3 generations during the production season in Konya (Çatlı and Ünlü, 2022).

The flight activity of the pest began on 2 June and continued until 11 October 2011 in Bursa in open field tomato growing areas, and also showed 3 distinct flight peaks during the growing season (Pehlevan and Kovanci, 2013). In Marmara Region, males of the pest were caught in traps for the first time on March 15 in 2016 and March 20 in 2017 in Çanakkale province (Polat, 2020), and it was determined that the pest gave 5 generations during the tomato production season (Polat et al., 2016; Polat, 2020).
In Southeastern Anatolia region, the pest was active from May to November in Şanlıurfa and gave 4 generations in open field conditions (Mamay and Yanık, 2012). In Diyarbakir province, the pest gave 3-5 generations in open field (Bayram et al., 2017).

Damage and Economic Importance

In Turkey, both in open field and greenhouse conditions, *T. absoluta* is considered as a key pest of tomato (Aksoy and Karaca, 2015; Polat et al., 2016; Ünlü et al., 2021). The larva can cause damage in all aerial parts of the tomato plant except the root and all growth stages (seedling, vegetative growth, flowering, fruit development). Larvae penetrate leaves, stems, or fruits. They feed by opening galleries between the two epidermises on the leaves of the tomato. These galleries are wide and conspicuous in the form of transparent spaces and affect the photosynthetic capacity of the plant. The pest prefers mostly immature tomato fruits and enters from the sepals. It is possible to see the black-colored feces of the pest in the galleries opened on the leaves and fruits. The direct feeding of the pest can significantly reduce yield and fruit quality. And also, secondary pathogens that may enter through mines made by the pest on fruits often cause a decrease in the commercial value of the fruit (Özkan et al., 2017; Ünlü et al., 2021).

Control Methods

In 2009, when T. absoluta invaded Turkey, farmers did not know how destructive this pest was and how to control it. And also, there were no registered insecticides for T. absoluta (Ugurlu Karaagac, 2012). Therefore, the pest caused serious yield loss. Currently, there are many insecticides registered for T. absoluta in Turkey (Anonymous, 2022c), and since it was detected in our country the main control method against this pest has been chemical control both in open-field and greenhouse conditions. Unfortunately, when the pest population starts to increase, farmers apply insecticide almost every week, both in open-field and greenhouse tomato cultivation (over 15 applications per cultivation period). However, the development of resistance against some insecticides has been reported in our country (Yalcin et al., 2015; İnak et al., 2021). On the other hand, sex pheromone-based monitoring/control strategies, such as mating disruption and mass trapping have been used to control T. absoluta (Polat, 2019; Erler and Bayram, 2021; Ünlü et al., 2021) and they have been considered as promising alternatives to control of tomato leafminer. Moreover, some biological control agents, such as Trichogramma evanescens, Trichogramma brassicae, Nesidiocoris tenuis, Beauveria bassiana, Isaria farinosa, and Purpureocillium lilacinum have been used to control T. absoluta and promising results were obtained (Keçeci and Öztop, 2017; Yüksel et al., 2017). Despite all these positive results, biological and biotechnical methods are still used on an experimental or small scale in Turkey. The consensus of all these studies is that chemical control alone is insufficient in the management of tomato leafminer, and therefore, this pest should be controlled within the scope of integrated pest management by using cultural measures, biological and biotechnical methods.

Distribution Map

Tuta absoluta spread rapidly in our country and was detected one year later in Iğdır, which is approximately 1500 km away from İzmir, where it was first detected in our country. Such a rapid spread of the pest may be due to the trade of tomato fruits. In addition to this, active (by flight) and passive (by wind) spread are also possible. In Turkey, it was determined that 39 provinces in different regions, especially in the Mediterranean and Aegean regions, were infested by *T. absoluta* in 2010 (Ugurlu Karaagac, 2012). The distribution map of *T. absoluta* in Turkey has been updated with the help of the literature and the information obtained from the relevant units of the Republic of Turkey Ministry of Agriculture and Forestry Directorates of Provincial Agriculture and Forestry. The updated distribution map of *T. absoluta* in Turkey has shown in Figure 1. In Rize and Kars provinces, where the pest has not been detected yet, tomato production is almost non-existent. Therefore, it can be said that the pest has invaded in all provinces where tomato cultivation is carried out in Turkey.

Figure 1. The updated distribution map of *T. absoluta* in Turkey

Host Plants

The major host plant of *T. absoluta* is tomato (*Solanum lycopersicum* L.) but it can also feed, develop and reproduce on other cultivated Solanaceous such as potato (*Solanum tuberosum* L.), eggplant (*S. melongena* L.), pepino (*S. muricatum* L.) and as well as on various wild weed species. However, here we discussed the hosts, which are the first records for both Turkey and the world, as a result of the studies carried out in Turkey. In the East Mediterranean Area of Turkey, *Convolvulus* spp. was recorded as the host of *T. absoluta* for the first time in the world by Karabüyük et al (2011). The pest was detected on potatoes (*Solanum tuberosum* L.) by Ünlü (2012) and this was the first record of potatoes as the host plant of *T. absoluta* in Turkey. Ogur et al (2014) determined that *Chenopodium album* L. is the host of the *T. absoluta* in Konya province and they declared that this is the first record of *C. album* as a host of the tomato leafminer in the world. Polat et al. (2015) determined two new weeds, *Sinapis arvensis* L. and *Sonchus oleraceus* L., as the host of the pest in Çanakkale province. In the Southeast Anatolia Region of Turkey, *Solanum woronowii* Pojark., *Physalis angulata* L., *Xanthium strumarium* L., *Amaranthus viridis* L. and *Sorghum halepense* L. were recorded by Bayram et al (2015) for the first time as hosts of *T. absoluta* in Turkey.

Conclusions

Tuta absoluta has been considered as a key pest of tomatoes in Turkey for 13 years, both in open-fields and greenhouses. Within this period, the pest continued its spread and invaded all of the main tomato-growing provinces in Turkey. A lot of research has been conducted about the biology, damage, new host plants, and especially new control methods. Nevertheless, in the control of the pest, further studies are still needed especially eco-friendly control methods like biotechnical and biological control. In particular, in order for these studies to be adopted by the farmers, the results should be shared with them. Although it is obvious that chemical control alone is not effective in the control of tomato leafminer, the main control method is still chemical control in our country. Therefore, one of the upcoming dangers in the management of *T. absoluta* is insecticide resistance. Integrated pest management strategies should be used both in greenhouse and open-field, in order to prevent insecticide resistance and be successful in the control of *T. absoluta*.

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PESTICIDE APPLICATION TECHNIQUES IN PRECISION AGRICULTURE

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Abstract

Agriculture is a vital sector that is directly concerned with society when it is studied economically, socially and environmentally, because people provide raw materials to other sectors as well as life, employment. In order to meet the food needs of the world population, which will be approximately 9.7 billion in 2050, production must be increased by 70%. Agricultural practices carried out in order to meet the growing population food needs bring various risks and problems. In particular, unconsciousness in fertilizer and pesticides practices or technical inaccuracies in practice have impacted natural balance. Today, with the increasing importance of food and environmental awareness, precision agricultural techniques have become priority issues. Precision agriculture allows farmers to take advantage of the power of science and technology to provide optimal control, as well as increase land productivity with the use of technological tools. In precision agriculture, remote sensing geographic information systems and variable application technologies are used together. Precision agricultural applications enable use from soil processing to harvesting in the agricultural area. Reducing the risks from the point of view of food and the environment that may arise as a result of improper and excessive use of pesticides, improving the quality and efficiency of production are important issues that should be focused on. In precision agriculture today, pesticides are done with techniques such as unmanned aerial vehicle (UAV), Variable rate application technologies (VRA), geographical information systems, global positioning system (GPS). In this study, pesticides application techniques that have found the area of use within precision agriculture and are subject to research have been evaluated.

Keywords: *Precision agriculture, pesticide, pesticide applications, sprayer, food safety.*

Introduction

Since humans have existed, they have had to meet their nutritional needs in order to continue their vital activities. The rapid increase in the world population in recent years presents the food problem as the biggest problem. Today, the agricultural sector increases its importance in terms of providing raw materials to many sectors, not just nutrition. As a result of the development of the industry in which agricultural products are used as raw materials, diversity in production has also increased. (Kirişçi et al., 1999).

Sustainable production is important for existing agricultural areas due to the possibility of increasing agricultural production areas or the difficulty of finding new agricultural areas. In terms of agricultural production, the sustainable productivity of the soil is closely related to the balance of plant nutrients in the chemical, physical and biological processes that take place in it. Today, the most basic goal is to reach the desired levels in terms of quality and quantity in production by using environmentally friendly techniques for agricultural production.

In terms of sustainable agriculture, it has been thought to benefit from the technological developments used in non-agricultural fields (industry, transportation, communication, medicine, etc.) in agricultural production in recent years (Tekin and Sındır, 2006). Advances in technology provide new possibilities in farm and land management (Goddard et al., 1995). Studies on the use of these technologies to increase economic and ecological efficiency in agricultural production are generally referred to as precision agriculture (Kirişçi et al., 1999). This new farming method is also expressed with different concepts due to its characteristics (Görücü et al., 1998). Basic elements of technologies used in precision agriculture; data collection, data processing and decision making, variable-level application can be grouped into three main groups (Blackmore et al., 1994). In order to achieve success in precision agriculture, strategies (Figure 1) must be developed and implemented correctly (Blackmore, 1996).

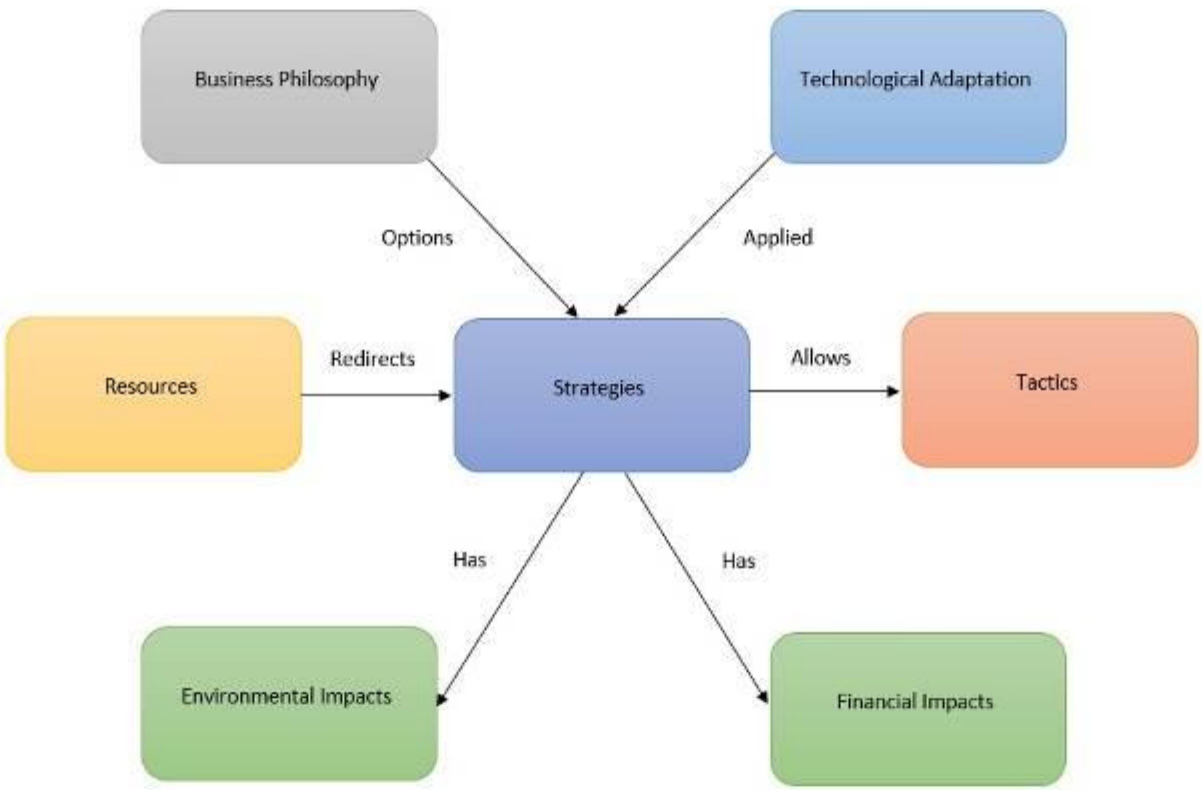

Figure 1. Strategy formation in precision agriculture

Obtaining the yield value constitutes the basis and the beginning of the data collection process, which is one of the basic elements of the technologies used in precision agriculture (Dodd et al., 1999). Data processing and decision making in precision agriculture; The product filled in the machine warehouse during the harvest is recorded with the appropriate sensors together with the geographical location information, and then the yield map is drawn (Keskin et al., 1999). Variable level implementation; The inputs are applied with two different methods: according to a map or according to the sensor on the machine (Keskin and Say., 2007). In order for the Precision Agriculture system to bring ultimate benefit, these technologies should be used.

Energy, water, seeds, fertilizers and pesticides are used as basic inputs to increase yield and quality in agricultural production. Among these inputs, pesticides carry the greatest risk. Therefore, pesticide applications should be done with the right parameters.

Pesticide production, which is the main element of agricultural struggle in the world, is increasing rapidly and new pesticides are being developed day by day. Chemical control should be applied sensitively, carefully and with the least loss due to the negative effects of pesticides on human health, environment and natural balance and increasing production costs (Dursun 2000).

As of the end of 2018, pesticide consumption in the world is 3.8 million tons per year; sales amount is approximately 58 billion USD. Pesticide consumption in Turkey is below the world average of 2.0 kg/ha and is low compared to developed countries (Yalçın and Turgut, 2016 Kartal, 2019). As of 2018, pesticide consumption in Turkey is 59.000 tons and the sales amount is approximately 2.5 billion TL (ZMO, 2019).

Many researches are carried out to reduce the use of pesticides in the world. In their study, Koç and Keskin (2001) developed a system that will keep the boom actively parallel to the field surface in the field sprayer.

Ishak and Rahman (2010) developed a system that detects weeds and applies pesticides in their study. The system also determines the density and emergence points of weeds. Depending on the density or percentage of weeds, the spray nozzles open or close. Habib et al (2007) used image processing method to classify weeds in their laboratory research. Classification of narrow and broad-leaved weeds was made and they achieved 97% success.

Precision farming and pesticide application techniques

Precision Agriculture systems bring together an advanced system approach by combining control, electronics, computer and database and account information.

In precision agriculture; global positioning system, geographic information system, variable rate application and remote sensing technologies are used. With these applications, instead of the usual applications made to the whole field, variable-level applications are made by determining the soil and plant characteristics of the smaller parts. Thus, agricultural production aiming at more economical and environmentally friendly production is carried out (Özgüven and Türker, 2010).

By reducing the use of inputs in precision agriculture, waste of resources can be prevented and at the same time, negative effects on the environment and natural life can be prevented. (Özguven and Karaman, 2012).

In precision agriculture, there have been significant changes in business operations compared to traditional agriculture. In precision agriculture, basic inputs such as seeds, pesticides, fertilizers and water are GPS, remote sensing, real-time sensors, etc. thanks to the field-specific applications. Efficient use of inputs is ensured, product quality and productivity increase despite decreasing production costs (Özgüven and Türker, 2010; Özgüven, 2018a).

The target of precision agriculture is not only an increase in yield, but also includes practices that will save on input usage without causing loss of efficiency (Vatandas et al., 2005).

In precision agriculture applications, many components (figure 2) interact with each other (Türker, 2001).

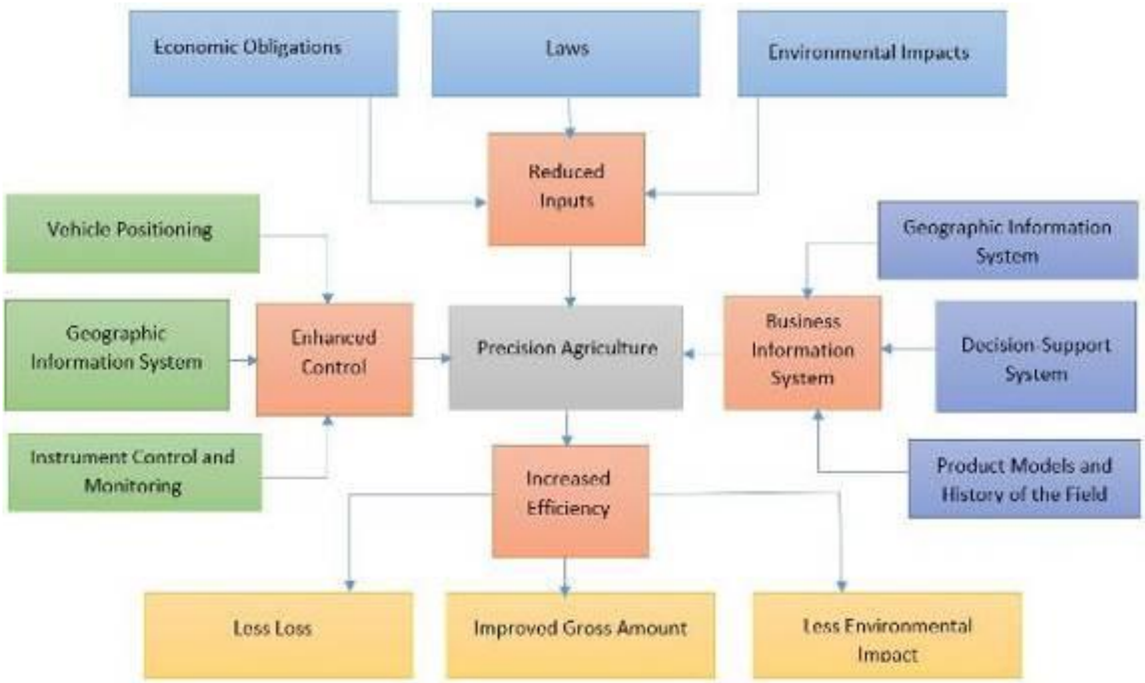

Figure 2. Precision agriculture and its components

Pesticide

pesticide; It is known as substances or mixtures of substances that prevent, destroy, remove or reduce the damage of harmful organisms in plant production.Considering that pesticides consist of chemical compounds and the risks they carry, care should be taken when using them (Figure 3). When pesticides are used unconsciously and excessively, they can lead to extremely dangerous results in terms of human, food, natural life and environmental health. The importance of using new technologies in pesticide applications suitable for precision agriculture is increasing day by day.

Figure 3. Pesticide application

GPS (The Global Positioning System)

The purpose of the GPS system is to determine the position of the GPS receiver by measuring the relative distances with the help of signals sent simultaneously from satellites with known orbits (Eren and Uzel, 1995).

It is a satellite-based system that provides three-dimensional location, speed and time information (Figure 4) from anywhere in the world, 24 hours a day, regardless of weather conditions. (Salgin, 2007).

GPS is very important in terms of providing basic spatial data of precision agriculture in the field (Çorumluoğlu et al., 2007).

Figure 4. Pesticide application with the help of GPS technology

GIS (Geographic Information System)

Geographic information systems (GIS), mapping software, economic analysis, modeling, decision support systems, data processing and appropriate decision-making is the technology used. GIS makes it possible to examine the relationships between knowledge levels in order to determine the cause and effect relationship and to make decisions based on this information (Tekin and Sındır, 2006).

VRA (Variable Rate Application Technologies)

Variable rate applications with smart machines in plant production are carried out both in soil cultivation and in planting, fertilization, pesticides and irrigation. Especially in recent years, with the increase in the use of robot technology in agricultural areas, the issue of variable rate agricultural applications has come to the fore. Agricultural inputs (seeds, water, pesticides) provide variable level application opportunity by placing electronic control systems on equipment (Figue 5).

Figure 5. VRA technology application in the field

UAV (Unmanned Aerial Vehicle) (Drone)

Pesticide and fertilizer applications in agricultural lands are very important to increase yield. The use of UAV is preferred because it is fast and effective in pesticide applications (Figure 6). However, remaining untreated area or spraying the same area over and over may reduce the yield. The direction and intensity of the wind during spraying may adversely affect the application. Faiçal et al. (2014) developed an algorithm to eliminate these effects in their study.

Ay and İnce (2015) developed a UAV system that can be used in the field. The system consists of an autopilot, ground control station and spray system that controls the UAV. With the developed system, the range of the UAV and the time it stays in the air are predetermined.

Figure 6. UAV use in pesticide applications

Electrostatic Charge Technique

In the electrostatic charging technique, spray droplets are charged with static electricity. As these charged spray droplets approach the plant, they create an opposite charge on the plant (Figure 7). Thus, it provides the formation of an electrostatic attraction force between the spray droplets and the plant surface and the collection of the spray droplets on the plant surface (Dursun et al., 2005). While the electrostatic charging technique increases the coating rate, it also reduces the drag, thus reducing environmental pollution. The most important advantage of this method is that it allows to reduce the drag that occurs when the spray droplets reach the target by approximately 50%. (Anonymous 2013i).

Figure 7. Use of electrostatic charging technique in pesticide applications

Air assisted sprayer Technique

The air produced with the help of the fan in the sprayers reaches the nozzles with the help of the air duct mounted on the spray system. In order to provide a homogeneous air flow, the air pipe is manufactured in a narrowing section towards the ends of the spray system. In these sprayers, the drift is lower (Dursun et al., 2005; Yağcıoğlu, 2008).

Conclusions

Precision farming practices ensure that the yield is at the highest level by informing the farmers on which field and what kind of application they should make, the amount of nutrients and water that the plant needs, the condition of the soil, the estimated harvest time, the detection of diseases and pests. In this way, farmers have the opportunity to observe and manage their agricultural lands. It is known that mistakes in pesticide use cause risks in terms of human, food and environment. In order to prevent these risks, researches on the determination of the right machinery and pesticide application techniques increase their importance day by day. Research in this area is generally aimed at preventing drift due to the adhesion of the pesticide on the target surface. Sensitive practices in terms of safe and sustainable agriculture have increased their importance in recent years. In particular, the evaluation of pesticide applications within the concept of precision agriculture highlights the researches on application techniques. In terms of safe food and sustainable agriculture, the use of pesticides is one of the most important issues that need to be emphasized in precision agriculture.

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THE USE OF RGB AND HYPERSPECTRAL IMAGING IN DETECTION OF CODLING MOTH AND ITS DAMAGES ON APPLE

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Abstract

Codling moth is a cosmopolitan pest that causes economically significant damage in apple production. The damage is visible on the fruits, which lose their organoleptic properties and market value. Therefore, the use of artificial intelligence offers a good perspective for the early detection of the pest and its damage in the field. Information and communication technology has contributed to the use of intelligent devices throughout the agricultural chain. In the context of precision agriculture, the artificial intelligence system is a comprehensive solution for the digitalization of agriculture. In practice, this system involves the creation of an information database, and in the case of pest monitoring, red-green-blue (RGB) and hyperspectral imaging cameras (HSI) can be used. These cameras record the occurrence of pests and damage in orchards. Later, these photos are processed using machine learning methods. Based on all the data, accurate models are developed to identify the target pest and facilitate monitoring and management. Inhibiting factors for the use of cameras can be the high market price, the lack of certain electronic components, and the required expertise. Nonetheless, high-precision classification models for pest monitoring represent the future of agriculture and offer a new opportunity to reduce economic losses caused by codling moth.

Keywords: *Precision agriculture, Artificial intelligence, RGB and HSI cameras, Cydia pomonella L., Apple production*.

Introduction

The codling moth (*Cydia pomonella* (Linnaeus, 1758)) is native to Eurasia but is now distributed worldwide (Ciglar, 1998, Franck *et al*., 2007). Its main host is apple (*Malus domestica* Borkh., 1803), one of the most widely grown fruit crops worldwide (Gardiner *et al*., 2007). It is the most important economic pest in apple production (Thaler *et al*., 2008). The larvae penetrate the apple fruit and enter the core, where they leave brown-colored holes filled with larval droppings (Maceljski, 2002). If no chemical treatment is used during production, codling moth can cause a 30-50% decrease in apple yield (Šubić, 2015), so the most important requirement for the market is the production of high-quality fruit without symptoms caused by this pest (Lacey *et al*., 2008). Although there are a variety of environmentally friendly IPM strategies (e.g., mating disruption, sterile insect technique, and attract-kill strategy), farmers mostly opt for insecticide treatments (Ju *et al*., 2021) Frequent insecticide applications are harmful to human health, the environment, and beneficial insects and affect food safety. In addition, codling moth has developed resistance to insecticides due to intensive chemical treatments (Kadoić Balaško *et al*., 2020). Because of all these problems, early detection and identification of damage is crucial so that farmers can respond in time and prevent huge production losses. The classical approach to monitoring insect pests is based on setting traps in orchards, which are then inspected by human personnel at regular intervals. This strategy involves high labor costs and provides low spatial and temporal resolution (Preti *et al*., 2021a). The application of different techniques, including the use of red-green-blue (RGB) and hyperspectral cameras (HSI) to monitor pests can bring many benefits. The ability to monitor pests in real time and online from a remote location allows continuous measurement of insect population dynamics in numerous traps with a limited need for human labor (Preti *et al*., 2021a). Since apples are grown on huge areas (according to FAOSTAT (2022) the harvested apple area in 2020 was 4.62 million ha), monitoring codling moth is a major challenge. Therefore, the use of artificial intelligence is an excellent solution for early detection of codling moth. This paper presents the use of RGB and HSI cameras as a tool for rapid and accurate codling moth monitoring in apple crops to improve apple production and food safety.

The use of RGB imaging in codling moth detection

RGB cameras are designed to produce images in the same spectrum as the human eye by capturing light in red, green, and blue wavelengths (400 to 700 nm) for accurate color representation. Just like the human eye, visible cameras require light. Their performance is also severely affected by fog, haze, smoke, heat waves and smog. This limits their applications to daylight and clear skies. Therefore, RGB cameras often need to be combined with illumination or thermal imaging cameras to operate at night or in adverse climatic conditions (Infiniti Electro-Optics, 2020).

RGB imaging is widely used in various fields, including agriculture, especially in monitoring agricultural crops (Osroosh *et al*., 2018). For example, these cameras are used to distinguish plants from weeds using various machine learning algorithms for data analysis, such as Convolutional Neural Network (CNN) and Artificial Neural Network (ANN) (Su 2020). RGB imaging has also recently been increasingly used to detect important economic pests to increase orchard prosperity while reducing the need for pesticides (Brunelli *et al*., 2019; Feng *et al*., 2022). RGB cameras are often integrated into so-called smart trap in orchards and the system is operated to automatically detect important pests and alert the farmer to apply targeted pesticide treatments.

Ding and Taylor (2016) used RGB images captured in pheromone traps installed at multiple sites to develop an automated codling moth monitoring system based on Deep Learning. This system counts and determines codling moth individuals based on images captured inside the trap. Schrader *et al*. (2022) presented a more affordable trap or RGB imaging system that can be integrated with existing and widely used delta pheromone traps for codling moth monitoring. This system consists of an RGB image sensor combined with a microcontroller unit and associated hardware for optimized power consumption and data acquisition. The advantage of this system is the ability to activate sleep mode to save battery power. The system can take a picture every day. This facilitates monitoring of codling moth population, which is one of the goals of RGB imaging in agriculture.

Albanese *et al*. (2021) improved existing smart RGB imaging traps for codling moth monitoring. They implemented sophisticated machine learning algorithms so that the smart trap can detect pests in orchards in a very short time without requiring cloud infrastructure, which is common in machine learning applications. In addition, Preti *et al*. (2021b) reported the development of a prototype smart trap using the latest technologies. The detection of codling moth individuals was

based on preliminary analyzes of photographs taken daily under field conditions. In this case, they showed that the smart trap system can provide higher temporal resolution of images compared to standard monitoring at a slightly higher cost.
Preti *et al*. (2021c) evaluated a commercial smart trap (Trapview; EFOS d.o.o., Slovenia) for codling moth monitoring. The smart trap was found to be reliable in terms of data transmission speed, photo quality, and battery life. The main drawback is the incorrect detection of multiple morphologically similar insects. Therefore, it is important to improve the algorithm for detection of the target pest. Pajač Živković *et al*. (2020) tested the same trap system and confirmed that the delta pheromone trap can be replaced by the smart trap with camera in codling moth monitoring, as the proper intervention thresholds were set during the growing season.

The use of hyperspectral imaging in codling moth detection

Hyperspectral imaging is a new technique that analyzes a broad spectrum of light instead of assigning only the primary colors (red, green, blue) to each pixel. The light incident on each pixel is split into many different spectral bands to provide more information about what is being imaged (Schneider and Feussner, 2017).
This is an emerging field that combines the advantages of optical spectroscopy as an analytical tool with two-dimensional visualization of objects through optical imaging. In HSI, each pixel of the image contains spectral information that is added as a third dimension of values to the two-dimensional spatial image, creating a three-dimensional data cube (Lee *et al*., 2002). A simple, well-known example of a three-dimensional data cube is the common RGB color image, where each pixel has the colors red, green, and blue. Hyperspectral data cubes can contain absorption, reflectance, or fluorescence spectrum data for each image pixel (Lu *et al*., 2014).
The use of HSI in industry is common. However, recent interest in HSI technology has also been seen in food quality and safety (Gowen *et al.*, 2007), pharmaceuticals (Lyon *et al*., 2002), healthcare (Vasefi *et al.*, 2016), and agriculture (Lu et *al.*, 2013, Benelli *et al*., 2020, Caballero *et al.*, 2020). In agriculture, HSI is used for many purposes, including monitoring important economic pests in agriculture (Lu *et al.,* 2013).
Considering the great economic importance of apple crops and the damage caused by codling moth, effective monitoring of its infestation is important for maintaining quality and fresh fruit such as apples. In addition, effective early detection of external and internal damage in apples infested with codling moth could significantly prevent postharvest losses and improve the quality of final products.
Rady *et al*. (2017) tested the efficacy of visible (VIS)/near-infrared (NIR) (HSI) hyperspectral imaging for detecting codling moth infestation in apples, as removal of fruit infested with codling moth is critical for consumption of apples, especially when fresh. A HSI was implemented to acquire hyperspectral images for fresh apples, stored at 4,10,17, and 27 °C, for 4 months. The authors thus demonstrated the potential of hyperspectral imaging as a nondestructive method for detecting codling moth infestation in apples. In addition, Ekramirad *et al.* (2021) used near-infrared (NIR) hyperspectral reflectance imaging in the 900-1700 nm wavelength range to detect codling moth infestation at the pixel level in three organic apple cultivars (Gala, Fuji, and Granny Smith). The results showed that the infested and healthy samples were classified at the pixel level with an overall accuracy of up to 97.4%, indicating the high potential of HSI in detecting and classifying latent codling moth infestation in apples of different cultivars.

While there are several research papers on the use of HSI, most of them are limited to laboratory use. The currently available equipment is large, expensive, and difficult to use. The main goal of research at HSI is to make HSI cheaper, more user-friendly, and more compact (Schneider and Feussner, 2017).

Conclusions

The efficiency of RGB and HSI for monitoring damage and early detection of pests has been increasingly explored recently. These methods have so far shown great potential in detecting codling moth and its infestation on apples. If the possibility of affordability of these systems can be considered, they could be available to the general population (small growers) in the future and used intensively for codling moth monitoring. The use of these systems could completely reduce chemical control measures and promote site-specific management, reduce the spread of infection from storage, and reduce the poor quality of the final product. This modern approach to agriculture can greatly facilitate the control of this pest and improve fruit production while protecting the environment and human health.

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APPLICATION OF MODIFIED QUECHERS METHOD FOR THE DETERMINATION OF NEW GENERATION PESTICIDES IN BLACKBERRIES

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Abstract

Blackberries (*Rubus fruticosus*) are fruits that contain vitamins, minerals, amino acids and essential dietary fiber. Blackberries are also considered a rich source of phenolic acids and anthocyanins, which play an important role in the prevention of chronic diseases. Unfortunately, pesticide residues in fruit products are becoming a major health problem for human consumption. However, simultaneous determination of pesticides in fruit is a challenging task due to the complex fruit matrix and the dynamic increase of new pesticides being introduced to the market. In this research, a modified QuEChERS sample preparation method was developed to simultaneously determine seven new generation pesticides, namely cyprodinil, pyrimethanil, chlorothalonil, folpet, fluopyram, pyriproxyfen and fenhexamid. Vortex-assisted solid-phase extraction and an additional clean-up step using 500 mg of primary secondary amine (PSA) were applied successfully. PSA was used to eliminate fatty acids, organic acids, and polar pigments and sugars. Calibration curve linearity was evaluated by spiking blank blackberries samples with the concentration levels: 0.01, 0.02, 0.03, 0.05 and 0.1 mg/kg. The linearity of the analytical response across the studied range of concentrations was excellent, obtaining correlation coefficients higher than 0.99. Matrix-matched calibration was established in order to compensate possible pesticide losses and the impact of matrix effects. The average recovery for all pesticides quantified by gas chromatography mass spectrometry (GC/MS) at 0.01 and 0.02 mg/kg fortifying levels were from 86 % to 109% (RSD < 8.2%). The fungicide cyprodinil was found in 8% of samples but the determined levels were in the range 0.050-0.105 mg/kg and below the maximum residue level established by Reg. (EU) 2021/1810. Other tested pesticides were below the quantification limit.

Keywords: *Pesticides, Blackberries, GC/MS, Extraction.*

Introduction

The increasing use of pesticides in agriculture, as well as long-term use and contamination of soil and water lead to the need for monitoring of fruits and vegetables. Pesticide contamination has been a concern for many years due to the risk of adverse effects on human and animal health (Jovanov et al., 2014). Also, the consequence of using pesticides is environmental pollution (Jin et al., 2012). Modern analytical instruments that use mass detection and tandem mass detection allow the simultaneous determination of a large number of pesticides. Gas chromatography (GC) is one of the most useful analytical techniques for the separation and quantitative determination of many compounds, especially pesticides residues. The main techniques for extraction and concentration of pesticide residues in fruit and vegetables are: liquid–liquid extraction (Narenderan et al., 2020), solid phase extraction (Banno and Yabuki, 2020), solid phase micro extraction and supercritical fluid extraction. Extraction of pesticide residues from different

matrices using QuEChERS method reduce the time for preparation and minimize of extraction steps with the use of acetonitrile for extraction in small amounts (usually volumes 10 ml or 15 ml). Furthermore, the individual acetonitrile or mixture with other organic solvents such as methanol, ethyl acetate, dichloromethane, acetone, acetic acid, hexane, toluene, petroleum ether, cyclohexane, diethyl ether can be used for extraction (Narenderan et al., 2020).
The aims of the present study were validation and determination of new generation pesticides in blackberry samples. Blackberries are produced on plantations in Serbia. Gas chromatographic technique with mass detection was used for detection and quantification.

Materials and methods

Each blackberry sample of 1 kg was homogenized in a blender. 10 g of homogenized blackberry samples were measured into the 50 ml extraction tube. Then 10 ml of acetonitrile were added. The extraction kit consisting of 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate and 500 mg of disodium citrate was added to the tube and vigorously stirred for 2 minutes on a vortex mixer at 2500 rpm. After centrifugation for 5 minutes at 4000 rpm (Hettich EBA 280, US) the 2 ml supernatant was transferred into the microcentrifuge tube containing 150 mg $MgSO_4$, 25 mg PSA (primary secondary amine), and 50 mg C18. An additional 50 mg of PSA was weighed and added to the original microcentrifuge tube. A gas chromatograph system Clarus 680 equipped with Clarus SQ8T mass spectrometer (PerkinElmer, Waltham, MA, USA) was used for sample measurement. A capillary column (Elite-5MS, 30 m, 0.25 mm I.D., 0.25 μm film thickness, 5% phenyl 95% dimethylpolysiloxane) was used to separate the analyte. For the matrix-matched calibration curves, blank blackberries samples were enriched with working standard solutions in the ranges: 01, 0.02, 0.03, 0.05 and 0.1 mg/kg. For recovery and precision, blank blackberries were spiked at 2 concentration levels (0.01 and 0.02 mg/kg).

Results and discussion

Under instrumental conditions GC-MS in scan mode was performed for the tested pesticides applicable to GC analysis, to obtain their full scan spectra and retention times. The full scan mode was selected in the range *m/z* 40–400 for scanning monitoring ions, the quantifier ions and qualifier ions with the highest sensitivity are given in the Table 1. The mass spectrum parameters were optimized to provide good chromatographic separation, identification and quantification of developed method. In the first step, tests of standard solutions were first performed, which were analyzed to obtain the mass spectrum of the entire full scan and to measure the retention times of pesticides. After that, ion selection with more than 15% abundance compared to base peak quantitative ions and three qualitative ions for quantification were selected for each compound.

Table 1. Mass spectrometry analysis parameters for determination of pesticides

Analyte	Quantify trace, m/z	Qualifier ions, m/z	Retention time, min
Pyrimethanil	198	77, 100, 199	13.93
Chlorothalonil	266	109, 264, 268	14.03
Cyprodinil	224	210, 225, 226	20.85
Fluopyram	173	145, 195, 223	21.15
Folpet	104	76, 147, 260	21.38
Fenhexamid	97	55, 177, 179	26.63
Pyriproxyfen	136	77, 96, 137	29.62

In order to increase the extraction efficiency, PSA was chosen as an additional purifying agent, because it has the property to clean the GC-MS background and is powerful for removing the matrix effect. It was found experimentally that the use of 75 mg of primary secondary amine (PSA) gives better recovery and less impact of the matrix effect. Therefore, this modified QuEChERS preparation procedure was used to analyze real samples. Rahman and associates using both PSA and C18 were provided a cleaner extract without hampering analyte determination (Rahman et al., 2018). Method was validated in terms of linearity, limit of quantification (LOQ), specificity, accuracy and precision based on validation guidelines SANTE 11312/2021.

Table 2. Validation parameters of modified QuEChERS method used for determination new generation pesticides in blackberries

Analyte	Calibration equation	Correlation coefficients, (r^2)	LOQ, (mg/kg)
Pyrimethanil	3.78421x-103.719	0.998918	0.004
Chlorothalonil	0.991633x-82.9846	0.994184	0.006
Cyprodinil	2.78314x-69.7201	0.998754	0.004
Fluopyram	1.44580x-82.3414	0.998686	0.003
Folpet	0.308032x-20.3144	0.999558	0.004
Fenhexamid	1.58941x-22.3156	0.995325	0.005
Pyriproxyfen	3.70898x-78.1291	0.999692	0.006

The limit of quantification (LOQs) in the blackberry matrix was obtained over the range of 0.003–0.006 mg/kg with a correlation coefficient (r^2) $\geq$ 0.99 (Table 2) and recovery of the method was over the range of 86% to 109% (Figure 1). The accuracy was expressed in terms of average recoveries of spiked blank matrix blackberry at 0.01 and 0.02 mg/kg concentration levels. Satisfactory recovery was achieved when additional PSA was used for the cleaning step. Precision is presented as relative standard deviation (RSD, %) of within laboratory reproducibility analyzes. The range of RSD ranged from 3.5 to 8.2 % for the tested pesticides. The presence of a matrix effect was not observed significantly on the basis of the difference between standard and spiked samples curves of the slope. Analysis of the variance test did not show that there were statistically significant differences. This contribution can be attributed to a significant amount of PSA during cleanup. The tested new generation pesticides belong to the class of fungicides, except for pyriproxyfen which belongs to the class of insecticides. Table 3 shows the molecular formulas of the pesticide.

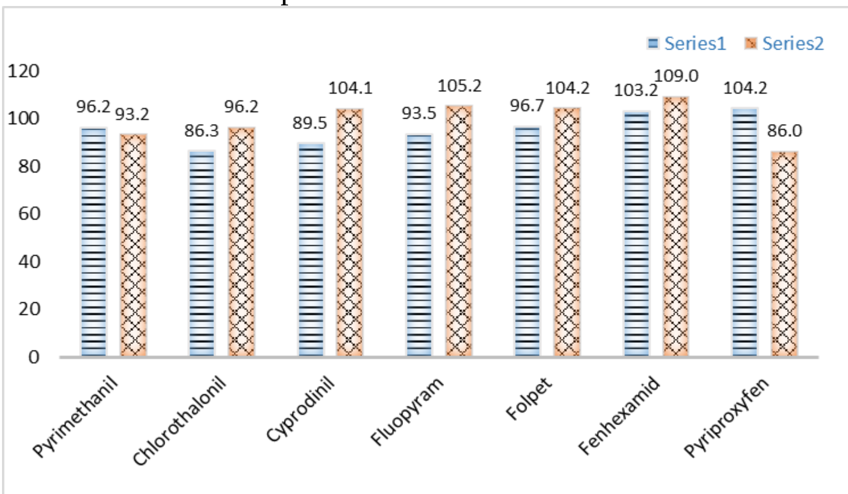

Figure 1. Evaluation of the mean recoveries at two fortification levels– series 1 - 0.01 mg/kg; series 2 – 0.02 mg/kg.

Table 3. New generation pesticides, their class and molecular formula

Analyte	Class	Formula
Pyrimethanil	Anilinopyrimidine fungicide	$C_{12}H_{13}N_3$
Chlorothalonil	Chloronitrile fungicide	$C_8C_{l4}N_2$
Cyprodinil	Fungicide	$C_{14}H_{15}N_3$
Fluopyram	Fungicide	$C_{16}H_{11}ClF_6N_2O$
Folpet	Phthalimide fungicide	$C_9H_4Cl_3NO_2S$
Fenhexamid	Fungicide	$C_{14}H_{17}Cl_2NO_2$
Pyriproxyfen	Insecticide	$C_{20}H_{19}NO_3$

The developed method was applied to the real samples of blackberries. Residues of new generation pesticides were determined in 50 blackberry samples collected in the 2021 harvest season. The following amount of cyprodinil was obtained in four blackberry samples: 0.050; 0.075; 0.085 and 0.105 mg/kg. The results obtained are below the maximum residue level (MRL) which is 3 mg/kg (Reg. (EU) 2021/1810) and will also be applicable 3 mg/kg determined according to SANTE / 10182/2022. In the other blackberry samples tested, the amount of cyprodinil was below the LOQ. Also, other pesticides tested were below the quantification limit (Table 2). The European Food Safety Authority (EFSA) presented data recommending that exposure to cyprodinil on blueberries, cranberries, currants and gooseberries will not result in a consumer exposure exceeding the toxicological reference value and proposed limits of 8 mg/kg. While the value for blackberries is still 3 mg/kg with an input value of 0.81 mg/kg for chronic risk assessment. Cyprodinil is an important fungicide used for the prevention and treatment of various fungal rots that can affect fruit plants, such as grapefruit (Zhang et al., 2015), blueberries (Munitz et al., 2013), etc. Consequently, the presence of cyprodinil in blackberries cannot be excluded. But it is important to control the safety of blackberries for consumers, as well as the compliance of the results of the present cyprodinil with the regulatory limit. The results are below the maximum residue limit and no evident risk to consumers was identified from the consumption of blackberries. It is important to mention that one of the necessary processes, i.e., washing the fruits, can reduce the number of pesticides (Kaushik et al., 2009). It was also confirmed that the approved agent Switch, a composition of cyprodinil and fludioxonil for fungicide destruction, was used. The presence of cyprodinil was confirmed by individual ion extraction in a selected ion monitoring (SIM) scan and by confirming the presence of ions and the ratio of ions to the area of the triphenyl phosphate standard (Figure 2). The ion intensity decreases in the order of 224 < 225 < 210 and the lowest in intensity is 226.

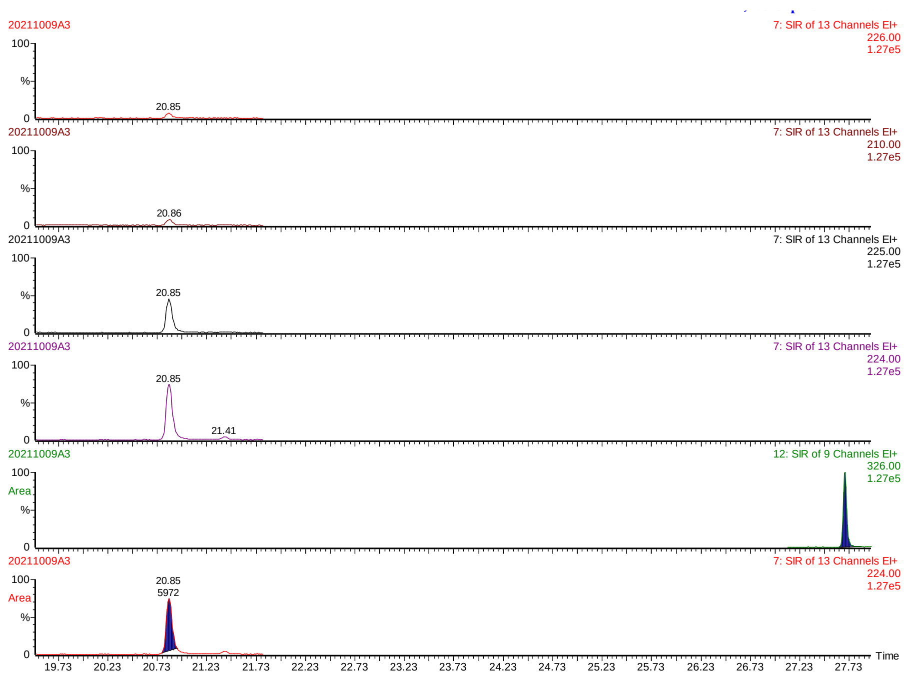

Figure 2. Cyprodinil peak and triphenyl phosphate in the selected ion monitoring (SIM), and also the area of individual cyprodinil ions.

Conclusion

A new method has been established for analysis the of seven pesticides in blackberries using gas chromatography. The effective, simple and fast method was validated by modifying the standard QuEChERS method. All seven new generation pesticides were simultaneously validated. The method shows satisfactory validation parameters. The use of PSA for cleanup step makes this methodology less susceptible to matrix effects during quantification. These results indicated that the newly established method was precise and reliable for the detection of pesticides in blackberries. Analysis of real blackberry samples obtained from the market showed the presence of cyprodinil in the analyzed samples. The results of the present study indicated the need to monitor the presence of pesticides as well as control the presence of fludioxonil.

Acknowledgments

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SANTE 11312/2021- Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed

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APPLICATION OF INSECTICIDES IN THE CONTROL OF COLORADO POTATO BEETLE (*Leptinotarsa decemlineata* Say)

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Abstract

Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is one of the most dangerous pests in potato fields. Nowadays it is worldwide present. *L. decemlineata* is an oligophagous due to its feeding on leaves of different plants such as potato, eggplant, paprika, tobacco, as well as many weeds from *Solanaceae* family. Larvae are much more harmful than adult insects. Successful potato cultivation in the world and our climate is possible only with efficient chemical crop protection against CPB. It is well known that this insect in its way, during a relatively short time, manages to develop resistance to almost every insecticide used so far. Therefore, this study aimed to evaluate the biological efficacy of plant protection products (PPP) based on acetamiprid (200 g/kg) and spinosad (240 g/l) in the control of adults, eggs, and larvae (L1-L3, and L4 larval stages) of CPB, and to determine whether there is a significant difference in efficacy of mentioned insecticides.

Field trials were conducted according to standard EPPO methods at two localities in the Republic of Serbia (Šajkaš and Mošorin, region of Vojvodina) in 2021, in the potato crops of Bellarosa and Rivera varieties. The insecticidal treatments were performed using back-sprayer "Solo" 473 P, with water consumption of 400 l/ha. According to the obtained results, it can be concluded that two days after the application of PPPs based on acetamiprid (200 g/kg) and spinosad (240 g/l), the number of L1-L3 larvae was significantly lower than the control, and the efficacy ranged from 95.6 to 100% at both localities. Larvae in stage L4, adults, and eggs were registered in very low numbers in all variants.

After seven days from the insecticides application, efficacy in L1-L3 larval stages ranged from 71,3 to 100%, and for the L4 stage from 83,8 to 100%, depending on the applied PPP. Adult insects and egg clusters were noticed in negligible numbers.

The high effectiveness of PPPs based on acetamiprid and spinosad, at both localities, indicates the sensitivity of *L. decemlineata* populations to the mentioned insecticides and the possibility of their successful use in the control of this pest in our agroecological conditions.

Key words: *Leptinotarsa decemlineata*, potato, acetamiprid, spinosad.

Introduction

Potato (*Solanum tuberosum* L.), a tuberous vegetable from *Solanaceae* family, is one of the most significant cultivated plants in the whole world. It has exceptional agrotechnical, biological, ecological, and economic importance. Young and physiologically riped tubers are used in human nutrition, and their high nutritional quality and a favorable ratio of nutrients are the most important characteristics of potatoes. However, it is at the very top in terms of losses caused by pests, diseases, and weeds, which is aggravating circumstance for the successful potato

production (Jakovljević, 1994; Lazić et al., 1998). In Serbia in the period 1970- 2014, the potato was grown in an area of about 86 887 ha (Ilin et al., 2014).
L. decemlineata is one of the most dangerous pests of potato. Nowadays it is worldwide present and potato productionwithout its control is practically impossible. *L. decemlineata* is an oligophagous due to its feeding on leaves of different plants such as potato, eggplant, paprika, tobacco, as well as many weeds from *Solanaceae* family. At the beginning of an attack, it makes small holes on leaves during feeding, and then it eats the whole leaf mass so that only the main leaf nerves remain. Larvae are much more harmful than adult insects. Due to the attack of CPB the number of formed tubers decreases, as well as their size. The damages can be huge if the destruction of the leaf mass occurs before flowering (Kereši et al., 2019). Therefore, successful and sufficient potato production is possible only with application of insecticides against CPB. On the other side, intensive application of insecticides has led to the rapid development of resistance of the CPB to insecticides which have been used for many years for its control. By controlling the degree of sensitivity, it is possible to detect this phenomenon and withdraw the particular insecticide from use. Another major problem is the increased number of treatments, due to which significant ecotoxicological and toxicological consequences can be manifested (Perić and Šestović, 2001). It is well known that this insect in its way, during a relatively short period, manages to develop resistance to almost every insecticide used so far. Therefore, this study aimed to examine the biological efficacy of plant protection products (PPP) based on acetamiprid (200 g/kg) and spinosad (240 g/l) in the control of adults, eggs, and larvae (L1-L3, and L4 larval stages) of CPB, and to determine whether there is a significant difference in efficacy of mentioned insecticides.

Material and methods

Field trials were performed at two localities Šajkaš and Mošorin during 2021 in the potato crops of Bellarosa and Rivera varieties, as a random block system according to the standard EPPO method for trial design and data analysis(Anonymus, 2012), the efficacy of examined insecticides in controlling CPB (Anonymus, 2004), and the phytotoxicity of these PPPs (Anonymus, 2014). The area of the basic plot was 25 m^2 (5 x 5 m). Treatments were performed on 18th May 2021 using the back sprayer “Solo” 473 P, with water consumption of 400 l/ha while plants were in the phenophase BBCH 31 (the beginning of rows closure). PPP based on acetamiprid (200 g/kg SP) and spinosad (240 g/l SC) were applied in the amount of 0.25 kg/ha and 0,1 l/ha, respectively. Immediately before the insecticide application adult insects, egg clusters, and larvae in larval stages L1-L3 were noted (Photo 1-2). Insecticide effects are shown over absolute and mean values for the number of CPB adults, egg clusters, and larvae (L1-L3 and L4). The deviation from the mean value was shown by the standard deviation (Sd±), and a significant difference (LSD 5%) was also determined. The efficacy was calculated according to Henderson&Tilton (Wentzel, 1963).

Photo 1-2. Adult insect and egg clusters at the back side of the leaf (left), L_1-L_3 larvae (right) (Photo: original)

Results and discussion

The results of efficacy of PPPs based on acetamiprid and spinosad in control of *L. decemlineata* in potato crops (localities Šajkaš and Mošorin) are shown in Tables 1-3. The number of CPB larvae in stages L1-L3 two days after the application of tested insecticides was at a significantly lower level compared to the control at both localities. In this case, the efficacy of PPPs based on acetamiprid and spinosad ranged from 96.9% to 98.6%, while the efficacy for control of L4 larvae two days after the treatment was 84.9-96.4 %, depending on the PPP and locality. Two days after the insecticide treatment the CPB adults and egg clusters were registered in small numbers. The efficacy of tested insecticides for CPB adults was in the range of 93.4-95.2% and for eggs 85.8-95.5%, at both localities. The larvae number (stages L1-L3 and L4) seven days after the application of acetamiprid and spinosad also was at a significantly lower level compared to their number in the control. Seven days after the treatment due to the high number of larvae in the control, in two trial replications, the total damage to the leaf mass has been noticed. While the efficacy of applied PPPs for L1-L3 larvae ranged from 73.2 to 100%, for the L4 larval stage it was 96.6- 100% depending on PPP and locality. The average number of adults and egg clusters seven days after the treatment was low in the control as well as in variants where insecticides were applied. For adult insects, the efficacy of tested insecticides ranged from 78.8 to 100%, and for eggs from 80.4 to 100%, at both localities. After the application of tested PPP based on acetamiprid and spinosad, there were no phytotoxic changes in the potato plants.

Table 1. The average number of L1-L3 larvae, adults, and egg clusters of L. decemlineata per plant immediately before the treatment (Šajkaš and Mošorin, 18th May 2021)

Insecticides (kg, l/ha)	**L_1-L_3** $\bar{x}$ ±Sd	**Adults** $\bar{x}$ ±Sd	**Egg clusters** $\bar{x}$ ±Sd
	Šajkaš		
acetamiprid (0.25)	**16.1**±2.1 a	**0.83**±0.1 a	**0.58**±0.1 a
spinosad (0.1)	**11.5**±4.6 a	**0.70**±0.2 a	**0.77**±0.2 ab
Control	**13.0**±3.5 a	**0.73**±0.2 a	**0.65**±0.1 ab
LSD 5%	18,24	0.32	0.27
F value	1.76	1.50	3.22
p	0.59	0.22	0.07
	Mošorin		
acetamiprid (0.25)	**7.4**±2.2 a	**0.60**±0.1 a	**0.55**±0.1 a
spinosad (0.1)	**7.27**±1.9 a	**0.47**±0.2 a	**0.45**±0.3 a
Control	**9.5**±2.2 a	**0.48**±0.2 a	**0.58**±0.2 a
LSD 5%	3.25	0.80	0.78
F value	0.49	1.22	1.33
p	1.24	0.95	1.22

$\bar{x}$ – average number; ±Sd - standard deviation, letters indicate significance level.

Table 2. The average number of L_1-L_3, and the L_4 larvae, adults, and egg clusters of *L. decemlineata* per plant two days after the treatment (Šajkaš and Mošorin, 20th May 2021)

Insecticides	**L_1-L_3**		**L_4**		**Adults**		**Egg clusters**	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
				Šajkaš				
acetamiprid (0.25)	**0.30**±0.3 a	**98.6**	**0.05**±0.1 a	**96.4**	**0.075**±0.1 a	**93.4**	**0.05**±0.1 a	**93.9**
spinosad (0.1)	**0.22**±0.3 a	**98.3**	**0.075**±0.1a	**94.6**	**0.05**±0.05 a	**95.2**	**0.05**±0.1 a	**95.5**
Control	**16.8**±1.8 b	/	**1.38**±1.2 b	/	**1.08**±0.3 b	/	**0.93**±0.3b	/
LSD 5%	1.42		0.95		0.83		0.25	
F value	112.4		1.32		2.14		11.6	
p	<0.05		<0.05		<0.05		<0.05	
				Mošorin				
acetamiprid (0.25)	**0.13**±0.1 a	**98.6**	**0.10**±0.2 a	**84.9**	**0.05**±0.1a	**94.4**	**0.075**±0.a	**88.4**
spinosad (0.1)	**0.30**±0.3 a	**96.9**	**0.075**±0.1a	**89.5**	**0.05**±0.1 a	**93.5**	**0.075**±0.a	**85.8**
Control	**12.5**±2.3 b	/	**0.58**±0.2 b	/	**0.78**±0.1 b	/	**0.68**±0.1b	/
LSD 5%	1.29		0.22		0.10		0.19	
F value	80.16		5.80		41.0		10.28	
p	<0.05		<0.05		<0.05		<0.05	

$\bar{x}$ – average number; ±Sd - standard deviation, letters indicate significance level, E%-efficacy.

Table 3. The average number of L_1-L_3 larvae, adults, and egg clusters of *L. decemlineata* per plant seven days after the treatment (Šajkaš and Mošorin, 25th May 2021)

Insecticides	L_1-L_3		L_4		Adults		Egg clusters	
	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%	$\bar{x}$ ±Sd	E%
Šajkaš								
acetamiprid (0.25)	**0.0**±0.0 a	**100**	**0.0**±0.0 a	**100**	**0.20**±0.1 a	**78.8**	**0.075**±0 a	**80.4**
spinosad (0.1)	**3.8**±1.1 a	**73.2**	**0.12**±0.1 a	**96.6**	**0.075**±0.1a	**90.6**	**0.0**±0.0 a	**100**
Control	**16**±4.7b	/	**3.53**±1.8 b	/	**0.83**±0.4 b	/	**0.43**±0.4 b	/
LSD 5%	4.37		3.28		0.42		0.22	
F value	11.77		2.07		3.99		3.79	
p	<0.05		<0.05		<0.05		<0.05	
Mošorin								
acetamiprid (0.25)	**0.25**±0.1a	**97.8**	**0.0**±0.0 a	**100**	**0.075**±0.1a	**82.9**	**0.0**±0.0 a	**100**
spinosad (0.1)	**0.45**±0.5a	**96.1**	**0.075**±0.1a	**97.3**	**0.00**±0.0 a	**100**	**0.00**±0.0 a	**100**
Control	**15**±5.0 b	/	**2.23**±0.6 b	/	**0.35**±0.1 b	/	**0.1**±0.1 ab	/
LSD 5%	6.38		0.77		0.33		0.15	
F value	77.42		7.73		3.20		1.57	
p	<0.05		<0.05		<0.05		0.21	

$\bar{x}$ – average number; ±Sd - standard deviation, letters indicate significance level, E%-efficacy.

Research conducted by Sanchez et al. (2006) indicates a high efficacy of acetamiprid in the control of *L. decemlineata* in Poland, which is in accordance with our research. Cross-resistance to acetamiprid and spinosad has also been reported in some tested populations of CPB (Sanchez et al., 2006). Spinosad as a contact-digestive insecticide has very favorable eco-toxicological properties and in much research it has shown high efficacy in controlling the CPB (Fabris, 2019), which is also in accordance with our results. In their study, Klein (2019) examined and confirmed the resistance of *L. decemlineata* to spinosad, which arose as a consequence of excessive use of spinosad in several examined fields which indicates the spread of the resistance allele. In contrast to the results obtained by Klein, in research conducted by Kowalska et al. (2021), it was determined that the CPB in laboratory conditions showed high sensitivity to spinosad, applied in three concentrations (0.2, 0.1, and 0.05%) at temperatures of 15, 20, and 25˚C.

Conclusions

Based on the performed field experiments and obtained results, the following conclusions may be reported:

- At the locality of Šajkaš immediately before the application of the tested insecticides in potato crops (phenophase BBCH 31- the beginning of row closure), a high number of CPB larvae (stages L1-L3, L4) was registered (11.5-16.1 larvae per plant). At the same time number of adults and egg clusters was low, and L4 larvae have not been found.
- Two days after the application of PPPs based on acetamiprid and spinosad the number of L1-L3 larvae was at a significantly lower level than in the control at both localities. The efficacy of tested PPPs in the control of L1-L3 larvae was 96.9-98.6%, while the efficacy in the control of L4 larvae two days after treatment was 84.9-96.4%

- Seven days after the application of PPP based on mentioned insecticides efficacy for L1-L3 larvae ranged from 73.2 to 100%, and for the L4 larvae from 96.6 to 100%, depending on applied PPP and locality.
- Used PPPs (acetamiprid 200 g/kg SP, and spinosad 240 g/l SC) have shown high efficacy in the control of CPB at both localities, and there was no significant difference in efficacy between these PPPs. They are at the same level of significance.
- The high efficacy of PPPs based on acetamiprid and spinosad at both localities indicates the sensitivity of the CPB population to mentioned insecticides and the possibility of their successful use in the control of this pest.

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IN VITRO ANTAGONISTIC ACTIVITY OF TRICHODERMA SPP. TO FUSARIUM OXYSPORUM AND FUSARIUM GRAMINEARUM

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Abstract

One of the major problems in agriculture are *Fusarium* species which cause fusariosis in wheat, corn, tomato, melon, watermelon, onion, peas, and beans. Also, *Fusarium* can synthesize thermostable mycotoxins which can lead to certain diseases if they were found in food. Currently, chemical fungicides are mostly used to prevent the occurrence of fusariosis disease, but the resistance of pathogens to such treatment is being more and more widespread. Soil microorganisms help in plant defense and growth. The rhizosphere fungi have an important role in the mutual exchange of nutrients with plants and they can establish specific interactions with plants. Such rhizosphere fungi are known as plant growth-promoting (PGP). These PGP fungi affect the better plant growth through the synthesis of certain phytohormones but they also have the function as biocontrol agents. They can inhibit the growth of phytopathogens through direct mechanisms of antibiosis, mycoparasitism, and competition.

The goal of this research was an investigation of the antagonistic effect of *Trichoderma harzianum* TR1 and *Trichoderma citrinoviride* 1V on *Fusarium oxysporum* and *Fusarium graminearum*. The antagonistic effect was examined through two tests: Dual culture test (DUAL test) and the effect of volatile organic compounds (VOCs) of *Trichoderma* strains on the growth of *Fusarium* strains. Also, the cell-wall degrading enzymatic activity of *T. citrinoviride* and *T. harzianum* was examined. The results showed that antagonistic activity of *Trichoderma* strains varies from moderate to high. Mycelial growth inhibition by *T. citrinoviride* was 44% for *F. graminearum* and 67% for *F. oxysporum*. *T. harzianum* inhibited *F. gramminearum* growth for 59% and *F. oxysporum* for 66%. Based on the results, it was concluded that *T.harzianum* and *T.citrinoviride* can be considered biocontrol agents for *F. oxysporum* and *F. graminearum*.

Keywords: *Fusarium oxysporum*, *Fusarium graminearum*, *Trichoderma harzianum*, *Trichoderma citrinoviride*, VOCs, *biocontrol agent.*

Introduction

The pesticides are very important in modern agriculture and economy and they are used to prevent spread of the plant disease. Some of the consequences of their excessive use are pollution of soil, surface water, groundwater, the emergence of resistance to pesticides, the emergence of diseases in humans and animals, etc. One of the possible solutions for solving this problem is using microorganisms as biocontrol agents. Biocontrol agents belong to the group of Plant Growth Promoting Microorganisms (PGPM) which have a number of positive effects on the plant such as the supply of nutrients to plants and phytopathogen suppression (Sousa et al., 2020). The use of biocontrol agents in agriculture are considered effective enough to compare vith chemisal fungicides (Hyder et al., 2017).

Fungi are among the most significant biocontrol agents, especially repsesentatis of the genus *Trichoderma* which have been shown as a good biofungicides. The *Trichoderma* strains have proven to be successful antagonists of *Fusarium sp*., which is one of the major pests in the field because it synthesizes persistent mycotoxins (Sharma I. P. and Sharma A. K., 2020). The genus *Trichoderma* have been known as biocontrol agents since 1920, it synthesize antibiotics and used for pest control as well as to increase yields (Chaparro et al., 2011). More than 60% of all biofungicides, are those that contain *Trichoderma* species (Abbey et al., 2019). The *Trichoderma* shows antagonistic interactions with phytopathogens based on several models like mycoparasitism, antibiosis and competition.
Volatile organic compounds (VOCs) are molecules of low molecular weight, lipophilic, easily volatile at room temperature and atmospheric pressure. Their basic role is to enable communication between fungi and other organisms. Over 300 VOCs of fungal origin have been identified so far and many of them are synthesized by different species of *Trichoderma* (Moya et al., 2018). A very important group of metabolites is peptaibol, which is synthesized in large quantities by fungi which are able to induce systemic resistance of the plant to pathogen attack (Mukherjee et al., 2012).
Trichoderma harzianum has proven to be the most effective biocontrol agent so far. It was noted that the antagonistic activity of *T. harzianum* (isolated from soil) versus *Fusarium oxysporum* is greater than 50% (Redda et al., 2018). The *T. citrinoviride* inhabits soil and it synthesizes enzymes that contribute to degradation phytopathogens cell wall.
Fusarium species are very important for agriculture as phytopathogens but also as mycotoxin producers and as opportunistic pathogens for humans. All *F. oxysporum* strains are saprophytes and can survive for a long time in soil with a lot of organic matter (rhizosphere). Interaction between *Trichoderma sp*. and *Fusarium sp*. is manifested through attraction, binding, twisting and lysis by hydrolytic enzymes or secondary metabolites (Mukherjee et al., 2012). During the interactions between *Trichoderma* sp. and *Fusarium* sp. it is forming apresoria which indicate mycoparasitism. The strains *T. virens*, *T. harzianum*, and *T. viride* produce about fifteen volatile compounds that have shown inhibitory effects on *F. oxysporum*.
The aim of this paper is determination the antagonistic activity of two representatives of the genus *Trichoderma*, *T. citrinoviride* and *T. harzianum* against *F. oxysporum* and *F. graminearum*. The ability to produce hydrolytic enzymes important in the manifestation of biocontrol potential was also investigated.

Materials and methods

Trichoderma citrinoviride TR1, *Trichoderma harzianum* 1V, *Fusarium oxysporum* and *Fusarium gramineraum* come from the collection of the Department for Microbial Ecology, Faculty of Agriculture, University in Belgrade, Serbia. The fungal strains were stored in Potato Dextrose Broth (PDB, Himedia, India) with 20% glycerol at -80°C. The working culture was maintained on Potato Dextrose Agar (PDA, Himedia, India), with occasional sieving on fresh medium, at a temperature of 25 °C.

1. Dual test

The nutrient media used in the dual test were: 1. Rose Bengal (RB) with the addition of streptomycin (streptomycin sulfate was added in the amount of 0.033 mg/1000 ml of media) which was prepared according to Pepper et al. (1995) and 2. PDA (Himedia, India). Fungal isolates were refreshed by sieving on PDA and RB medium and incubating at 25 °C (Binder, Lithuania) for 3 (*Trichoderma sp*.) and 5 days (*Fusarium sp*.). Then, 0.5 x 0.5 cm mycelial discs were cut from the periphery of the colony, and placed on new PDA plates. The following treatments have been set: 1. *T. harzianum* 1V vs *F. oxysporum* 2. *T. harzianum* 1V vs *F. graminearum* 3. *T. citrinoviride* TR1 vs *F. oxysporum* 4. *T. citronoviride* TR1 vs *F. graminearum.* Each test was repeated twice. The control was represented by individual cultures of *F. graminearum*, *F. oxysporum*, *T. citronoviride* TR1, *T. harzianum* 1V on RB and PDA. The incubation lasted 5-7 days at 25 °C (Binder, Lithuania). The diameters of the colonies of *F. oxysporum* and *F. graminearum* were measured in a dual test and control Petri plates and the percentage of growth inhibition (MGI) was obtained according to the formula:

MGI (%) = ((DC − DT)/DC)) × 100, where MGI is mycelial growth inhibition, DC is the average diameter of a fungal colony of the control group, and DT is the average diameter of a fungal colony of the treatment group (Mohareb et al., 2017).

The antagonistic levels were classified as low (MGI 50%); medium (50% < MGI ≤ 60%); high (60% < MGI ≤ 75%); and very high (MGI > 75%) (De la Cruz et al., 1992).

2. Biochemical Characterization of* Trichoderma spp. *Antifungal Activity

The biochemical characterization of *Trichoderma* spp. antifungal activity included the determination of cell wall-degrading enzymes and the production of siderophores. A semiquantitative determination of cell-wall degrading enzymes (lipase, esterase-lipase, N-acetyl-β-glucosaminidase and β-glucosidase) was performed using an API ZYM kit according to the manufacturer’s protocol (BioMereux, Craponne, France).

The presence of cellulase was determined using carboxymethyl cellulose (CMC) agar method in three repetitions (Romsaiyud et al., 2009). Siderophore production was detected on the Chrome azurol S (Sigma-Aldrich, St. Louis, USA) agar medium in three repetitions (Gezgin et al., 2020). The chrome azurol S (CAS) agar plates were inoculated with 5-mm-diameter mycelia discs of three *Trichoderma* isolates and incubated at 28 °C for 72 h. The appearance of yellow-orange halo zones around colonies was considered as a positive result.

3. The Effect of Trichoderma VOCs on Mycelial Growth of F. oxysporum i F. graminearum

The effect of VOCs produced by *Trichoderma* strains on the mycelial growth of *F. oxysporum* and *F. graminearum* was tested using the method of confronted cultures without contacts of the two mycelia (Dennis and Webster, 1971). The two Petri dishes containing 20 mL of PDA or RB were individually inoculated with 5-mm-diameter mycelia discs of a pathogen (*F. oxysporum* and *F. graminearum*) and an antagonist (*Trichoderma* spp.). Inoculated plates were sealed with Parafilm®, arranged to face each other and incubated at 25 °C in a microbiological incubator (Binder, Tuttlingen, Germany) in the dark, until fungi in the control plates (plates with individual fungi, negative control) reached edges of plates. The experiment was repeated three times. The effects of volatile metabolites were estimated through MGI.

4. Statistical Analyses

The data were subjected to ANOVA followed by Tukey’s HSD post-hoc comparison tests to determine if there were statistically significant differences between the means ($p = 0.05$). All statistical analyses were performed using Statistica 12.0 (StatSoft, Tulsa, OK, USA).

Results and discussion

About 60% of biocontrol agents are based on different strains of the genus *Trichoderma* which was first time isolated in 1794 from soil and compost (Sood et al., 2020).

The both species of *Trichoderma* were shown the ability to inhibit mycelia growth of *F. oxysporum* and *F. graminearum* (Table 1).. The highest degree of *F. oxysporum* inhibition was recorded in the dual test with *T. citrinoviride* TR1 on RB medium (67%) while the percentage of inhibition on PDA was slightly lower. According to Sookchaoy et al., (2009) scale such activity was characterized as high in both cases. The T. *harzianum* 1V also had shown a high level of antagonistic activity. The highest degree of *F. graminearum* inhibition was observed with T. *citrinoviride* TR1 on PDA and it was characterized as a high degree. The other effects of *Trichoderma* representatives were characterized as a moderate antagonistic activity. The only exception is the response of *F. graminearum* to *T. citrinoviride* TR1 on RB and in this dual test the lowest degree of pathogen growth inhibition was recorded. This result emphasizes the importance of environmental conditions on the final result achieved by the presence of biocontrol agent.

Table 1. Growth inhibition of *F. oxysporum* and *F. gramminearum* by *T. citrinoviride* TR1 and *T. harzianum* 1V

Treatment	Percentage of inhibition RB	Percentage of inhibition PDA
T. citrinoviride TR1/*F. oxysporum*	67%	61%
T. citrinoviride TR1/*F. graminearum*	44%	63%
T. harzianum 1V/ *F. oxysporum*	66%	62%
T. harzianum 1V /*F. gramminearum*	59%	60%

In agriculture, the genus *Trichoderma* was used for control phytopathogens such as *Fusarium* (Sivan et al., 1986). Fan et al., (2020) proved that the *T. citrinoviride* strain Snef1910 shows antagonistic activity *in vitro* against pathogens that cause diseases of wheat, cotton, melon and other plants. This strain inhibits the growth of *F. graminearum* by 60.76%, *F. oxysporum* by 49.28%, *Fusarium monihforme* by 21.73%, and *Fusarium roseum* by 25.20%. Some literature data showed that antagonistic activity of *T. harzianum* versus *F. oxysporum* could be greater than 50% (Redda et al., 2018).

The zones of inhibition growth and increased colony pigmentation were observed in response of the phytopathogen to the presence of a biocontrol agent. The transparent inhibition growth zone (0.4 cm) was observed on RB medium with *T. citrinoviride* TR1 and *F. graminerarum*, which indicates antagonistic activity of diffuse non-volatile compounds and the same was also observed on PDA medium with *T. harzianum* 1V. The increased pigmentation of *F. graminearum* colonies near the zone of inhibition growth was also noticed. Both of the *Trichoderma* species have high antagonistic activity against *F. oxysporum*. The transparent zone of inhibition growth was noticed on all media in response of phytopathogens to the presence of diffuse non-volatile compounds, mostly originating from *T. citrinoviride*.

Similar with our result, some authors showed that certain strains like *T. harzianum* Q710613 can inhibit *F. graminearum* by 73-75% so it possesses high antagonistic activity on PDA medium (Tian et al., 2016), *T. harzianum* can inhibit the growth of *F. oxysporum* by 53% (moderate antagonistic activity) (Sundaramoorthy and Balabaskar, 2013) whereas *T. citrinoviride* inhibits *F. oxysporum* growth by 50% and 56%, with mild to moderate antagonistic activity (Redda et al., 2018).

The effect of VOCs on the growth of *F. graminearum* and *F. oxysporum*

The VOCs emitted by *Trichoderma* in the presence of *F. oxyposrum* and *F. graminearum* resulted in inhibition of phytopathogen mycelia growth in the range of 13–36% for *F. oxysporum* and 26–41% for *F. graminearum* (Table 2). The antagonistic activity between *T. harzianum* 1V and *T. citrinovirie* TR1 differs by two phytopathogens. Thus, *T. harzianum* 1V showed a better percentage of mycelial growth inhibition for *F. oxysporum* and *F. graminearum* compared to *T. citrinoviride* TR1. Macroscopically observed, increased pigmentation was noticed on the RB medium for phytopathogens in the central part of the colony while it was lost at the edges of colony.

Based on the results, it is concluded that the degree of inhibition caused by VOCs varies and that all obtained values could be classified into low antagonistic activity. However, it is undeniable that inhibition is present.

Table 2. Growth inhibition of *F. oxysporum* and *F. gramminearum* by *T. citrinoviride* TR1 and *T. harzianum* 1V volatile organic compounds

Treatment	Percentage of inhibition PDA	Percentage of inhibition RB
T.citrinoviride /F.oxysporum	13%	23%
T. citrinoviride /F. graminearum	39%	32%
T.harzianum / F.oxysporum	36%	31%
T. harzianum /F.gramminearum	26%	41%

Semiquantitative analyses of *Trichoderma* spp. enzymatic profiles showed the ability of these fungi to produce lipase and esterase-lipase and β-glucosidase at a moderate level as well as high amounts of N-acetyl-β-glucosaminidase (Table 3). The results showed appearance of bright area around the colonies that confirmed the cellulolytic activity of *T. harzianum* 1V and *T. citrinoviride* TR1.

Table 3. Biochemical characteristics of Trichoderma spp.

Enzymes	*Trichoderma citrinoviride* tr1	*Trichoderma harzianum* 1v
Lipase	2	2
esterase-lipase	2	2
N-acetyl-β-glucosaminidase	3	3
β-glucosidase	2	3
cellulase	+	+
siderophores	+	+

1—low production, 2—moderate production, and 3—high production according to the API ZYM reading color scale; + positive react

The results showed appearance of bright area around the colonies that confirmed the cellulolytic activity of *T. harzianum* 1V and *T. citrinoviride* TR1. The production of those enzymes is especially important since chitin and glucan, common constituents of fungal cell-walls, are susceptible to chitinase, N-acetyl-β-glucosaminidase, and β-glucosidase (Karličić et al., 2021). Also, N-acetyl-β-glucosaminidase is already well known as inhibitor of *Botrytis cinerea* spore germination (Lorito et al., 1994), Literature data confirm *T. citrinoviride* as producer of strong cellulases (Park et al., 2019). The CAS assay confirmed the ability of *Trichoderma* spp. to produce siderophores which are an important mechanism of biocontrol (Chen et al., 2021).

Conclusion

Both tested biocontrol agents (*T. citrinoviride* TR 1 and *T. harzianum* 1V) showed a high percentage of antagonistic activity against phytopathogens (*Fusarium oxysporum* and *Fusarium graminearum*). The results of cellulolytic activity showed that *T. citrinoviride TR 1* and *T. harzianum 1V* produce cellulase. In the DUAL test, different results were obtained depending on the medium on which they were grown. As a response of the phytopathogen to the presence of the biocontrol agent, transparent zones of inhibition and increased pigmentation of the phytopathogen colony were observed. The test of inhibition by VOCs originating from *T. citrinoviride TR1* and *T. harzianum 1V*, showed positive results and their percentage of antagonistic activity was low. Mycelial growth inhibition values vary from 25.89% to 40.77% for *F. graminearum* and from 12.77% to 36.36% for *F. oxysporum*. The *T. citrinoviride TR1* and *T. harzianum 1V* have been shown to be very potent biocontrol agents with high antagonistic activity when it comes to non-volatile compounds and as bicontrol agents with low antagonistic activity when it comes to volatile organic compounds.

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UTILIZATION OF EDIBLE NANOEMULSION COATINGS IN MEAT AND MEAT PRODUCTS

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Abstract

Due to the increasing consumer demands for minimally processed food, the meat industry has tended to replace traditional techniques (heat treatment, salting, curing, etc.) with non-thermal processes to preserve meat quality. Among active packaging technologies, the use of edible coatings in the meat industry has important advantages such as being environmentally friendly, economical, long shelf life, good barrier properties, and carriers for bioactive compounds. The addition of functional agents has demonstrated its effectiveness in extending the shelf life of fresh and processed meat products. However, despite this effectiveness, there are few edible active coatings available in the market. This deficiency is mainly due to the low stability of the active ingredients during production processes and storage. Emulsion technology is a simple, economical, and effective method of keeping the hydrophilic phase and lipid in a stable system. The addition of lipophilic components can be enhanced coating stability by preparing the coating solution in emulsion form. Reducing the droplet size of emulsion to nano size is increase the functionality of edible coating. Droplet size and distribution of nanoemulsions have a significant impact on functionality, stability of the emulsion as a carrier, and more homogeneous distribution of the lipophilic material. Nanoemulsions have been widely used in the food industry in recent years as an innovative approach to transporting functional agents such as fatty acids, polyphenols, antioxidants, antimicrobials, etc. This paper will provide a review of development techniques of edible nanoemulsion coatings and use as a substitute for synthetic polymers and their applications in the meat industry.

Keywords: *Meat products, Active packaging, Edible coating, Edible nanoemulsion coating.*

Introduction

The coating prepared with natural and environmentally friendly materials to slight quality changes in meat and meat products has widely increased in recent years. The coating is an effective method applied for the preservation of foods from chemical reactions and microbial growth, and synthetic and/or natural materials can be used as coating material. Non-biodegradable synthetic coatings cause significant ecological problems. An alternative to replace synthetic coatings is using biodegradable coating made from natural materials (Gat et al., 2020). The development of new technologies to improve the carrier properties of edible films and coatings is an important topic for future research (Heydari et al., 2020).

The edible coating is defined as a thin layer that is applied to the food surface to create a barrier between the food and the external environment and can be eaten as part of the entire product. The ideal edible coating is a layer that can reduce moisture loss from the food surface and act as a barrier to gas, changing the atmosphere around meat and providing a partial barrier against water movement (Maringgal et al., 2020). Edible coatings have an important role to preserve the quality of food and extend shelf life. The edible coating is applied in three ways, by immersing meat and meat products in a coating solution, spraying, and rubbing over food (Maringgal et al., 2020).

Edible coatings can be produced with natural components such as proteins, polysaccharides, and lipids, or their combinations (Mojaddar Langroodi et al., 2021). Collagen, gelatin, milk proteins, seed proteins, and proteins obtained from vegetable sources are used as a coating material in meat and meat products. Protein-based edible coatings showed good hydrophilic properties, however, had poor water vapor permeability and are highly sensitive to moisture. Lipid-based coatings usually consist of wax, acylglycerol, or fatty acids. Hydrophobic material-based edible coatings not only reduced the defects of water, light, oxygen, and other external factors on food quality during storage, but can also reduce the water evaporation rate of the food. However, the production of these coating materials generally requires solvents or high temperatures, and the films/coatings produced in this way have poor mechanical properties (Bhagath and Manjula, 2019; Yousuf et al., 2017).

Polysaccharides are high in plants, are generally water-soluble, have good mechanical properties, and have no allergic effects. Polysaccharides such as cellulose derivatives, chitosan, starch, pectin, gums, alginate, and algae are used as coating materials. The important advantage of using polysaccharide coating materials is their chemical structures are defined for each monomer. This allows the coating properties to be predicted and controlled. In addition, polysaccharides have a low moisture barrier due to their hydrophilic nature, and the addition of lipids reduces the high moisture permeability of hydrocolloids (Ju et al., 2019; Arnon-Rips, 2018).

The functional properties required for edible coatings are different (prevention of oxygen, water, and carbon dioxide transport in food) and mainly related to the composition of individual foods; for example, it must decrease oxygen for protecting products that are sensitive to oxidative reactions or prevent moisture loss in other food. Besides, edible coatings must be organoleptically acceptable. In this regard, the use of essential oils, extracts, and other natural ingredients has been developed for their potential to inhibit chemical damage, and microbial growth and extend shelf life. Today, nanotechnology provides an opportunity to develop and transport these natural ingredients with antimicrobial and antioxidant properties. Also, nano-size (<1000 nm) of natural ingredients causes gas transport and target-specific release, while improving mechanical resistance, transparency, functionality, and antioxidant and antimicrobial activity. Application of nanotechnology offers greater surface area per mass and increases stability and bioactivity of nanosystems, while the incorporation of hydrophobic and/or biologically active substances that protect the visual characteristics and extend the shelf life has been possible (Ju et al., 2019; Zambrano-Zaragoza et al., 2018).

Edible nanoemulsion coating

The incorporation of lipophilic active ingredients into foods is limited due to their low water solubility, low stability, and their odor. Emulsification of hydrophobic active compounds can help to homogeneous dispersion in the water-based edible coating. Oil in water (O/W) emulsion is a promising system for loading(encapsulation) these bioactive compounds into the edible coating. At the same time, it can minimize the effects of bioactive compounds on the sensory properties, and it also ensures the protection of these compounds. The application of edible coatings containing active nanoemulsions is an emerging approach to producing foods with optimal functionality. The use of nanoemulsion not only improves the functionality of the emulsion as a carrier but also allows a more homogeneous distribution of the lipophilic material (Chen et al., 2020; Demisli et al., 2020).

Nanoemulsions are colloidal systems including dispersion of oil droplets (50–500 nm) in an aqueous phase, such that each oil droplet is surrounded by a thin layer of emulsifier agent which makes this type of emulsion kinetically stable. The lipophilic phase of nanoemulsions consists of the bioactive lipids (e.g., essential oil, oily flavor, oily color, vitamin, etc.), or oily solutions that provide antimicrobial, antioxidant, and nutraceutical activity, flavor, etc. These ingredients include essential oils from plants (e.g., sage, mint, oregano, clove, limonene), fatty acids (e.g. omega-3 fatty acids), carotenoids (lycopene, β-carotene, etc.), antioxidants (polyphenols tocopherols, flavonoids), phytosterols (stigmasterol, etc.) and quinones (coenzyme Q_{10}) (Zambrano-Zaragoza et al., 2018).

The advantages of the nanoemulsion system are the smaller size of lipid droplets (as shown in Figure 1), an increase in solubility of the bioactive lipids that cause to reduce in the amount of disperse phase, increasing bioavailability, the longer activity of the bioactive component, target-specific, effective and controlled release (Sutradhar and Amin, 2013). Because of these advantages, nanoemulsions are of great interest for studies and applications in different fields such as health, cosmetics, food, agrochemicals, pharmaceuticals, and biotechnology.

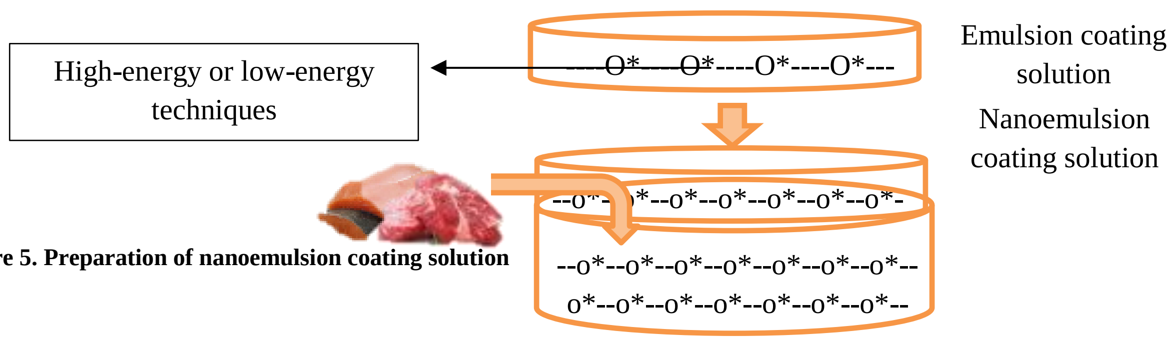

Figure 5. Preparation of nanoemulsion coating solution

Nanoemulsions can be produced using high-energy or low-energy techniques. High-energy methods are generally used for the preparation of nanoemulsions including high-pressure homogenizers, microfluidizers, and ultrasonic generators. In addition, several studies have been carried out using low-energy methods for nanoemulsion production using the chemical potential of the components under special conditions (Serdaroğlu et al., 2015). The optical transparency and kinetic stability of nanoemulsions depend on thermodynamic conditions such as composition, temperature, and pressure, as well as preparation methods (Esquena and Solans, 1998). Therefore, it is possible to prepare nanoemulsions with specific droplet sizes using different preparation techniques.

Utilization of nanoemulsion-based edible coatings in meat and meat products

As can be seen from Table 1 nanoemulsion-based edible coatings have significant potential in maintaining the quality of meat and meat products.

Table 1. Utilization of nanoemulsion-based edible coatings in meat and meat products

Meat products	Nanoemulsion coating materials	Results	References
Fresh meat (storage at 4°C, 30 days)	Arabic gum (40%), MCT (20%), and chitosan (40%) + Thyme essential oil (2%)	• Slight increase in pH, TVB-N, TBARS, and microbial growth • Extended shelf life	Snoussi et al., 2022
Lamb loin (storage at 4°C, 30 days)	Pectin + Curcumin nanoparticles and ajowan (*Carum copticum*) essential oil combined with gamma irradiation	• Decreased the count of *mesophilic* and *psychrotrophic* bacteria, *lactic acid bacteria*, *Enterobacteriaceae* • Reduced lipid and protein oxidation, TVB-N, met-myoglobin formation, and color deterioration • Extended shelf life from 5 to 25 days	Fallah et al., 2022
Camel meat (storage at 4°C, 20 days)	Basil seed gum + Resveratrol and clove essential oil	• High antioxidant activities of nanoemulsion-based films • Reduced lipid and protein oxidation, and increased overall acceptability	Ansarian et al., 2022
Pork meat (storage at 4°C, 12 days)	Chitosan + Thymol or thyme essential oil	• Low TVB-N, pH, and TBARS values • Protected against color degradation, inhibited unpleasant odors • Controlled the population of microbial flora and inoculated foodborne pathogens	Wang et al., 2022
Hairtail (*Trichiurus haumela*) fish (storage at 4°C, 18 days)	Chitosan + Eugenol EO	• Better preservative effects compared to samples coated with chitosan nanoemulsion only • Delayed lipid and protein oxidation	Liu et al., 2021
Beef fillet (storage at 4°C, 20 days)	*Lepidium sativum* seed gums + Red onion (Allium cepa, L.) Extract	• Reduced pH, PV, TBARS, microbial growth • Desirable sensory attributes • Extended shelf life from 4 to 16 days	Sarvinehbaghi et al., 2021
Fresh chicken meat (storage at 4°C, 20 days)	Corn starch + *Zataria multiflora* essential oil fortified with cinnamaldehyde	• Higher antimicrobial activities than conventional forms • Reduced Total viable count, *Psychrotrophic* count, *Lactic acid Bacteria*, *Enterobacteriaceae* count, Mold and yeast count and inoculated *L. monocytogenes*	Abbasi et al., 2021
Turkey fillet (storage at 4°C, 12 days)	Alginate + Trachyspermum *ammi* essential oil	• Prevented the growth of *L. monocytogenes*	Kazemeini et al., 2021
Pork meat patty (storage at 4°C, 12 days)	Chitosan + Fennel essential oil/ Cinnamaldehyde	• High zeta potential, translucent appearance, the smooth surface, and uniform distribution of the particle • Samples coated by nanoemulsion had the lowest TVC, TBARS value, and TVB-N content • Maintained flavor and texture and extended storage life to 10 days	Sun et al., 2021
Donkey meat (storage at 4°C, 10 days)	Carboxymethyl chitosan, ε-polylysine, and a mixture of clove essential oil	• Nanoemulsion increased the stability of the coating • Reduced lipid and protein oxidation, improved texture, and inhibited microbial growth	Zixiang et al., 2021
Pork slice (storage	Chitosan (1%) -gelatin (3%) + Tarragon essential oil (1%)	• Nanoemulsion contributed to the sustainable release of TEO and	Zhang et al., 2021

at 4°C, 16 days)		improved antioxidant, antibacterial, and sensory properties	
Pork meat (storage at 4°C, 12 days)	Chitosan + Thymol or thyme essential oil	• Improved color parameters • Extended shelf life up to 6 days due to the slight increase of pH and total volatile count	Liu and Liu, 2020
Pork loin (MAP, storage at 4°C, 20 days)	Pectin+ Oregano essential oil and resveratrol	• Nanoemulsion coatings extended shelf life through inhibition of lipid and protein oxidation and maintained meat tenderness • Increased preservative function and stability by smaller particle size	Xiong et al., 2020
Yao meat products (storage at 4°C, 20 days)	Soy protein isolate + Mixture of star anise essential oil, polylysine, and nisin	• Reduced pH and TVB-N content • Extended shelf life to 16 days • Improved color, odor, and overall acceptance	Liu et al., 2020
Chicken fillet (storage at 4°C, 10 days)	Poly lactic acid/ nanochitosan + *Polylophium involucratum* essential oil	• Reduced peroxide values, TVB-N, and microbial population • Desirable antibacterial, antioxidant and sensory attributes • Extended shelf-life to 10 days with desirable sensory properties	Javaherzadeh et al., 2020
Beef Fillet (storage at 4°C, 16 days)	Alginate(3%) + *Zataria multiflora* Boiss Essential Oil(0.25,0.5,1%)	• Inhibition of inoculated E. coli O157: H7 growth • High antibacterial effect, the lowest bacterial growth in samples containing 1% ZMEO	Alavi et al., 2020
Turkey meat (storage at 4°C, 18 days)	Chitosan + *Zataria Multiflora* Boiss and *Bunium persicum* Boiss essential oils	• The highest reduction rate of total viable bacteria, total *psychrophilic, Pseudomonas spp., Enterobacteriaceae, lactic acid bacteria,* and yeast and mold count • Increased shelf life (15-18 days)	Keykhosravy et al., 2020
Fish fillet (storage at 4°C, 16 days)	Alginate + *Zataria multiflora* Boiss essential oil	• The highest reduction rates of TVC, TPC, hydrogen sulfide-producing bacteria count, and *Enterobacteriaceae*	Khanzadi et al., 2020
Turkey meat (storage at 4°C, 20 days)	Gelatin/Hydroxypropyl-β-Cyclodextrin + Nettle Essential Oil o	• Lower weight loss, TVB-N, mesophilic, psychrophilic bacteria, molds, and yeasts compared to samples coated with cellophane	Adeli Milani et al., 2020

To summarize Table 1, there are various researches on using edible nanoemulsion coatings in beef, chicken, pork meat, lamb meat, turkey meat, camel and donkey meat, and several kinds of seafood. Generally, Arabic gum, chitosan, soy protein isolated, pectin, gelatin, alginate, etc., and various essential oils such as thyme, oregano, clove, anis, *Zataria multiflora*, etc., and extracts such as red onion have been used as nanoemulsion coating materials. These studies indicate that preparing coating in the form of nanoemulsion causes more stability of coating due to the smaller size of oil droplets that increase more surface surrounded by emulsifier and uniform distribution compared to conventional forms. Nanoemulsion form of coating has increased antioxidant, antibacterial, generally functionality of bioactive substance that incorporated within.

Conclusion

Owing to growing interest in using innovative techniques, particularly the application of nanotechnology has increased to improve the safety, health, and quality of food products.

Nanoemulsions specially oil-in-water nanoemulsions are easily formulated using ingredients and technologies and are delivery systems for hydrophobic bioactive substances in foods, including antioxidants, antimicrobials, vitamins, flavors, colors, etc. Moreover, it can possible to incorporate multiple bioactive agents into a single nanoemulsion system which can have synergistic effects, and it could be combined with other strategies (hurdle technology). Nanoemulsion-based edible coatings can positively affect the physicochemical, microbial, and/or sensory properties of meat products. These coatings ensure stable, safe, and healthy meat products by decreasing lipid and protein oxidation, TVB-N as enzymatic and bacteriological activities indicator, controlling water and gas transportation, and improving sensory characteristics.

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NATURAL ALTERNATIVE TO SYNTHETIC NITRITE: THE USE OF PRE-CONVERTED NITRITE IN MEAT PRODUCTS

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Abstract

Nitrite has historically been used in meat products for its biological and functional roles. Beneficial effects of the addition of nitrites to meat products are increased shelf life, improvement of quality characteristics as well as microbiological safety. The nitrates and nitrites are mainly responsible for the development of the distinct flavor, the stability of the red color, as well as the protection against lipid oxidation in cured meat products. It contributes to the development of characteristic color development and stability. Moreover, nitrite is a bacteriostatic agent that prevents the growth of *Clostridium botulinum* and spore germination. Despite many recent advancements in the meat industry, consumers continue to change preferences owing to health issues. In 2015, the International Agency for Research on Cancer identified processed meat products and red meat as possible carcinogens. Therefore, researchers have focused on developing processed meat products that are not harmful to health by eliminating synthetic nitrite additives or replacing them with natural materials. Nitrite is challenging to replace using simple antioxidants or antimicrobial substances because it can serve multiple functions. Food manufacturers have explored the use of vegetable powder, vegetable extract, and nitrite (NO_2^-) converted from nitrate (NO_3^-) with nitrate-reducing bacteria (pre-converted) as a substitute for synthetic nitrite. Natural nitrate sources origin (direct use of nitrate-containing vegetable powders or juices) could be used in long-ripened meat products, which allows converting nitrate to nitrite by microorganisms with nitrate reduction activity. However, in meat products that have short production processes and are heat treated, nitrate reduction will not be achieved due to the lack of sufficient time. Accordingly, industries prefer to use the ‘cultured,’ ‘pre-fermented,’ or ‘pre-converted’ nitrate-containing plant source, which has already been incubated with nitrate-reducing bacteria to produce nitrite. Pre-converted plant powder is simple to use because specific nitrites can be applied. Also, pre-converted vegetable products typically contain about 15,000–20,000 mg/kg of nitrite. This review briefly overviews pre-converted nitrite as a natural curing agent in meat products.

Keywords: *Pre-converted, Nitrate-reducing bacteria, Clean label meat products.*

Introduction

Nitrite and nitrate are important food additives in cured meat products. These additives have been used since about 3000 BC. Saltpeter (potassium nitrate), a natural salt contaminant, has historically been used as it contributes to the pinkish-red color in salted meats. At the end of the 19th century, nitrates were discovered as an effective ingredient. It was also learned that a complex series of reactions involving nitrates were responsible for the formation of meat color. Nitrite is contributed to the microbiological safety of cured meat products; therefore, several countries have regulated nitrite and nitrate use since the 1920s, but it was mainly during the

1970s that the regulation became regular, still with considerable differences between countries (Cassens et al., 1990; Bernardo et al., 2021). Sodium nitrite has many beneficial functions: Preservative properties, antimicrobial properties, and pigment fixation.

- Lipid and protein oxidations are the most significant changes affecting meat quality and shelf life. One of the remarkable properties of nitrite is its ability to retard the development of oxidative changes during storage, and the subsequent warmed-over flavors developed upon heating meat and meat products (Jo et al., 2020).
- Nitrite is a bacteriostatic agent that prevents the growth of *Clostridium botulinum* and spore germination (Lebrun et al., 2020).
- Nitric oxide reacts with myoglobin and forms nitrosomyoglobin (dark red color). Further thermal treatment converted nitrosomyoglobin into nitrosohemochrome (pink color), which gives cooked sausage characteristics (Yong et al., 2019).

The adverse effects of nitrite as a meat additive were first recorded in the early 1950s and 1960s when N-nitroso compounds (NOCs) were initially discovered that were formed endogenously from nitrites and nitrates are group 2A carcinogens (probably carcinogenic to humans; Monographs (IARC, 1989). Food authorities, namely the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO, 2021), and local food safety authorities, such as the European Union “EFSA’s expert Panel on Food Additives and Flavorings” (EFSA, 2021) or the United States “USDA Food and Drug Administration” (FDA, 2021) estimated the use of nitrate and nitrite as a food additive. According to scientific knowledge, these compounds are approved as food additives, but discussions continue based on their potential risks and benefits (EFSA, 2017). The scientific community and consumers continue to be concerned about it (Higuero et al.,2020; Fraqueza et al., 2020). Many consumers negatively perceive nitrite as a food additive in processed meat products. Thus, natural meat products have been made of natural ingredients, such as vegetable powder, with high nitrate content.

The vegetables can be grouped according to their nitrate level: celery, cress, lettuce, spinach, and rucola (<2500 mg/100 g); Chinese cabbage, endive, leek, and parsley (1000 to <2500 mg/100 g); turnip, savoy cabbage, and cabbage (500 to <1000 mg/100 g); carrot, cucumber, pumpkin, and broccoli (200 to <500 mg/100 g); and potato, tomato, onion, eggplant, mushroom, and asparagus (<200 mg/100 g) (Gassara et al., 2016; Schullehner et al., 2018). These concentrations are expressed as the fresh weight. When the product is dried, it is possible to obtain vegetable extracts with a much higher nitrate content. Celery is the most extensively studied plant and has been used commercially because it does not significantly affect the sensory properties of meat products (Sebranek et al., 2012).

This review aims to explain the role of natural nitrate sources used in meat products. Pre-converted strategies to replace synthetic nitrite are summarized, and their effects on potential advantages and disadvantages are discussed.

Pre-converted process

Pre-converted nitrite is formed before adding meat products, and the selection of proper starter culture and incubation conditions is essential for producing stable and abundant nitrite (Sebranek et al., 2012). Meat products are generally incubated with nitrate sources and starter culture (Figure 1). However, nitrite generation by this process is often slow, and incubation at the appropriate temperature for culture can reduce the quality of meat products (Sindelar et al.,

2007). To overcome these limitations, pre-converted nitrite can be added directly to meat products (Kim et al., 2017a; Krause et al., 2011).
Although the incubation temperature is an essential factor when reducing nitrate to nitrite, the incubation time is a more critical factor because excessive incubation time induces a decrease in the nitrite content of pre-converted nitrite sources (Kim et al., 2019; Sindelar et al., 2007). This decline in nitrite content may be due to a decrease in the pH value of the pre-converted nitrite solution. The acidic condition decreases the activity of nitrate reductase and the release of nitrite to NO easily (Paik and Lee, 2014). Several authors have recently studied pre-converted nitrite sources in meat products (Table 2).

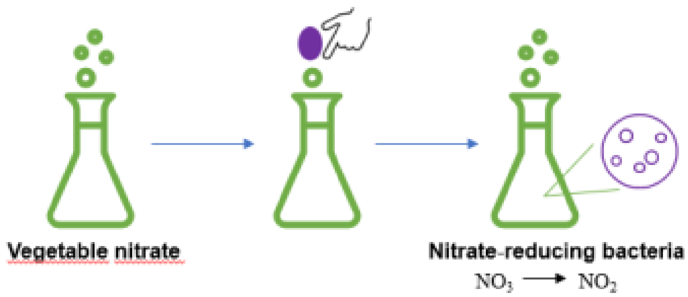

Figure 1. The pre-converted process with the use of vegetable nitrate sources.

Starter culture

Starter cultures containing lactic acid bacteria, micrococci, yeast, and molds are generally added to meat during fermentation. Starter cultures protect against pathogen microorganisms and control the fermentation process (Löfblom et al., 2017). Table 1 shows the use of starter culture in meat products as an alternative to nitrite. The significant roles of lactic acid bacteria, yeasts, and molds are to decrease the pH and improve the flavor of meat products (Sunesen and Stahnke, 2003). However, these starter cultures may not help reduce nitrate to nitrite (Ammor and Mayo, 2007). However, cultures of coagulase-negative cocci such as *Micrococcus* variants, *Staphylococcus xylosus*, *Staphylococcus carnosus*, and others can reduce nitrate to nitrite (Bosse et al., 2016).
S. carnosus was originally isolated from fermented sausage and is still used as a culture in producing fermented meat products. *S. carnosus* used to reduce nitrate to nitrite can generate catalase more significantly in the presence of nitrate. Since catalase is an important antioxidant enzyme, which degrades hydrogen peroxide, the use of the starter culture could contribute to additional antioxidant protection (Talon et al., 1999; Ko et al., 2017). Consequently, the antioxidant effect of the starter culture used in natural meat products may be greater than that of conventional meat products without the starter culture. In addition, *S. carnosus* cannot produce nitrite well at low temperatures (6°C), and medium temperatures (24°C) are more suitable than high temperatures (38°C) for the amount of nitrite produced. Still, the reduction rate increases at high temperatures compared to medium or low temperatures (Krause et al., 2011).

Table 1: Starter cultures used in meat products as a nitrite alternative.

Starter culture	Meat product	References
Lactobacillus fermentum	Cured fermented sausage	(Møller et al., 2003)
Lactobacillus fermentum	Chinees sausage	(Zhang et al., 2007)
Staphylococcus xylosus	Meat dough	(Li et al., 2013)
Staphylococcus carnosus, Staphylococcus simulans, Staphylococcus saprophyticus	Sausage	(Gøtterup et al., 2008)

Table 2. Pre-converted nitrite sources and their effect on meat products.

Meat product	Nitrite alternative	Effect		References
		+	-	
Ground, cooked, and sliced ham	Celery powder+ *Staphylococcus carnosus*	-Reduced residual nitrite	-Low color stability	Krause et al. (2011)
Cured pork loin	Pre-converted celery powder	-Prevented the growth of *Listeria monocytogenes*	-Darkening in color -Increased residual nitrite content	Horsch et al. (2014)
Vacuum-packed turkey meat	Pre-converted celery juice powder and cherry juice powder	- Decreased number of anaerobic bacteria Increased redness value in combined use	-Increased vegetable flavor and lower taste scores	Djeri and Williams. (2014)
Meat emulsion	Pre-converted red beet + *S. carnosus*+ ascorbic acid	-Increased redness values with combined use -Low residual nitrite content	-Higher TBARS values with extract use	Choi et al. (2017)
Pork sausage	Pre-converted spinach, lettuce, celery, and red beet+ *S. carnosus*	-Low residual nitrite content	-Low redness value -High TBARS values	Hwang et al. (2018)
Cured pork loin	Spinach juice and pre-converted spinach juice	- Decreased residual nitrite content - Increased sensory acceptability with the use of fermented spinach juice	-Low taste scores	Kim et al. (2019)
Fermented smoked sausage	Lyophilized celery+*S. xylosus* or *S. xylosus* and *P. pentosaceus* mixture	- Sausages with *S. xylosus* revealed less residual nitrate content -Positive effect in reddish color	- The reddish color was less intense in sausages with lyophilized celery’s addition	Eisinaite et al. (2020)
Cooked ground pork product	White kimchi powder, acerola juice powder, celery powder + *S. carnosus*	- Formulas with acerola juice powder presented no difference in the redness of cured color	- Supplementary ingredients with buffering capacity for pH are required	Choi et al. (2020)
Fermented cured sausage	Radish and beetroot powder+ *Staphylococcus carnosus*	- Vegetable powder addition lowered humidity and aw, increasing the weight loss of the sausages	-Beetroot’s powder addition negatively affected the sausage’s color.	Ozaki et al.(2021)

Conclusion

Nitrite and nitrate can represent a risk to consumer health. It is not an immediate risk but a result of an ensemble of factors that might favor the formation of carcinogenic nitrosamines. However, epidemiological studies have reported that the risk of cancer formation or toxic effects of nitrate taken with diet from natural sources is not observed. Pre-converted nitrite, called natural nitrite, may not be harmful to humans. This review discussed pre-converted nitrite technology as a natural curing agent in processed meat products. Residual nitrite content may be the most challenging problem. The residual nitrite contents of meat products supplemented with natural nitrite were found to be lower than those of meat products supplemented with synthetic nitrite. Furthermore, the flavor of vegetable powder in meat products may not be acceptable to consumers. Still, the appropriate addition of natural nitrite sources do not produce undesirable flavor. The use of vegetable extracts has antimicrobial and antioxidant properties, so they are probably the most studied alternatives. Overall, the use of pre-converted nitrite is expected to have critical applications in the natural curing of meat products.

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TECHNOLOGICAL PROPERTIES OF MEATBALLS FORMULATED WITH DIFFERENT LEVELS OF DRIED TOMATO POWDER

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Abstract

In recent years, the development of functional meat products with properties that can promote well-being is one of the fastest-growing areas of research. Enhancing the functionality of meat products by adding natural additives rich in dietary fiber is a prominent strategy. Therefore, this study was carried out to observe the effects of using dried tomato powder (4%, 8%, and 12%) on some quality parameters of meatballs. The increase in the tomato powder (TP) ratio to 12% in meatball samples caused the moisture content of the samples to decrease. Protein amounts of the treatments were not statistically significant, while fat content was reduced by adding tomato powder. The fibers in the tomato powder swelled, thus, preventing moisture and fat release from the matrix and providing higher cooking efficiency. The addition of 4% TP did not affect fat retention while other inclusion levels resulted in higher retention of fat in the meat system. The incorporation of tomato powder had minimal changes in meatball diameters, treatments added with 12% TP and 4% TP had the lowest change in diameter and thickness, respectively. In the early stages of storage, the use of TP did not affect the TBA value, however, lycopene in the tomato powder was responsible for the lower TBA values in the later stages of storage. As a result, appropriate amounts of dried tomato powder could be a worthy attempt to entitle functional meat products without any detrimental effects on sensory properties.

Keywords: *Tomato powder, Meatball, Cooking properties, Oxidation.*

Introduction

In recent years, consumers' interest in healthy meat product formulations has increased. From this point of view, researchers have focused on changes in product formulations in the meat industry. Nonmeat ingredients are widely incorporated as fillers, binders, or extenders to increase the water holding capacity and yield and improve the sensory properties in low-fat meat products, including sausage and patties. Functional properties, sensory acceptability, and cost are the major criteria for choosing non-meat ingredients. Especially in the last 30 years, meatball type reshaped grounded meat products very important developments on both sectoral and scientific scales have been recorded. A variety of plant sources such as plum puree (Yıldız Turp and Serdaroğlu, 2010), potato puree (Ergezer et al., 2014), pumpkin powder (Serdaroğlu et al., 2018), carrot powder (Kerimoğlu Öztürk et al., 2021) have been used as binders and extenders in comminuted meat products. Tomatoes are rich sources of a variety of nutritional compounds and antioxidant components such as the carotenoid lycopene, vitamin C, and a range of polyphenols and dietary fiber (Bartikiene et al., 2015). Consumers have already become aware of the potential importance of the protective properties of these antioxidants. Tomato peel is an agro-industrial waste, that contains dietary fiber with numerous physiological effects and various nutritional compounds. The benefits of the use of tomato by-products in meat products are mainly their antşoxidant properties, red color, and rich in bioactive compounds (Dominquez et al., 2020). Wang et al., (2015) showed that tomato peel powder is an excellent fat substitute in sausages reduced the animal fat, improves the fatty acid composition profile, and decreased the lipid oxidation. Tomato peel powder can be an appropriate fat replacement in low-fat emulsion-type sausages because it improves the texture and increased the sensory scores. The objective of this research was to evaluate the effects of dried tomato powder on proximate composition, cooking parameters, and oxidative changes of meatballs.

Material and Methods

Beef as boneless rounds was obtained from the local butcher in Izmir, Turkey. All subcutaneous fat and inter-muscular fat were removed from the muscles and used as the fat source. Lean and fat were ground through a 3 mm plate grinder. Sun-dried tomatoes were obtained from the local market, in order to facilitate grinding, an additional drying process was applied at 70C for 4 hours in the oven. In this research, 4 different meatball samples were produced with (0, 4, 8, or 12 %) different amounts of tomato powder. With grounded meat and ingredients (Table 1) meatball dough was prepared by mixing and the prepared mixture was shaped using 7 mm diameter Petri dishes, and meatballs were packed in polypropylene containers and stored at -18°C for 6 months.

Table 1 Meatball formulations

Treatment	**Meat (g)**	**Salt+spices (g)**	**Tomato powder (g)**	**Water (g)**
C	6279	105+56	0	560
T4	5999	105+56	280	560
T8	5719	105+56	560	560
T12	5439	105+56	840	560

The moisture and ash contents were measured by using AOAC (1990) procedures. Fat content was determined according to the method of Flynn and Bramblett (1975). Protein content was determined according to Anonymous (1979). Meatballs were cooked in a pre-heated Teflon-coated pan for 4 min each side. Percent cooking yield was determined by calculating weight differences for samples before and after cooking. Fat retention was calculated according to the method described by Murphy et al.,((1975) Diameter and thickness changes were measured following to our previous methods (Serdaroglu et al., (2005). Secondary oxidation products were determined using the method of Tarladgis et al., (1960). Trained panelists participated in sensory analyses. Appearance, texture, flavor, and overall acceptability were scored through 9-like extremely to 1-dislike extremely. Meatballs were served warmed to panelists with randomly coded digits. Water and bread were provided to panelists to rinse the palate between the samples. For statistical analyses, One-way ANOVA (Minitab, 2003) was applied for chemical composition, cooking measurements, and sensory analyses. In the case of TBA analyses, two-way ANOVA was applied (Minitab, 2003). Significant means were separated using the least significant difference (P<0.05) test (LSD).

Result and Discussion

Chemical analyses of the meatballs added with tomato powder are presented in Table 2. The inclusion of tomato powder in the meatball formulation affected the moisture and fat contents of the samples (P< 0.05). The highest moisture content was obtained in control samples while the lowest moisture was recorded for T12 samples, this effect was due to the increase in dry matter with the addition of TP. Tomato powder resulted in a decrease in fat content. It may be due to the dilution of the oil ratio in the mixture.

Table 2. Chemical analyses of the meatballs

Treatment	Moisture %	Protein%	Fat%
C	58.1±0.70a	27.7±0.30	12.5±0.20b
T4	52.5±0.90b	30.7±0.45	10.3±0.01a
T8	52.9±0.82b	28.2±1.15	11.2±0.64a
T12	50.7±0.41b	27.0±0.80	11.8±0.88a

[a-b] Different letters in the same row indicate a significant difference (*p* < .05). Data were presented as the mean ± standard deviation.

Cooking properties in terms of cooking yield, fat retention, diameter, and thickness changes could be seen in Table 3. The highest cooking yield (73%) was found in T12 samples, T4 samples had a similar cooking yield to the control samples. However, Modzelewska-Kapıtuła (2019) found that the addition of tomato powder to meatloaf formulation had no impact on the cooking losses of the products. Keeping the fat in the matrix of meat products is an important feature in terms of the sensory quality and acceptability of the product (Anderson and Berry, 2001). Fat retention values of meatball samples are shown in Table 3. The fat retention of the samples varied between 59.9% and 79.5%. Fat retention values of T4 did not differ from the control sample, the other treatments had higher fat retention values when compared to the control. Cooking meatballs results in significant dimensional changes (Serdaroglu et al., 2005). A significant (P< 0.05) decrease in diameter change with increased contents of TP was detected. Our study showed that the addition of tomato powder significantly affected (P <0.05) the thickness change of the meatballs. After cooking thickening was detected in all treatments, due to the swelling, meatball thickness increased between 12.8% and 16.2%. A probable explanation is that the șexcellent water-holding capacity of the tomato fiber is present in high amounts. In our previous research meatballs prepared with corn flour had lower thickness changes (Serdaroğlu and Degirmencioglu, 2004).

Table 3 Cooking parameters of meatballs

Treatment	Cooking yield %	Fat retention %	Diameter change %	Thickness change %
C	62.2±0.36c	59.9±0.29b	17.6±0.01a	-15.3±0.41b
T4	59.±0.49c	59.8±0.40b	12.6±0.23b	-12.8±0.41c
T8	68.8±0.41b	79.5±0.01a	11.4±0.56b	-16.2±0.01a
T12	73.9±0.02a	78.7±0.09a	9.4±0.45 c	-14.7±0.18b

[a-c] Different letters in the same row indicate a significant difference (*p* < .05). Data were presented as the mean ± standard deviation.

Figure 1 shows the changes in TBA values of meatballs during frozen storage. Our study showed that the addition of tomato powder significantly affected (P <0.05). Initial TBA values varied between 0.54–0.47 mg ma/kg and no significant differences were recorded between the samples. TBA values increased in all samples during the storage. TP x storage interaction was found to be effective on TBA values (P <0.05). 1st, 2nd, and 3rd month TP level was not affected on TBA values, whereas on 4th, 5th, and 6th months TBA values of the TP added samples were found to be lower. This finding is not surprising because of the Antioxidant character of tomato powder containing a high amount of lycopene. In the 6th month TBA values of C, T4, T8, and T12 treatments were recorded as 1.63, 1.63, 0.98, and 0.94 mg ma/kg, respectively. The antioxidant activity of tomato products such as tomato paste and tomato powder is dose-dependent, which could be attributed to lycopene's activity. Reformulation of patties with tomato paste at all levels (5, 10, and 15%) and tomato powder (0.25, 0.5,0.75, and 1%) resulted in significant lower TBA values than control samples during refrigerated storage (Kim et al., 20013; Garcia et al., 2009). Also, Candogan (2002) reported that the inclusion of 5%, 10%, and 15% tomato paste in beef patties decreased the TBA values due to the antioxidative activity of lycopene in tomato paste.

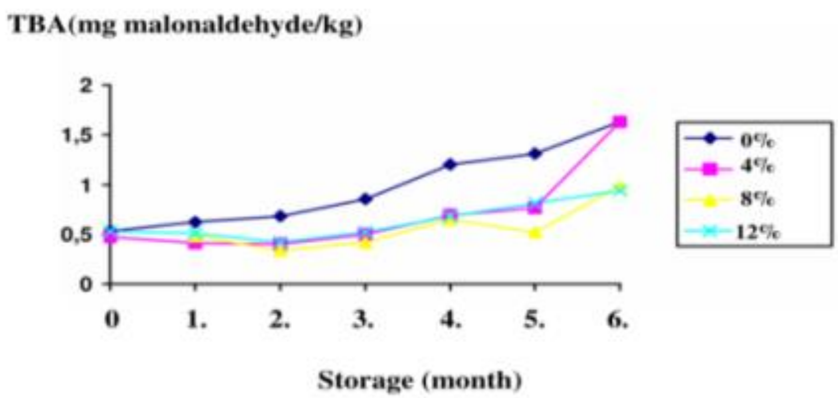

Figure 1 Changes TBA values of meatballs during storage at -18°C

The appearance scores of the meatball samples after production vary between 7.9-6.0. The addition of TP negatively affected the appearance scores (P <0.05). The darkening color of the TP added samples during cooking caused the panel to evaluate the appearance of these samples with low scores. After production, the flavor score was 8.1 in the control samples, and 6.9, 6.1, and 5.9 in the 4, 8, or 12 TP added samples, respectively. The addition of TP significantly affected the texture scores of the samples (P <0.05).

Table 4. Sensory scores of meatballs

Treatment	Appearance	Texture	Flavor	Overall acceptability
C	7.9	7.9	8.1	7.9
T4	6.8	7.6	6.9	7.6
T8	6.1	5.9	6.1	6.1
T12	6.6	5.5	5.9	6.8

While T4 samples did not differ from the control, 8% and 12% TP added samples had lower texture scores than the control samples. The use of tomato powder at an increasing rate caused the texture to harden during cooking because the water was not increased in the formulation, and the texture of these samples was evaluated with low scores by the panel. Overall acceptability was slightly lower in T8 and T12 samples the most acceptable sample was that with 4% TP. The addition of 3% flaxseed powder and 3% tomato powder to the sausage formulation resulted in the most desired product in terms of sensory properties.

Conclusions

In conclusion, tomato powder addition improved the cooking parameters and oxidative stability of meatballs. Fat content was reduced by adding tomato powder. Meatballs with 4% added tomato powder was the most acceptable. These results also indicated that the meatballs with low doses of tomato powder were more acceptable the than that with higher doses.

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ORGANIC AGRICULTURE

CONTENT OF MINERALS IN APRICOT FRUITS FROM THE URBAN AREA OF THE CITY OF MOSTAR, BOSNIA AND HERZEGOVINA

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Abstract

Apricot cultivation in the area of the city of Mostar has a long tradition. With the urbanization of the city in the early 1960s, larger cassia plantations disappeared, but individual trees remained in a large number of private backyards. The aim of this study was to examine the content of mineral substances in apricot fields from the urban area of the city of Mostar. For that purpose, three localities in the urban zone of the city were chosen, namely, Brankovac, Mazoljice, and Donja Mahala. All three tree sites from which the samples were taken were of different ages. Analysis of the content of K, Ca, Mg, Fe, Zn, Na, and Cu was performed by the method of atomic absorption spectrophotometry in the reference laboratory of the Federal Agro- Mediterranean Institute in Mostar. Since, according to our information, similar tests on apricot fruits from the area of Herzegovina and the City of Mostar have not been done so far, the obtained results are compared with the literature data. The results showed that the content of Na and Cu is extremely low, while the content of Fe is extremely high in relation to the reference value. The influence of localities, actually the age of the tree, on the content of K, Ca, Mg, and Fe showed statistical significance. The influence of localities on the Zn content had no statistical significance, and since the sodium and copper content was extremely low, they could not serve as a statistical feature.

Keywords: *cultivar, locality, mineral matter, concentration, statistical analysis.*

Introduction

Apricot, *Prunus armeniaca* L., is a nutritious fruit, rich with bioactive matter, which have a positive effect on human health. (Alajil et al, 2021). It is one of the most important fruits because almost all of its parts are edible, and it is grown in temperate climates (Milatović, 2013). The most prized part of this species is its fruit. In addition to carbohydrates, proteins, phenols, and vitamins, minerals also play a significant role in the positive impact on human health. Apricot fruit is a source of nine minerals, Zn, Ca, Cu, Fe, Mg, Na, Mn, P, and K (Heghedűş-Mîndru, 2014). Their content can be influenced by both the variety and the geographical area in which it is grown (L. D. Elia et al, 2011). Apricot cultivation in the Mostar area has a long tradition, among other things, due to the geographical area, ie the sub-Mediterranean climate that favours its cultivation.

With the urbanization of the city in the early 1960s, larger apricot plantations disappeared, but individual trees remained in a large number of private backyards. The aim of this study was to examine the content of mineral substances K, Ca, Mg, Fe, Zn, Na, and Cu in apricot fields from the urban area of the city of Mostar. For that purpose, three localities in the urban zone of the city were chosen, namely, Brankovac, Mazoljice, and Donja Mahala. According to the Pedological Map of the Federal People's Republic of Yugoslavia from 1959, this area is marked as a recent alluvial deposit, and according to the Pedological Map of the SFRY from 1976, it is marked as a settlement and is still managed to this day. The most commonly grown variety in this area was Hungary, and among older orchards, it is the most common today. Since this research has not been done before, the obtained results are compared with the literature data for the average values of these elements measured on fresh fruit.

Method and material

As a material for this research, apricot fruits from three localities of the urban zone of the City of Mostar were used: Brankovac, Mazoljice, and Donja Mahala. Samples were taken from trees within private estates, ie. backyards. According to the owner, the Hungarian variety is the best in all three localities, but the age of the trees is different. Samples were taken during the month of June 2020., and in that period the tree from the Brankovac site was six years old, at the Mazoljice site the tree was 16 years old, and at the Donja Mahala site, the tree age was 39 years.


Brankovac locality

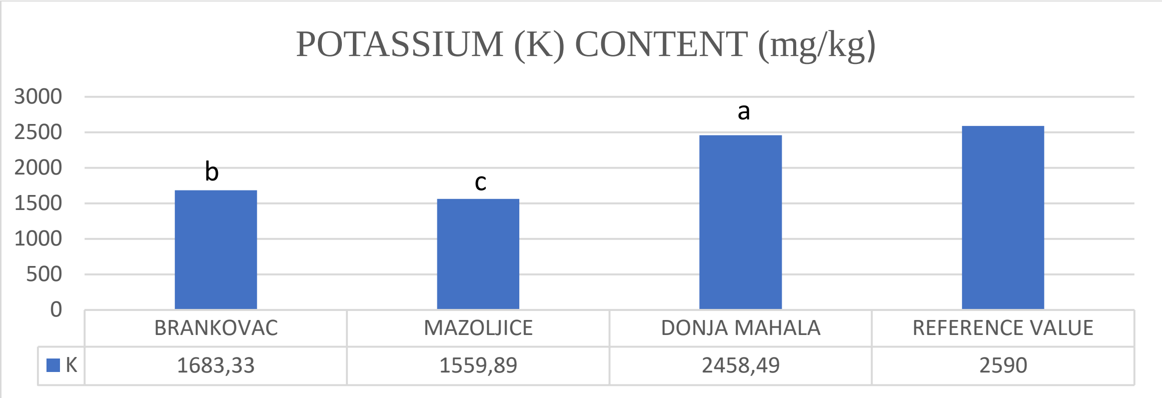

Mazoljice locality


Donja Mahala locality

From all three localities, average samples of apricot fruit were taken at the moment of their full physiological maturity and suitability for consumption. The average sample from all three localities weighed between 800 and 1000 grams. The samples were analysed for the content of the following minerals: K, Ca, Mg, Fe, Zn, Na, and Cu.
The analyses were performed in the reference laboratory of the Federal Agro-Mediterranean Institute in Mostar by Atomic Absorption Spectrophotometry. Since there has been no such research conducted so far, ie there are no data related to the content of these elements in apricot fruit from Mostar, the values obtained for each tested mineral were compared with the values for the average content of these elements (Milatović, 2013), which were used as reference values.
The results were statistically processed using variance analysis and the Tukey-Kramer test.

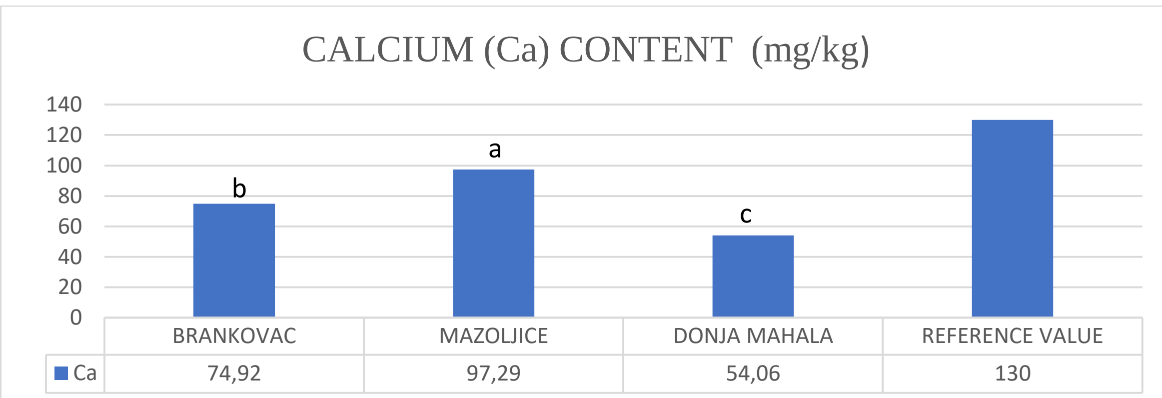
Results and discussion

Graphs 1 to 5 and Tables 1 and 2 show the results of analyzes for each examined element in relation to the reference value, as well as the results of statistical processing obtained by analysis of variance and the Tukey-Kramer test as statistical significance obtained by comparing the results in relation to localities ($p \leq 0.5$).



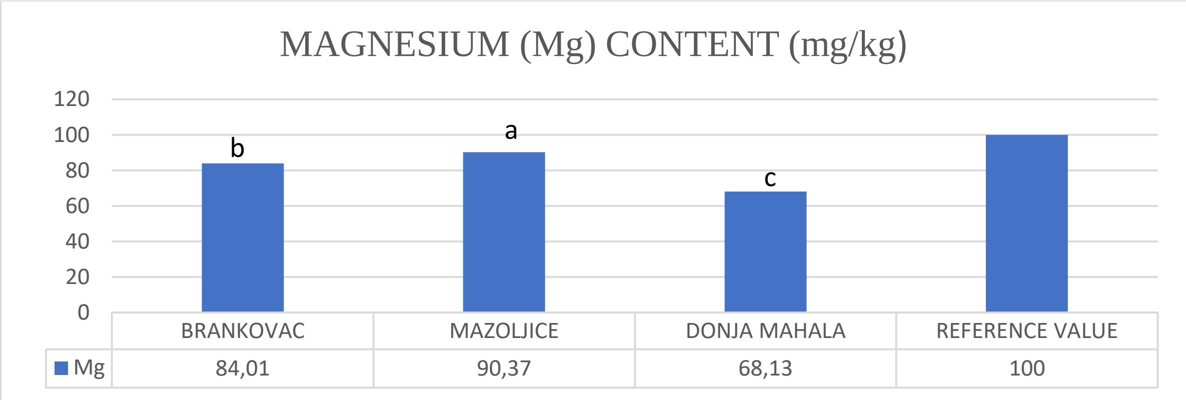
Graph 1. Potassium (K) content in apricot fruits from all three localities expressed in mg / kg

Fresh apricots are rich in potassium as an electrolyte, potassium generally helps maintain proper fluid balance, helps muscle work, and helps regulate heart rate. Getting enough potassium every day can help maintain normal blood pressure and can reduce the risk of stroke (Antoniod et al., 2018). The content of potassium in the examined samples was the highest at the locality Donja Mahala, and the lowest at the locality Mazoljice. In relation to the reference value, in the samples from the locality Donja Mahala potassium was 96%, at the locality Brakovac 64.99%, and at the locality Mazoljice 60.23%. Analysis of variance and Tukey-Kramer test showed the statistical significance of potassium content in apricot fruits between all three localities.



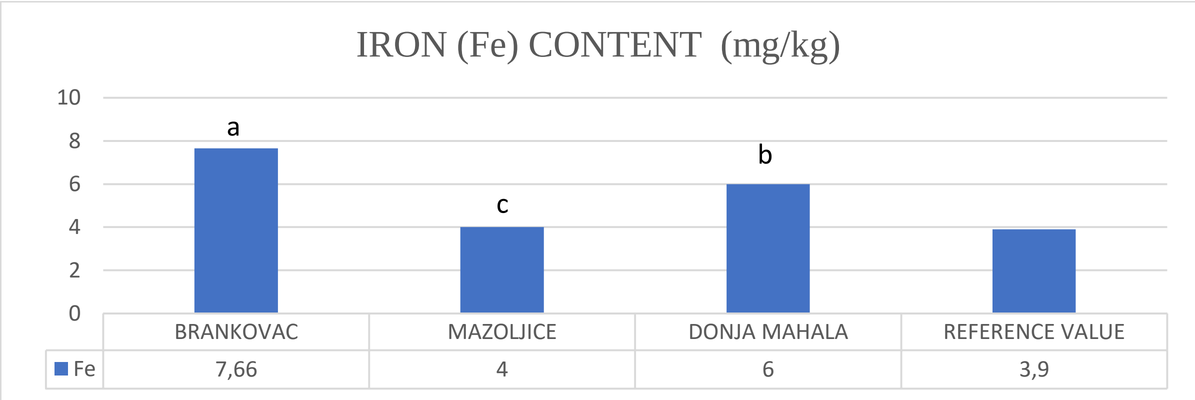
Graph 2. Calcium (Ca) content in apricot fruits from all three localities expressed in mg / kg

In addition to magnesium, calcium is considered one of the main minerals in apricot fruits (Drogoudi et al., 2008). Calcium nourishes bones, nerves, and muscles, balances blood pressure, and protects against allergies. The calcium content in the examined samples was the highest in the locality of Mazoljica and the lowest in the locality of Donja Mahala. In relation to the reference value, in the samples from the locality Mazoljice calcium was 74.83%, at the locality Brankovac 57.63%, and at the locality Donja Mahala 41.58%. Analysis of variance and Tukey-Kramer test showed the statistical significance of calcium content in apricot fruits between all three localities.



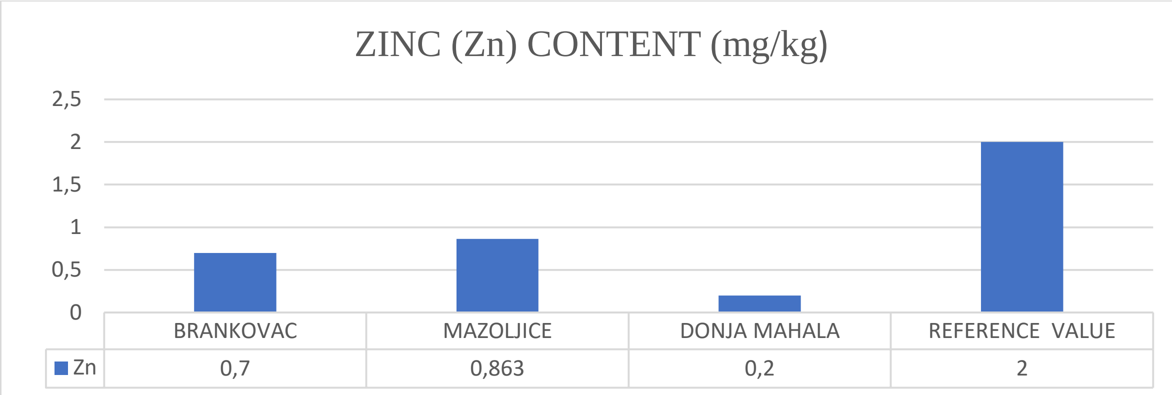
Graph 3.Magnesium (Mg) content in apricot fruits from all three localities expressed in mg / kg

Magnesium is vital in chlorophyll and metabolism and in the synthesis of carbohydrates, lipids, and proteins. The deficiency of this element leads to a decrease in the concentration of carotenoids (Negrea et al., 2012). The content of magnesium was the highest in the locality of Mazoljica and the lowest in the locality of Donja Mahala. In relation to the reference value, in the samples from the locality Mazoljice there was 90.37% of magnesium, in the samples from the locality Brankovac 84%, and from the locality Donja Mahala 68.13%. The results coincide with the data on magnesium content for different varieties and hybrids of apricot grown in Europe (Drogoudi et al., 2008). Analysis of variance and Tukey-Kramer test showed the statistical significance of magnesium content in apricot fruits between all three localities.



Graph 4. Iron (Fe) content in apricot fruits from all three localities expressed in mg / kg

The iron content was significantly higher than the reference value. The largest was in apricot fruits from the Brankovac locality and the smallest was from the Mazoljica locality. In relation to the reference value, in the samples from the Brankovac locality there was 196.41% of iron, in the samples from the Donja Mahala locality 153.84%, and in the Mazoljica locality 102.56%. Although the bioavailability of iron in fruits and vegetables is low compared to foods of animal origin, the presence of vitamin C-containing fruits, including apricots, may contribute to its more efficient absorption in the human body (R. Hurrell et al 2010). Analysis of variance and Tukey-Kramer test showed the statistical significance of iron content in apricot fruits between all three localities.



Graph 5. Zinc (Zn) content in apricot fruits from all three localities expressed in mg / kg

Zinc is one of the important minerals involved in many biochemical processes in the body and is especially important in times of flu and colds (Roohani et al., 2013). The zinc content was the highest in the locality of Mazoljice and the lowest in the locality of Donja Mahala. In relation to the reference value, in the samples from the locality Mazoljice zinc was 43.15%, in the samples from the locality Brankovac 35%, and in the locality Donja Mahala 10%. Analysis of variance and Tukey-Kramer test showed that there are no statistically significant differences between zinc content in apricot fruits from different localities.

Table 1. Sodium (Na) content in apricot fruits from all three localities expressed in mg/kg

Locality	Brankovac	Mazoljice	Donja Mahala	Reference value
Na Content	< 0,100	< 0,100	< 0,100	**10**

The sodium content was less than 0.1 mg/kg of fresh weight of the edible part of the fruit at all three localities, which is less than 1% of the reference value.

Table 2. Copper (Cu) content in apricot fruits from all three localities expressed in mg/kg

Locality	Brankovac	Mazoljice	Donja Mahala	Reference value
Cu Content	< 0,100	< 0,100	< 0,100	**0,8**

The copper content was less than 0.1 mg/kg of fresh weight of the edible part of the fruit at all three localities, which is less than 12% of the reference value.

Conclusion

Analyzing the obtained results, it can be concluded that the main characteristic of apricot fruits from the urban zone of the city of Mostar is extremely high iron content, which was significantly higher at all three examined localities in relation to the reference value. At the same time, the sodium and copper content was significantly lower than the reference value. Statistical significance of the influence of localities, actually the age of the tree, on the content of the observed parameters was recorded for potassium, calcium, magnesium, and iron. The same was not recorded for zinc content, and since the sodium and copper content was extremely low, they could not serve as a statistical characteristic. In the end, it can be said that, since this is the first study of this species on apricot fruits in this area, it is recommended that such research be continued so that apricots, which have a very long tradition of cultivation in Herzegovina and Mostar, receive confirmation of nutritional value as for human health valuable foods.

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THE INFLUENCE OF SOME BIO-PRODUCTS ON GERMINATION AND PROTECTION OF BASIL SEEDS

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Abstract

Basil (*Ocimum basilicum* L.) is an annual herb with medicinal, edible and economical values. To reach a yield potential, among other things, seed high germination percentage and seed vigor are required. The aim of the study was to discover the most efficient biopreparations with a beneficial effect on basil seed vigor, germination and seed health. Testing was conducted with two plant protection products, permitted in organic production ('Exstrasol F', 'Polyversum'), and three herbal preparations (fermented valerian extract, fermented extract of yarrow, and fermented LAB mix). Seed germination testing followed the standard procedure suggested by the Rule book on seed quality control, while seed health was examined by the filter paper method. The seeds were treated with 15 ml of bio-product solution, while the same amount of distilled water was used as a control. The experiment was conducted in three replications, and the seeds were observed on the 4th and 14th day following the treatment. The seed germination was increased in all treatments, in comparison to control. The treatments with fermented extract of yarrow had the highest effect on seed vigor. Based on the symptoms, the presence of *Alternaria* sp. was confirmed on the seed surface. Compared to control, the infection of seeds was reduced in all treatments, but the fermented extract of yarrow had the highest effect on disease reduction.

Keywords: *Ocimum basilicum L., bio-products, fermented extracts, germination, seed health, Alternaria* sp.

Introduction

Basil (*Ocimum basilicum* L.) is an annual herbaceous, a thermophilic plant species belonging to the Lamiaceae family. It is an ornamental, spicy, and medicinal plant (Stepanović and Radanović, 2011), used as fresh or dried for flavoring food, making tea, and liqueurs. The above-ground part of the plant (*Basilici herba*) is used in traditional and homeopathic medicine for the treatment of a number of diseases, and it is also used as a carminative, sedative, lactagogue and to improve appetite as well (Filipović et al., 2016). Basil essential oil (*Basilici aetheroleum*) is well-known for its bactericidal, fungicidal, antiviral, repellent, antioxidant, antidiarrheal, chemopreventive, and radioprotective properties (Kišgeci et al., 2009). It is mostly grown in Italy at about 80 ha, then in France on 30 ha and in Israel on 20 ha. In Serbia, basil has not been grown on larger areas in the past, but in recent years it has been grown more, especially in the Vojvodina region (Kišgeci et al., 2009; Stepanović and Radanović, 2011). Basil is often grown by direct sowing due to their good germination energy and total germination. The effect of lower

temperature on basil growth decreasing is well known (Walters and Currey, 2019), but the effect of bio-products in mentioned conditions has not been well characterized.
In an attempt to increase seed germination various “bio-products” has been commercially used (Filipović et al., 2021). The bio-products based on plant extracts can prevent the appearance of phytopathogenic fungi, as well. 'Exstrasol F' is a bio-product composed of rhizosphere nitrogen-fixing bacteria (*Bacillus subtilis*) intended to improve seed germination. The 'Polyversum' is based on the fungus *Pythium oligandrum* and is recommended for protection of seeds from economically important phytopathogenic fungi such as *Fusarium* spp., *Pythium* spp., *Peronospora belbahrii and Alternaria alternata* are mainly causing decay of cultivated plants and damping of seedlings (McLeod et al., 2006; Taba et al., 2009; Garibaldi et al., 2011).
The aim of this study was to examine the influence of some herbal preparations on germination and health of basil seeds on lower temperature.

Material and methods

The study was conducted in the laboratory of Agricultural Research and Development of the Institute for Medicinal Plants Research "Dr Josif Pančić" in Belgrade (Serbia). The seeds of basil (*O. basilicum* L.) cv. Genovese, produced at the experimental field of the Institute, have been subjected to tests on seed vigor, germination, and seed health, during 2022. Two bio-products (from the Lists of plant protection and plant nutrition products and the list of soil improvers, permitted in organic production) and three herbal preparations (created in the Institute), are used in the treatments of basil seed.

Testing of biopreparations on the seed germination
The energy (EG) and total seed (TG) germination were examined as suggested by the Rule book on seed quality control („Official Gazette of the Republic of Yugoslavia“, no. 23/2009, 64/2010, 72/2010 and 34/2013). The seed germination testing was conducted with 100 seeds on filter paper in Petri dishes, in triplicates. The seeds were treated with 15 ml of previously prepared solutions: 'Exstrasol F' (recommended concentration 0.20 ml/kg), 'Polyversum' (recommended concentration 0.50 g/kg), fermented valerian extract, fermented extract of yarrow, and fermented LAB mix (5 ml herbal preparations being dissolved in 45 ml of distilled water). The same amount of distilled water (15 ml) was used in the control treatment. The number of germinated seeds was counted by the use of a binocular loupe on 4th and 14th day from the day of setting up the experiment (ISTA, 2010). Preliminary research on germination at a temperature of 22^{o}C was conducted in triplicates, as well. Germination was 94%. The experiment was conducted in a humidity chamber at T 19/16°C day/night regime.

Table 1. Bio-products used in the treatments of basil seed.

Bio-product	Active substances		Short business name	
	name	**content**	**manufacturer**	**representative**
Exstrasol F	*Bacillus subtilis* strain Č13	1 x 10^{8} CFU/cm^{3}	BioGenesis, Bačka Topola and Jugo Hem, Leskovac	-
Polyversum	*Pythium*	3% (1 X 10^{6} - 10^{7} oospore/g)	Biopreparaty,	Vins 2000,

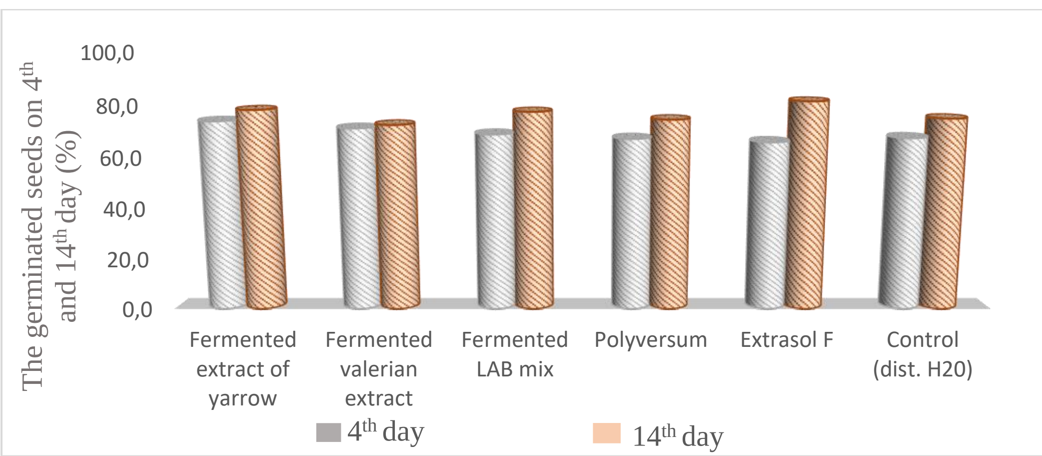
	oligandrum		Czech Republic	Belgrade
LAB mix	herbal preparation for protection and plant nutrition	fresh aerial parts of chamomile 30%, horsetail 20%, valerian 15%, nettle 15% and comfrey 20%	Institute for Medicinal Plants Research “dr J. Pančić”, Belgrade	

The seed health status of basil

The seed health status was examined by the filter paper method. The experiment was conducted on previously sterilised (110°C/1h) and moistened filter paper, using 100 unsterilised seeds, in triplicates. Macroscopic and microscopic seed examinations were performed 4th and 14th day following incubation. An Olympus CX43 microscope (Olympus, Hamburg, Germany) was used to observe the microscopic characteristics of the phytopathogenic fungi developed on the seeds, and the photographs were taken with an Olympus EP50 (Olympus, Hamburg, Germany).

Results and discussion

The efficacy of five bio-products, with different active substances (Tab. 1), was tested in recommended concentration, on the energy germination and the total germination of basil seeds. Observed was conducted on 4th and 14th experimental days (Graph 1).



Graph 1. The energy germination and the total germination of basil seeds observed on 4th and 14th days of the experiment (%).

Germination was higher then 20% at T 22°C than at T 19/16°C, which is in agreement with previous studies where it was concluded that higher temperatures (>20 °C) accelerate the growth and development of basil (Walters and Currey, 2019). Although germination at lower temperatures was significantly lower it was still on a satisfying level according to the Rule book on seed quality control („Official Gazette of the Republic of Yugoslavia“, no. 23/2009, 64/2010, 72/2010 and 34/2013).

In the treatments at T 19/16°C with herbal preparations and biofungicides, the highest energy of germination was achieved with 'Fermented extract of yarrow' (averagely 73.7%) followed by

'Fermented valerian extract’ and 'Fermented LAB mix' for 2.7% less. However, the biofungicides 'Exstrasol F' and 'Polyversum' exhibited weaker effects for 8% and 6.7% than the control.

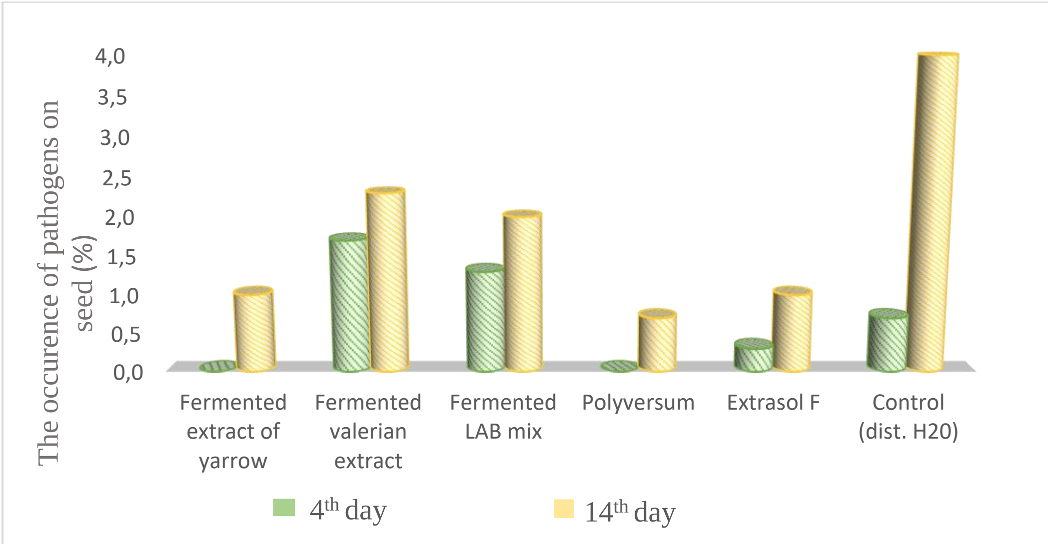
The seeds treated with 'Exstrasol F' and 'Polyversum', had higher total germination values (on average by 15.6% and 7.3%) compared to those achieved after 4th day. After 14th days, the herbal preparations ''Fermented extract of yarrow' and 'Fermented LAB mix' had a similar effects on total germination seeds (78% and 77.3%). The product 'Fermented valerian extract' had a weaker effect (2.4%) compared to the control (Picture 1).

a) b) c)

Picture 1. Total germination: a) Control treatment; b) 'Polyversum'; c) LABmix

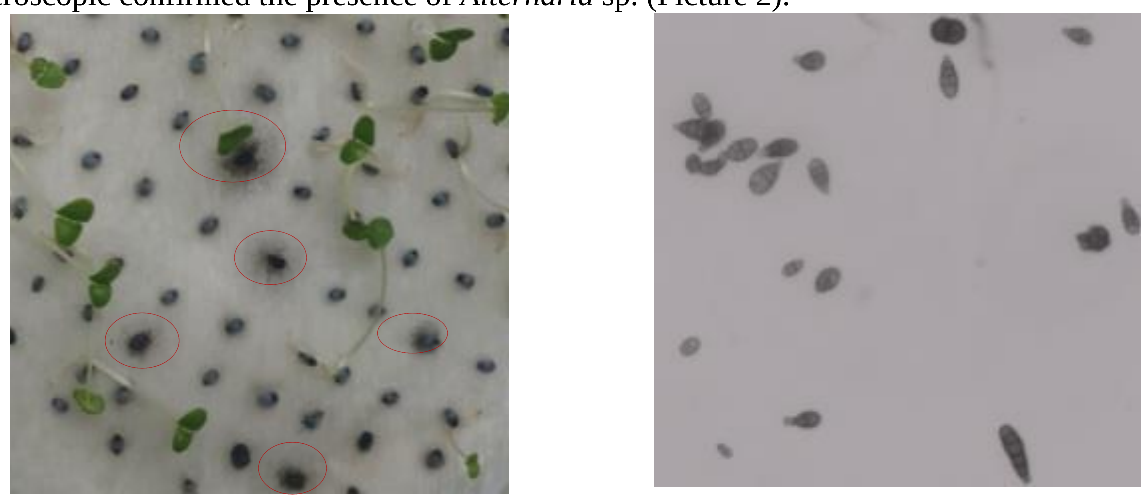
In earlier research, 'Exstrasol F' and 'Polyversum' also, had a positive effect on the energy germination and total germination of German chamomile seeds. Also, 'LAB 4' (fresh aerial parts of chamomile 50% and valerian 50%) had an effect on the EG and TG of chamomile seeds (32.3% and 60.8%) (Filipović et al., 2021). Filipović et al. (2014) tested EG and TG on seeds of white oregano (*Origanum heracleoticum* L.) and marjoram (*Origanum vulgare* L.) with the use of herbal preparation 'LAB 1' (fresh herbs of chamomile (20%), horsetail (20%), valerian (20%), yarrow herb (10%), nettle (10%), comfrey (10%) and licorice (10%)) which has a beneficial effect on tested characters, as well.

The efficacy of five bio-products tested in recommended concentration, on the occurence of basil pathogens, observed on 4th and 14 th experimental days, are given in Graph 2.



Graphs 2. The occurence of pathogens on basil seed observed on 4th and 14th days of the experiment (%).

Macroscopic examinations revealed the symptoms of dark mycelia on the seed surface, while the microscopic confirmed the presence of *Alternaria* sp. (Picture 2).



a) b)

Picture 2. The symptoms of diseases: a) dark mycelia on the seed surface; b) *Alternaria* sp.

'Polyversum' and 'Fermented extract of yarrow' prevented the occurrence of seed infection after 4th day. A similar effect was achieved with 'Exstrasol F' (0.3%). The most infected seeds were recorded after 14th days, in the treatments with 'Fermented valerian extract' (2.3%), 'Fermented LAB mix' (2.0%) and in the control treatment (4.0%). The pathogens of the genus *Alternaria* appeared on the seeds of fennel and chamomile (Filipović et al., 2021).

Conclusions

The several biopreparations showed a positive effect on germination and reduction of pathogens on *Ocimum basilicum* L. Herbal preparations 'Fermented extract of yarrow', 'Fermented valerian extract' and 'Fermented LAB mix' could be recommended for improvement of the germination energy and total germination of basil seeds, while the 'Polyversum' and 'Exstrasol F' could be recommended as one of the measures to prevent the occurrence of basil seed disease.

Acknowledgement

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EFFECTS OF ORGANIC FERTILIZERS APPLICATION ON FRUIT QUALITY IN MELONS

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Abstract

The need for consumption of vegetable fruits obtained from organic production has increased significantly in the last ten years. Melons (melons and watermelons) play a significant role in human nutrition. Compared to conventional melon production, the biggest problem in organic production is lower yield. This can be overcome by creating melon and watermelon varieties that are more suitable for growing in the organic production system. In this two-year field experiment, 5 melon, and 5 watermelon genotypes were used. The trial was conducted in an open field in the Smederevska Palanka. The effect of the application of four different commercial organic fertilizers on five characteristics of melons was observed. Mineral fertilizer was included in the trial as a control. The aim of the study was to determine the best melon and watermelon genotypes for the organic system of production. The greatest positive effects of the application of organic fertilizers were determined in the watermelon genotype Fairfax. Compared to the control, the fruit weight was increased by 15%, the sugar content up to 5%, while the thickness of the rind was decreased by 11%. In melon has been observed a smaller effect of the application of organic fertilizers, especially for the observed traits: weight of fruit and total sugar content. For Passport (melon genotype) was recorded 22% higher weight of fruits harvested from plants treated with organic fertilizers than in the control. The results showed that the Charentais, Passport (melon), Fairfax, and Greybelle (watermelon) are the genotypes that could be recommended for organic production systems.

Keywords: *Cucumis melo* L., *Citrullus lanatus (Thunb.) Matsum. et. Nakai), sustainable agriculture, conventional production.*

Introduction

Melon and watermelon are popular annual vegetable species belonging to the family *Cucurbitaceae*. Annually, in the world producers harvest about 130 million tons of fruits of these two vegetable species from about 4 million ha (FAO, 2020). The crop ratio in the production of melons and watermelons is 1: 3, in favor of watermelons. In the Republic of Serbia, melons are grown on approximately 20,000 ha, with a total production of 400,000 t fruits per year (Girek et al., 2014). Melons have a long history of growth. The center of origin of watermelon is Northeast Africa, while the center of origin of melons is Asia. In the past, watermelons were used as a source of drinking liquid, while melons were used exclusively in the form of young fruits (Paris, 2016). The development of civilization and raising people's awareness regarding nutrition has also affected the quality of the fruits of these vegetables. Today, when more and more attention

is paid to a healthy diet, the demands of consumers on the market are formulating the method of production and quality of watermelon fruits (Kyriacou et al., 2018; Dalorima et al., 2021).
Organic production is most commonly associated with production on small, family farms, and the average size of these farms is about 3.5 ha (Zrakić et al., 2017). In the last 20 years, the production in the organic production system has increased about 7 times in the world (Mpanga et al., 2021). In the Republic of Serbia, the transition from the conventional to the organic cultivation system goes slower. The total area under the organic vegetable growing system in Serbia in 2019 was only 184 ha, and the share of all organic fields in total arable agricultural land was only 0.61% (Simić, 2020). One of the reasons is the yield, which is 19 - 25% lower in the organic cultivation system compared to the conventional cultivation system (Seufert, 2019). One of the reasons for the lower yields is the fact that most of the existing varieties were not bred for the organic growing system and were not tested for specific conditions of the organic growing system during the breeding process (Park et al., 2018).
Today, many breeding companies have breeding programs that focus exclusively on creating varieties intended for the organic cultivation system (Boyhan et al., 2019;). New varieties are tested for resistance to specific diseases and pests, yield, fruit quality characteristics, and tolerance to local climatic conditions. In such programs, the most important is the availability of genetic material of old varieties and local populations that already have the adaptability to agro-climatic conditions of a particular region (Szamosi, 2005).
The aim of this study was to examine the influence of different commercial organic fertilizers with different nutrient ratios on the qualitative properties of fruits in melons. Also, to identify varieties that could be recommended for cultivation in the organic production system. One of the goals was to single out genotypes that would be a good starting material in melon and watermelon breeding programs for the creation of new varieties designed for the organic cultivation system.

Materials and methods

The two-year experiment was conducted in Smederevska Palanka (latitude 44°21'22.46"N, longitude 20°57'08.97"E, elevation 101 m). Five different melon genotypes (1 - Fiata, 2 - Cerovača, 3 - Galia, 4 - Charentais, 5 - Passport) and five watermelon genotypes (1 - Crimson sweet, 2 - Fairfax, 3- Greybelle, 4 - Mramorka, 5 - Dunay) were used in this study. Four commercial organic fertilizers available in Serbia with different nutrition ratios were used: Italpollina (4% N : 4% P_2O_5 : 4% K_2O), DCM Ekomix (9% N : 3% P_2O_5 : 3% K_2O), Guanitto (6% N : 15% P_2O_5 : 3% K_2O) i Duetto (5% N : 5% P_2O_5 : 8% K_2O). One-fifth of plants fertilized with mineral fertilizer NPK (15% N : 15% P_2O_5 : 15% K_2O) were considered the control in this experiment. All ten genotypes were seeded in the first decade of April.
Melons seedlings were produced in clay pots (diameter 10 cm), in the greenhouse of the Institute for vegetable crops. When the plantes reached the phase of 7 permanent leaves, they were transplanted to the open field. The experiment was set up in three replications using a complete randomized block design. Each replicate was composed of 50 rows (5 fertilizers x 10 melon and watermelon genotypes; 10 plants per row – 100 x 150 cm). Two weeks after transplanting, the plants were fertilized. The fruits were harvested successively. For watermelon 5 traits were observed: 1. Fruit weight, 2. Fruit length, 3. Fruit width, 4. Pericarp thickness, and 5. Sugar content. In melon 6 traits were observed: 1. Fruit weight, 2. Fruit length, 3. Fruit width, 4.

Pericarp thickness, 5. Mesocarp thickness, and 6. Sugar content. The agronomic measures that were implemented were in accordance with the organic system of cultivation.
All the obtained results were statistically analyzed. The differences between fruits harvested from plants fertilized with commercial organic fertilizers and controls were determined. All results were statistically analyzed using the Fisher's Least Significant Difference (LSD) test (Fisher, 1935). Also, the most important traits were singled out and the correlation between traits was determined using PCA analysis. The principal components method was analyzed using the statistical program Statistica 8.0 (StatSoft, 2007).

Results and discussion

The results in Tables 1 and 2 show that commercial organic fertilizers had a significant effect on all observed traits in both watermelon and melon.

Table 1. Average values of five observed watermelon traits and comparison of effect of commercial organic fertilizers and mineral fertilizer NPK on these observed traits (%)

Genotype	Treatment	Fruit weight – g (%)	Fruit length – cm (%)	Fruit width – cm (%)	Pericarp thickness – cm (%)	Sugar content - ^{0}brix (%)
1	Control	4159.52	20.76	18.49	1.11	8.23
	I	4083.52 (↓1.83)	20.53 (↓1.14)	18.34 (↓0.83)	1.07 (↓3.61)	9.26 (↑12.51)
	II	4256.32 (↑2.33)	21.01 (↑1.20)	18.86 (↑1.99)	1.15 (↑2.97)	8.20 (↓0.40)
	III	4097.59 (↓1.49)	20.66 (↓0.48)	18.39 (↓0.53)	1.10 (↓1.61)	8.59 (↑4.32)
	IV	4144.56 (↓0.36)	20.79 (↑0.14)	18.38 (↓0.62)	1.09 (↓1.81)	8.74 (↑6.20)
2	Control	4078.33	32.58	15.41	1.11	7.92
	I	4696.94 (↑15.17)	33.97 (↑4.26)	15.63 (↑1.44)	0.99 (↓10.64)	7.97 (↑0.59)
	II	4456.21 (↑9.27)	33.55 (↑2.98)	15.75 (↑2.22)	1.01 (↓9.02)	8.02 (↑1.23)
	III	4653.64 (↑14.11)	33.96 (↑4.23)	15.90 (↑3.17)	1.03 (↓7.17)	8.26 (↑4.28)
	IV	4201.21 (↑3.01)	32.06 (↓1.60)	15.77 (↑2.38)	1.03 (↓6.63)	8.37 (↑5.64)
3	Control	2466.90	17.46	16.01	1.04	9.18
	I	2919.15 (↑18.33)	18.94 (↑8.47)	16.57 (↑3.47)	1.01 (↓2.85)	9.56 (↑4.15)
	II	2472.76 (↑0.24)	18.19 (↑4.23)	15.98 (↓0.20)	0.96 (↓7.47)	9.19 (↑0.15)
	III	2356.95 (↓4.46)	17.53 (↑0.41)	15.80 (↓1.32)	0.96 (↓7.90)	9.83 (↑7.06)
	IV	2534.76 (↑2.75)	18.03 (↑3.27)	16.00 (↓0.06)	1.00 (↓3.21)	9.66 (↑5.26)
4	Control	3273.95	16.83	18.61	0.70	8.97
	I	3506.62 (↑7.11)	17.07 (↑1.47)	18.97 (↑1.92)	0.74 (↑5.46)	9.04 (↑0.78)
	II	2959.86 (↓9.59)	15.97 (↓5.08)	18.19 (↓2.24)	0.69 (↓0.82)	8.87 (↓1.15)
	III	2790.29 (↓14.77)	15.93 (↓5.31)	17.85 (↓4.06)	0.68 (↓2.80)	9.46 (↑5.47)
	IV	3083.46 (↓5.82)	16.63 (↓1.16)	18.22 (↓2.11)	0.71 (↑1.10)	9.29 (↑3.58)
5	Control	3590.43	19.85	17.87	0.96	7.90
	I	3750.74 (↑4.46)	20.38 (↑2.68)	18.24 (↑2.09)	0.92 (↓4.41)	8.29 (↑4.99)
	II	3415.90 (↓4.86)	19.24 (↓3.08)	17.48 (↓2.18)	0.88 (↓8.83)	7.96 (↑0.73)
	III	3511.75 (↓2.19)	19.66 (↓0.97)	↓0.74	0.88 (↓8.68)	7.91 (↑0.16)
	IV	3710.00 (↑3.33)	20.74 (↑4.47)	18.01 (↑0.80)	1.02 (↑6.04)	7.82 (↓1.06)
		$lsd_{0.05}$ = 24.86	$lsd_{0.05}$ = 0.09	$lsd_{0.05}$ = 0.12	$lsd_{0.05}$ = 0.02	$lsd_{0.05}$ = 0.05

% - the difference between control and treatment in percent; ***1*** *- Crimson sweet,* ***2*** *- Fairfax,* ***3****- Greybelle,* ***4*** *- Mramorka,* ***5*** *– Dunay;* ***I*** *- Italpollina,* ***II*** *- DCM Ekomix,* ***III*** *– Guanitto,* ***IV*** *- Duetto*

In watermelon, the best results were recorded on plants fertilized with commercial fertilizer Italpollina. With the application of this fertilizer in 4 out of 5 genotypes were recorded higher yields (from 4.46 - Dunay to 18.33% - Greybelle) than in the control. The largest increase in fruit length was also recorded on plants fertilized with Italpollina. A higher decrease in the thickness of pericarp was observed in fruits harvested from plants of genotype Greybelle fertilized with organic fertilizers, in relation to the control. This is an extremely good result, considering that one of the important tasks of every watermelon breeder is to create watermelon varieties with the thinnest possible fruit pericarp (Jiao et al., 2015).

The sugar content measured on the mesocarp of the fruit harvested from the plant which was fertilized with organic fertilizers was higher (especially with Italpollina), in all genotypes, compared to control. The largest increase in sugar content compared to the control was recorded in the genotype Crimson sweet (12.51%). For early-maturing genotypes, Fairfax and Greybelle, were recorded the highest overall positive effect of all four commercial organic fertilizers.

In melon genotypes, a smaller positive effect of the use of commercial organic fertilizers was recorded. The greatest positive effect of the application of organic fertilizers, compared to the control, was determined for Passport genotype (for all observed traits).

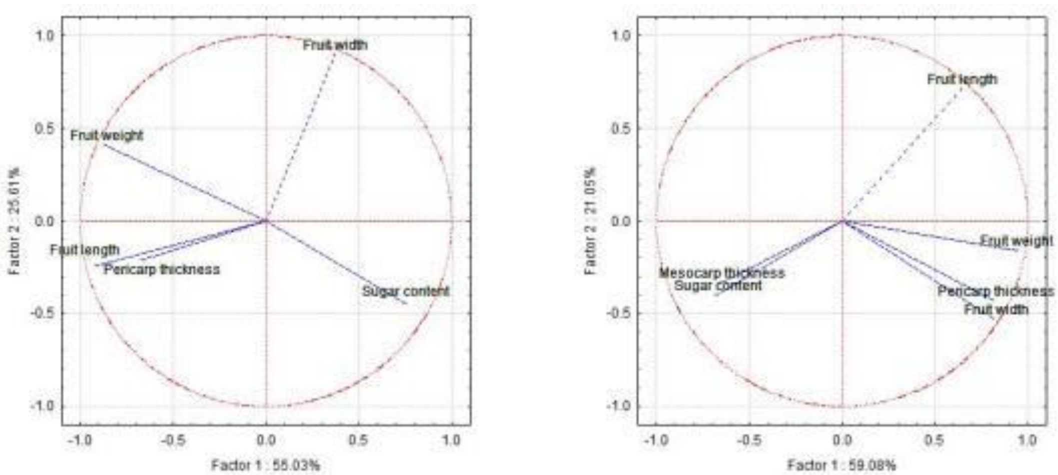
Table 2. Average values of six observed melon traits and comparison of effect of commercial organic fertilizers and mineral fertilizer NPK on these observed traits (%)

Genotype	Treatment	Fruit weight – g (%)	Fruit length – cm (%)	Fruit width – cm (%)	Pericarp thickness – cm (%)	Mesocarp thickness – cm (%)	Sugar content - ^{0}brix (%)
1	Control	1597.14	13.73	15.53	0.87	2.77	13.30
	I	1453.33 (↓9.00)	13.30 (↓3.12)	14.88 (↓4.21)	0.71 (↓18.24)	2.93 (↑5.54)	10.85 (↓18.42)
	II	1492.35 (↓6.56)	13.63 (↓0.72)	14.39 (↓7.31)	0.66 (↓24.73)	2.80 (↑1.03)	11.80 (↓11.28)
	III	1498.13 (↓6.20)	13.53 (↓1.48)	15.06 (↓3.03)	0.65 (↓25.41)	2.96 (↑6.74)	10.38 (↓21.99)
	IV	1426.00 (↓10.72)	13.66 (↓0.47)	14.11 (↓9.12)	0.72 (↓17.84)	2.88 (↑3.77)	10.53 (↓20.86)
2	Control	2381.88	19.61	16.95	1.04	2.58	6.50
	I	1995.40 (↓16.23)	18.56 (↓5.35)	16.18 (↓4.54)	1.03 (↓0.53)	2.62 (↑1.75)	7.47 (↑14.87)
	II	1773.38 (↓25.55)	17.26 (↓11.97)	15.88 (↓6.32)	1.06 (↑2.34)	2.33 (↓9.54)	8.60 (↑32.31)
	III	2166.21 (↓9.05)	19.01 (↓3.05)	16.31 (↓3.77)	0.97 (↓6.94)	2.83 (↑10.08)	8.55 (↑31.54)
	IV	2187.76 (↓8.15)	19.04 (↓2.91)	16.73 (↓1.30)	0.98 (↓5.39)	2.74 (↑6.39)	7.32 (↑12.62)
3	Control	1643.57	18.39	13.29	0.76	2.64	10.22
	I	1366.59 (↓16.85)	17.51 (↓4.74)	13.07 (↓1.60)	0.63 (↓16.55)	2.55 (↓3.34)	9.89 (↓3.23)
	II	1512.42 (↓7.98)	18.06 (↓1.78)	13.45 (↑1.25)	0.62 (↓18.62)	2.66 (↑0.82)	10.67 (↑4.40)
	III	1439.13 (↓12.44)	17.43 (↓5.20)	13.45 (↑1.25)	0.57 (↓24.77)	2.63 (↓0.47)	9.50 (↓7.05)
	IV	1380.83 (↓15.99)	17.53 (↓4.64)	13.04 (↓1.87)	0.65 (↓14.15)	2.66 (↑0.59)	9.21 (↓9.88)
4	Control	1250.00	17.20	12.48	0.44	2.87	9.70
	I	1299.67 (↑3.97)	17.59 (↑2.29)	12.63 (↑1.19)	0.39 (↓11.50)	2.81 (↓1.86)	9.25 (↓4.64)
	II	1441.96 (↑15.36)	18.63 (↑8.31)	13.01 (↑4.30)	0.47 (↑5.27)	2.76 (↓3.82)	9.21 (↓5.05)
	III	1375.16 (↑10.01)	17.93 (↑4.27)	12.71 (↑1.83)	0.44 (•0)	2.86 (↓0.15)	10.70 (↑10.31)
	IV	1517.07 (↑21.37)	17.24 (↑0.24)	13.32 (↑6.76)	0.44 (•0)	2.80 (↓2.45)	9.90 (↑2.06)
5	Control	1205.00	14.78	12.95	0.33	2.95	8.60
	I	1308.40 (↑8.58)	14.98 ()↑1.36	13.22 (↑2.05)	0.55 (↑69.85)	3.04 (↑3.19)	9.36 (↑8.84)
	II	1326.59 (↑10.09)	14.99 (↑1.46)	13.27 (↑2.46)	0.41 (↑27.27)	3.17 (↑7.40)	10.80 (↑25.58)
	III	1270.71 (↑5.45)	14.45 (↓2.18)	13.29 (↑2.59)	0.48 (↑47.99)	3.26 (↑10.41)	10.08 (↑17.21)
	IV	1389.44 (↑15.31)	15.27 (↑3.33)	13.72 (↑5.96)	1.04 (↑221.37)	3.07 (↑4.14)	8.88 ()↑3.29
		$lsd_{0.05}$ = 18.65	$lsd_{0.05}$ = 0.12	$lsd_{0.05}$ = 0.07	$lsd_{0.05}$ = 0.03	$lsd_{0.05}$ = 0.03	$lsd_{0.05}$ = 0.15

% - the difference between control and treatment in percent; ***1*** *- Fiata,* ***2*** *- Cerovača,* ***3*** *- Galia,* ***4*** *- Charentais,* ***5*** *- Passport;* ***I*** *- Italpollina,* ***II*** *- DCM Ekomix,* ***III*** *– Guanitto,* ***IV*** *- Duetto*

The positive effect of the application of commercial organic fertilizers, which was determined in this experiment, is in line with the results of Davis et al. (2007) where was determined a higher content of sugar and lycopene in watermelon fruit grown in an organic cultivation system in comparison to the conventional cultivation system. The positive effect of the application of the organic cultivation system was also found in watermelon and melon (Curuk et al., 2004), pepper (Berova and Karanatsidis, 2008), and potatoes (El-Sayed et al., 2015). Although many studies and practices have shown that due to limited inputs in the organic cultivation system, the quality of vegetable fruits is lower than those harvested in conventional production, this is not always the rule.

In order to determine the relationship between the observed traits, the obtained results were analyzed using principal component analysis. Based on this analysis, it was found that PC1 explains 59.08% of the total variability of the observed traits in melon, and 55.03% in watermelon, while PC2 explains 21.05% of the total variability in watermelon and 25.61% in melon. In watermelon fruit weight, fruit length, and sugar content explain the most PC1 variability, while fruit width explains the most PC2 variability. In melon, fruit weight, fruit width, and pericarp thickness explain the most variability of PC1, while the most PC2 variability explains trait fruit length. Based on the results shown in Graph 1a, we can conclude that the sugar content and fruit weight in watermelon are negatively correlated, while the fruit length and the pericarp thickness are in a high positive correlation. In melon, fruit length is significantly negatively correlated with mesocarp thickness and sugar content, which are mutually positively correlated (Graph 1b).



Graph 1– Projection of the traits based on eigenvalues of PC1 and PC2 (a – watermelon; b – melon)

Conclusion

The usage of commercial organic fertilizers can positively impact the quality of fruit traits in melons and watermelons. On watermelon genotypes, Fairfax and Greybelle, and on melon genotypes, Charentais and Passport, all four commercial organic fertilizers had the best positive effect and these genotypes can be recommended for inclusion in organic farming systems. Italpollina had the greatest positive effect on observed fruit quality traits in watermelon, in all five genotypes. In the organic cultivation system, the size of the fruit in watermelon is negatively correlated with the sugar content in the mesocarp of the fruit. In melons, mesocarp thickness and sugar content are positively correlated. These results are important when the breeder makes breeding plans and in a process of creating new varieties of watermelon and melon intended for the organic cultivation system.

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THE EFFECT OF DEHYDRATION PROCESSES ON TOTAL PHENOL, ANTIOXIDANT ACTIVITY, FATTY ACID COMPOSITIONS AND POLYPHENOLICS OF WHITE MULBERRY FRUITS

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Abstract

In this study, the effect of dehydration process on oil contents, total phenol, total flavonoid contents, total carotenoids and antioxidant activity values, phenolic compounds and fatty acid compositions of mulberry fruits dehydrated by microwave and oven systems was investigated. While moisture contents of mulberry fruits are reduced from 83.90% (control) and 18.55% (oven), the oil contents of mulbery fruits treated were measured between 6.75% (oven) and 7.40% (control). In addition, while total phenolic amounts of mulberry fruits dried by microwave and oven systems increase from 177.97 mg GAE/100g (control) to 416.88 mgGAE/100g (microwave), antioxidant activity values of dehydrated mulberry fruits also increased from 55.14% (control) and 89.43% (microwave). Also, carotenoid contents of mulberry fruit samples randeg from 0.21 µg/g (control) to 0.45 µg/g (microwave) while total flavonoid contents of mulberry fruits dried by microwave and oven systems are determined between 180.50 (control) and 1082.17 mg/100g (microwave). The highest carotenoid, phenolic content, flavonoid contents and antioxidant activities were determined in mulbery fruits dehydrated by microwave oven. Phenolic constituents found at the highest levels in mulberry fruits dehydrated by microwave and oven systems were gallic acid, 3,4-dihydroxybenzoic acid, (+)-catechin, 1,2-dihydroxybenzene and syringic acid. In general, the amount of phenolic constituents of mulberry fruits dehydrated by microwave was identified higher than those in mulberry fruits dehydrated by oven drying system. Linoleic acid contents of oils of dehydrated mulberry fruits varied between 75.05% (control) and 76.66% (microwave) while oleic acid contents of mulberry fruit oils were determined between 9.37% (microwave) and 9.87% (oven). Results showed significant differences depending on dehydration systems compared to control group.

Key words: *Mulberry fruit, oil, bioactive compounds, phenolics, fatty acids.*

Introduction

Mulberry (Morus, Moraceae), which has different properties in terms of chemical composition, nutritional value, pehnolic components and antioxidant properties, is widely grown in temperate, subtropical or tropical regions of the world and in a wide variety of climatic, topographic, and soil conditions (Liang et al.2020). Soluble bioflavonoids with high antioxidant properties may be responsible for the medicinal properties of mulberry fruit (Naderi et al. 2004). Apigenin, lueolin, quercetin, caffeic acid, gallic acid, rutin, chlorogenic acid and kaempferol have been identified in the fruit of the mulberry belonging to the family of Moraceae (Chu et al. (2006). Mulberry plant (Morus spp.), which was cultivated thousands of years ago, generally grows in temperate and subtropical regions of the northern hemisphere and also in the tropics of the southern hemisphere, considering the climate, topographic and soil conditions (Imran et al., 2010; Natic et al. 2015). The fruits of the mulberry plant, which has historically been used in silkworm, deciduous, fast-growing (about 10-20 m), and recently attracted great attention, have been reported to be a good source of health-beneficial compounds. (Hassimotto et al. 2007; Ercişili and Orhan, 2007; Yang et al.2010; Singhal et al. 2010). Tsai et al. (2005) studied on The antioxidant activity of mulberry crude extract against the ABTS radical cation. Treat fewer, protect liver from damage, facilitate discharge of urine and lower blood pressure of mulberry fruit have been reported in folk medicine (Gerasopoulos and Stavorulakis (1997). Recently, studies on the bioactive properties and phenolic components of mulberry fruit have intensified (Bae et al. 2007; Kostic et al. 2013; Jang et al. 2015; Natic et al. 2015;Yang et al. 2016). Recently, the production and consumption of mulberry fruits have been increasing rapidly due to their taste, flavor and biological values. Mulberry fruits have been reported to have odontalgic, anthelmintic, expectorant, hypoglycemic, and emetic effects as an worm agent, a remedy for dysentery, and a laxative (Naderi et al.2004; Ercisli and Orhan, 2007; Liang et al.2020). Morus alba is known because of its use in sericulture (Lim et al. 1990). According to Yaltirik (1982), the Anatolia has growing available conditions for growing high quality mulberyy fruit and plants. Mulberry fruits are used in an unique delicious fruit with a refreshing acid taste (Kostic et al. 2013). Due to the its nutritional value, the mulberry fruits are consumed nowadays, both fresh and processed in the form of juices, syrups, jams, marmalade, beverages, natural food dyes or dried fruits (Ionica et al. 2017; Gündoğdu et al. 2011; Güngör and Şengül, 2008). Mulberry fruits of the same species may contain different amounts of bioactive components and different antioxidant properties due to climatic and growing factors. Mulberry fruits are used as a nutritional element in diet fruit, fruit juices, marmalades, liquor and various food industries. (Liang et al.2020). White mulberries are known as an important nutritional source for protecting the immune system due to rich in flavonoids (Butt et al. 2008). The texture, color, phenolic profile, flavor and appearance of most herbal materials can change with dehydration and / or roasting, and the taste, flavor and aroma and shelf life of the products obtained increase (Nicoli et al. 1999; Chandrasekara and Shahidi, 2011; Kim et al., 2011). However, studies regarding effect of drying on quantification of bioactive properties, antioxidant activity, fatty acid composition and phenolic compounds of mulbery fruits are very limited. The aim of present study was to illustrate the effect of dehydration process on oil contents, total phenol, total flavonoid contents, total carotenoids and antioxidant activity values, phenolic compounds and fatty acid compositions of mulberry fruits dehydrated by microwave and oven systems.

Material and Methods

Material

The fruits of *Morus alba* were picked randomly at the biologically ripened stage from five trees of the same age (about 20 years) from Konya distinct in Turkey in July 2019. The harvested fruits were taken to the laboratory in plascic bags for analytical assays. The fruits were stored at -18 oC until analysis.

Methods

Heat treatment

The white mulberry samples were heated in a conventional oven at 60°C; in a microwave oven at 360W until the moisture content decreases below 20%. The heated samples were ground into powder using a grinder before analysis.

Determination of moisture content

The moisture amounts of morus fruits were calculated by drying at 105°C in an oven (Nüve FN055 Ankara. Turkey) up to a constant weight.

Oil content

Each of the ground mulberry samples (about 10 g) was individually weighed, and extracted 5h using petroleum ether in the Soxhlet apparatus. The temperature applied in the extraction process was 50 ^{o}C. At the end of the extraction period, petroleum ether was removed in the evaporator at 50 ^{o}C. The oil content of the mulberry fruit was determined by calculating the data obtained (AOAC (1990).

Carotenoid content

Method stated by Silva da Rocha et al. (2013) was applied for extraction of carotenoids. After adding 25 ml of acetone to about 2 g of the ground sample, the mixture was vortexed for 10 minutes and filtered. The filtrate was then taken into a separatory funnel and fractionated with 20 ml of petroleum ether. It was then washed with 100 ml of distilled water to remove acetone from the mixture. These steps were repeated twice. The supernatants collected were Whatman No. 1 filtered on filter paper and the volume of the Extracts made up to 25 ml with petroleum ether. The absorbance of the samples obtained was measured at 450 nm.

Extraction procedure

The extraction process of mulberry fruit was carried out according to method stated by Pehluvan et al. (2015) with some modifications. After adding 10 ml of methanol: water (80:20; v/v) to approximately 1 g of the ground sample, the sample was sonicated in an ultrasonic bath for 30 minutes at room temperature. After sonication, the sample was centrifuged at 6000 rpm for 10 minutes. The supernatant obtained was concentrated by evaporation under vacuum at 40 ^{o}C. The volume of the extract obtained was made up to 10 ml with a methanol: water (80:20; v/v) mixture in volume and each extract was filtered through a 0.45 µm nylon filter.

Total phenolic content

The Folin-Ciocalteu method was applied to identify total phenolic amounts of mulberry extracts according to method described by Yoo et al. (2004). After adding 1 ml of FC on approximately 2 g of mulberry extract, the mixture was mixed for 5 minutes and at the end of this time 10 ml of Na_2CO_3 was added. The solution in the tubes was then mixed again and the final volume made up to 25 ml with deionized water. After holding the samples for 1 hour, their total phenolic content (mg gallic acid equivalent (GAE) / 100 g) was measured in a spectrophotometer at a wavelength of 750 nm. All determinations were made three times.

Total flavonoid content

The colorimetric method specified by Hogan et al. (2009) was used to detect the total flavonoid content of the extracts. 0.3 ml $NaNO_2$, 0.3 ml $AlCl_3$ and 2 ml NaOH on approximately 1 ml of extract were added and mixed. The absorbance value of the resulting mixture was measured at 510 nm. Results are expressed as mg catechin / g fresh weight.

Antioxidant activity

DPPH (1.1-diphenyl-2-picrylhydrazyl) method described by Lee et al. (1998) was applied to measure the free radical scavenging activity of samples. After the extract was mixed with 2 ml of methanolic DPPH solution, the mixture was shaken vigorously. After shaking, the samples were kept at room temperature for 30 minutes. At the end of this period, the absorbance values of the samples were measured at 517 nm using a spectrophotometer. All detections were made three times.

Determination of phenolic compounds

A Shimadzu-HPLC equipped with a PDA detector and an Inertsil ODS-3 (5µm; 4.6 x 250mm) column was used for analysis of phenolic compounds. A mixture of 0.05% acetic acid in water (A) and acetonitrile (B) were used as the mobile phase. The flow rate of the mobile phase and the injection volume were 1 ml/min at 30 °C and 20 µl. The peaks were recorded at 280 and 330 nm using a PDA detector.

Fatty acid composition

Fatty acid methyl esters of the mulberry oil esterificated according to ISO-5509 (1978) method were analysed gas chromatography (Shimadzu GC-2010) equipped with flame-ionization detector (FID) and capillary column (Tecnocroma TR-CN100. 60m x 0.25mm. film thickness: 0.20µm). The temperature of injection block and dedector and mobile phase were 260ºC and nitrogen with 1.51 ml/min flow rate, respectively. In addition, total flow and split rates were 80 ml/min and 1/40, respectively.

Statistical Analyses

Analysis of variance was determined by using JMP version 9.0. All analyses were carried out three times. The results obtained are mean±standard deviation of independent mulberry fruit dehydration types (Püskülcü and İkiz, 1989).

Results and Discussion

The physico-chemical and bioactive compounds of mulberry fruits dried by microwave and oven systems are given in Table 1. Dehydration process showed differences among bioactive properties of mulbery fruits dried by both microwave and oven treatments compared to control (fresh fruit) group. While moisture contents of mulbery fruits are reduced from 83.90% (control) and 18.55% (oven), the oil contents of mulbery fruits dehydrated were measured between 6.75% (oven) and 7.40% (control). In addition, while total phenolic amounts of mulberry fruits dried by microwave and oven systems increase from 177.97 mg GAE/100g (control) to 416.88 mgGAE/100g (microwave), antioxidant activity values of dehydrated mulberry fruits also increased from 55.14% (control) and 89.43% (microwave). Also, carotenoid contents of mulberry fruit samples randeg from 0.21 µg/g (control) to 0.45 µg/g (microwave) while total flavonoid contents of mulberry fruits dried by microwave and oven systems are determined between 180.50 (control) and 1082.17 mg/100g (microwave). As seen in Table 1, total phenolic, flavonoid contents and antioxidant activities of mulberry fruits dehydrated by microwave and oven treatments increased compared to control (fresh fruit) group. Also, it was observed a linear relation among bioactive compounds of mulberry fruits. The highest total carotenoid, phenolic content, flavonoid contents and antioxidant activity values were found in mulbery fruits dehydrated by microwave oven. This case can be probably due to not to destroyed bioactive compounds from heat applied by microwave oven. In previous study, *Morus alba*, *Morus nigra* and *Morus rubra* contained 71.5%, 72.6% and 74.6% moisture (Ercişili and Orhan, 2007). Kabus-Cisowska et al. (2020) determined 553-2833 µg/gtotal flavonoid, 895-2176 µg/g total phenolic acid and 1.33-3.55 mmol Trolox/g antioxidant activity value in *Morus alba* fruit extracts obtained by different solvents. Ercişili and Orhan (2007) determined 181-1422 mgGAE/100g total phenol and 29-276 mgQE/100g (fw) total flavonoids in three mulberry species. Total phenolic, total flavonoid contents and antioxidant activity values of mulberry fruit extracts from different ripening stages changed between 2.72 and 11.33 mgGAE/g, 6.76 and 10.83 mgRU/g and 10.08 and 24.80 µg/ml, respectively (Yang et al. 2016). Total phenolic contents of four mulberry fruit species changed between 880 mgGAE/100g (fw) (*Morus nigra*) and 1650 mgGAE/100g (fw) (Morus alba) (Imran et al. 2010). The total phenolic content was determined as 1515.9 mgGAE/100g (fw) in Chinese origin mulberries (Lin and Tang, 2007). Total phenolics and antioxidant activities of mulberry (*Morus nigra* and *Morus alba*) extracts obtained by methanol were determined as 164 and 119 mgGAE/g to 1.25 and 0.75 mmolTrolox/g, respectively (Arfan et al. 2012). Mulberry fruit extracts obtained by 70% ethanol contained 0.95-2.57 mgGAE/g total phenolic (Katsube et al. 2006). Total phenol and total flavonoid contents of mulberry extracts were found between 959.9 and 2570.4 µgGAE/g to 5.6 and 65.4 µg/g, respectively (Bae et al. 2007). The total phenolic contents and antioxidant activity values of White mulberry genotypes varied between 50.29 and 76.29 mgGAE/100g to 70.29 and 75.48%, respectively (Natic et al. 2015). Total phenolic, flavonoid and antioxidant activity values of *Morus nigra* fruit extracts obtainedby water, ethanol/water (50/50;v/v) and ethanol changed between 90.26 and 118.84 mgGAE/100g; 141.70 and 183.90 mgCE/100g and 168.71 and 283.10 mg TroloxEquivalent/100g, respectively (Kostic et al. 2013). Black mulberry fruit extract obtained by ethanol contained 867 mg GAE/100g total phenol (Chun et al. 2011). Total phenolic contents and antioxidant activities (DPPH) of mulberry fruit genotypes changed between 1874.35 and 2977.30 mgGAE/g to 16.87 and 26.80%, respectively (Okatan, 2018). The amounts of total phenolic, flavonoid and antioxidant activity varied from one species to another from 436.93 mgGAE/100g in red mulberry to 924.55 mgGAE/100g in black mulberry, from 72.285 mgQE/100g in red mulberry to 241.215 mgQE/100g (fw) in black mulberry and from 454.97 mmol Trolox/100g in white mulberry to 505.50 mmol Trolox/100g in red mulberry (Ionica et al. 2017). Different values of total phenolic content have been reported by Kostic et al. (2013) who found 118.84 mgGAE/100g in the aqueous extract of black mulberry and Ercişili and Orhan (2007) found 1422 mgGAE/100g. Memon et al. (2010) determined 48.13 mgGAE/100g (fw) total flavonoid in white mulberry fruit. Geçer et al. (2016) reported that antioxidant activity value of White mulberry fruit was 6.17 µmolTE/g. It has been stated that the differences in the total phenol content of the plant materials may be due to the extraction medium used and the polarity of the extraction solvents, and the extraction time of the plant material (Shi et al. 2005). These differences in total phenol and flavonoid contents of mulberry fruits can be probably due to genetic deviation, cultivation and collection time (Scalzo et al. 2005; Hakkinen and Torronen, 2000). Antioxidant activities of mulberry cultivars and genotypes varied between 6.17 and 21.13 µmolTE/g (Gündoğdu et al. 2017). Güngör and Şengül (2008) determined 18.16 and 19.24 µmolTE/g antioxidant activity values in White mulberries. The health importance of berry fruits, which have a strong relationship between free radical scavenging and phenolic contents, has recently increased due to their high antioxidant activity. (Sanchez et al.2014). It has been reported that the compound responsible for the antioxidant capacity of fruits and vegetables originates from phenolic acids (Jin et al., 2017). Results obtained exhibited partly changes compared to results of previous studies. These fluctuations can be probably due to genotype variations, growing conditions, harvest time and maturation and applied analytical conditions and solvent properties used.

Table 1. Some chemical properties of mulberry samples

	Moisture content (%)	Oil content (%)	Carotenoid content (µg/g)
Control	83.90 ± 0.38**a	7.40 ± 1.13a	0.21 ± 0.00b
Microwave	19.40 ± 0.00b**	7.00 ± 0.28ab	0.45 ± 0.00a
Oven	18.55 ± 0.00c	6.75 ± 0.35c	0.16 ± 0.00b
	Total phenolic content (mg/100g)	**Total flavonoid content (mg/100g)**	**Antioxidant activity (%)**
Control	117.97 ± 0.02c	180.50 ± 0.02c	55.14 ± 0.06c
Microwave	416.88 ± 0.04a	1082.17 ± 0.04a	89.43 ± 0.00a
Oven	286.67 ± 0.03b	498.00 ± 0.06b	71.29 ± 0.01b

*standard deviation;** values within each column followed by different letters are significantly different at $P < 0.05$.

The phenolic compounds of mulberry fruits dehydrated by microwave and oven treatments are presented in Table 2. According to results, gallic acid, 3,4-dihydroxybenzoic acid, (+)-catechin, and 1,2-dihydroxybenzene were the dominant phenolic comppounds of mulberry fruits dehydrated by microwave and oven systems. Statistically significant differences were established among the amounts of mulberry fruits dried by both drying systems. While gallic acid amounts of dehydrated mulberry fruits change between 106.95 mg/100g (control) and 1436.09 mg/100g (oven), 3,4-dihydroxybenzoic acid contents of dried mulberry fruits were reduced from 20.39 mg/100g (control) to 4.76 mg/100g (oven). While (+)-catechin amounts of mulberry fruits dehydrated are reduced from 35.37 mg/100g (control) to 9.13 mg/100g (control), 1,2-dihydroxybenzene amounts of mulberry samples were decreased from 14.42 mg/100g (control) to 6.70 mg/100g (oven). Syringic acid contents of mulberry fruit samples varied between 9.43 mg/100g (oven) and of 41.23 mg/100 g (microwave) while the amounts of caffeic acid of dehydrated mulberry fruits are determined between 1.04 (oven) and 13.19 mg/100g (microwave). While rutin trihydrate amounts of mulberry fruits dehydrated by microwave and oven systems vary between 2.26 mg/100 g (oven) and 4.89 mg/100g (microwave), quercetin contents of dehydrated mulberry samples varied between 2.61 mg/100g (control) and 4.09 mg/100 (oven). In addition, trans ferulic acid and apigenin-7-glucoside amounts of mulberry fruits dehydrated were reduced from 1.98 (control) to 0.48 mg/100g (oven); from 1.87 (control) to 0.40 mg/100g (oven) together with heating, respectively. While kaempferol contents of dehydrated mulberry fruits change between 0.61 mg/100g (control) and 1.56 mg/100g (microwave), isorhamnetin contents of mulberry fruits dehydrated by microwave and oven treatments were determined between 0.44 mg/100g (control) and 1.21 mg/100g (microwave).

Table 2. Phenolic compounds of mulberry samples

Phenolic compounds (mg/100g)	Control	Microwave	Oven
Gallic Acid	106.95 ± 0.54*c	862.72 ± 5.52b	1436.09 ± 7.44a
3,4-Dihydroxybenzoic Acid	20.39 ± 0.57a**	7.98 ± 0.28b	4.76 ± 0.63c
(+)-Catechin	35.37 ± 0.51a	16.47 ± 0.86b	9.13 ± 0.38c
1,2-Dihydroxybenzene	14.42 ± 0.05a	8.63 ± 0.99b	6.70 ± 0.23c
Syringic Acid	27.77 ± 0.97b	41.23 ± 0.24a	9.43 ± 0.25c
Caffeic Acid	8.03 ± 0.05b	13.19 ± 0.74a	1.04 ± 0.09c
Rutin trihydrate	4.48 ± 0.10b	4.89 ± 0.89a	2.26 ± 0.14c
p-Coumaric Acid	0.64 ± 0.15a	0.53 ± 0.18b	0.19 ± 0.04c
trans-Ferulic Acid	1.98 ± 0.55a	1.38 ± 0.61b	0.48 ± 0.12c
Apigenin 7 glucoside	1.87 ± 0.46a	1.60 ± 0.41b	0.40 ± 0.07c
Resveratrol	0.25 ± 0.05b	0.49 ± 0.06a	0.22 ± 0.04c
Quercetin	2.61 ± 0.52a	2.65 ± 0.16a	4.09 ± 0.54b
trans-Cinnamic Acid	0.18 ± 0.04c	0.22 ± 0.04b	0.26 ± 0.05a
Naringenin	0.79 ± 0.16a	0.56 ± 0.05c	0.65 ± 0.10b
Kaempferol	0.61 ± 0.07c	1.56 ± 0.60a	0.65 ± 0.10b
Isorhamnetin	0.44 ± 0.07b	1.21 ± 0.17a	1.20 ± 0.35a

*standard deviation;** values within each row followed by different letters are significantly different at P < 0.05.

As seen in Table 2, gallic acid, rutin trihydrate, quercetin, and *trans*-cinnamic acid contents of mulberry fruits increased by the microwave and oven drying. In addition, when compared to control, syringic acid, caffeic acid, resveratrol, kaempferol, and isorhamnetin contents of mulberry fruits dehydrated by microwave increased, and then reduced in oven drying system. In general, the amount of phenolic constituents of mulberry fruits dehydrated by microwave oven were identified higher than those of results of mulberry fruits dehydrated by oven drying system. In previous study, Yang et al. (2016) determined 639.269-134.829 µg/g rutin, 9.823-28.568 µg/g quercetin-3-o-galactoside, 0.011-0.081 µg/g quercetin, 0.003-0.013 µg/g kaempferol, 0.268-0.383 µg/g caffeic acid, 136.8-517.667 µg/g chlorogenic, 0.068-0.159 µg/g p-hydroxybenzoic acid in mulberry fruit extracts obtained obtained from different ripening stages. In other study, *Morus alba* fruit extracts obtained by different solvents contained 23-66 µg/g epigallocatechin, 32-169 µg/g gallocatechin, 64-222 µg/g quercetin, 432-2376 µg/g rutin, 12-62 µg/g caffeic acid, 122-433 µg/g chlorogenic acid, 104-1265 µg/g ferulic acid, 33-64 µg/g gallic acid, 44-86 µg/g o-coumaric acid, 43-155 µg/g p-coumaric acid, 24-58 µg/g p-hydroxybenzoic acid, 106-255 µg/g protocatechuic and 43-76 µg/g vanillic acid (Kabus-Cisowska et al. 2020). Arfan et al. (2012) reported that *Morus nigra* and Morus alba fruit extracts contained 10.8 to 23.3 mg/g and 7.4 to 17.7 mg/g chlorogenic acid and 12.3 to 43.0 mg/g and 14.0 to 34.3 mg/g rutin, respectively. In another study, Natic et al. (2014) reported that White mulberry fruits grown in Vojvodina (North Serbia) contained 0.38-0.42 mg/kg gallic acid, 1.02-6.64 mg/kg gallocatechin, 2.64-2.72 mg/kg protocatechuic acid, 0.45-0.67 mg/kg epigallocatechin, 0.43-2.65 mg/kg chlorogenic acid, 0.41-0.84 mg/kg p-hydroxybenzoic acid, 0.11-0.21 mg/kg caffeic acid, 1.72-13.46 mg/kg rutin, 0.24-0.35 mg/kg p-coumaric acid, and 0.57-0.83 mg/kg ferulic acid. Mulberry fruits contained 1.36 to 5.89 mg/100g ellagic acid, 32.06 to 133.60 mg/100g rutin, 2.33 to 11.25 mg/100g quercetin, 21.83 to 40.90 mg/100g gallic acid, 2.28 to 10.54 mg/100g catechin, 43.20 to 97.59 mg/100g chlorogenic acid, and 6.14 to 17.97 mg/100g caffeic acid (Okatan, 2018). Gündoğdu et al. (2017) determined 0.03-3.86 mg/100g vanillic acid, 10.54-118.23 mg/100g rutin, 0.98-10.42 mg/100g quercetin, 12.85-36.85 mg/100g gallic acid, 1.13-9.85 mg/100g catechin, 21.09-2.44 mg/100g caffeic acid, 11.91-1.16 mg/100g syringic acid and 5.67-0.70 mg/100 g p-coumaric acid in mulberry germplasm. Ionica et al. (2017) determined 1.14 mg/100g gallic acid, 1.79 mg/100g vanillinic acid, 5.08 mg/100g chlorogenic acid, 0.14 mg/100g syringic acid, 0.76 mg/100g coumaric acid, 1.94 mg/100g rutin, 9.56 mg/100g ellagic acid in black mulberry fruits. The phenolic compounds imparting taste in ripening berry fruits were affected by genetic factors and pre-harvest conditions (Zadernowski et al. 2005). Differences in results obtained can be probably due to location, harvest time, ripening conditions and climatic factors compared to results of previous studies.

Table 3. Fatty acid composition of mulberry oils

Fatty acids (%)	Control	Microwave	Oven
Myristic	ND*	0.08 ± 0.00	ND
Palmitic	7.82 ± 0.44*c	8.76 ± 0.11a	8.43 ± 0.03b
Stearic	3.41 ± 0.10b**	3.34 ± 0.00c	3.58 ± 0.01a
Oleic	9.76 ± 0.09b	9.37 ± 0.02c	9.87 ± 0.03a
Linoleic	75.05 ± 2.50c	76.66 ± 0.12a	76.39 ± 0.25b
Arachidic	0.10 ± 0.00c	0.15 ± 0.00b	0.18 ± 0.02a
Linolenic	0.62 ± 0.12c	0.73 ± 0.00a	0.66 ± 0.06b
Behenic	0.15 ± 0.02a	0.07 ± 0.00b	ND
Arachidonic	0.18 ± 0.07a	0.08 ± 0.00c	0.10 ± 0.00b

*ND: Not dedected

standard deviation; **values within each row followed by different letters are significantly different at P < 0.05.

The fatty acid compositions of the oils of mulberry fruits dried by microwave and oven drying systems are shown in Table 3. It was observed statistically significant differences among the amounts of fatty acids of the oils of fresh and dried mulberry fruits ($p<0.05$). Results showed differences among fatty acid contents depending on drying types. While palmitic acid contents of mulberry fruit oils vary between 7.82% (control) and 8.76% (microwave), stearic acid contents of mulberry oil samples were determined between 3.34% (microwave) and 3.58% (oven). In addition, linoleic acid contents of oils of dehydrated mulberry fruits varied between 75.05% (control) and 76.66% (microwave) while oleic acid contents of mulberry fruit oils change between 9.37% (microwave) and 9.87% (oven). As seen in Table 3, palmitic, linoleic, arachidic, linolenic acids of the oils mulberry fruit dried by both drying systems increased compared to control group. In addition, stearic, oleic, and arahidonic acids of the oils of mulberry fruits dried by microwave were reduced and then partly increased compared to control group. Myristic and behenic acids were not identified in mulberry fruit oil dehydrated by oven. Ercişli and Orhan (2007) reported that mulberry fruit oils contained 0.98 to 2.5% myristic, 12.06 to 24.79% palmitic, 4.27 to 6.19% stearic, 10.49 to 14.75% oleic, 43.39 to 61.85% linoleic acids. Results showed similarity compared to literature values. As seen, generally mulberry fruit oil is rich in linıleic acid. So, mulberry fruits are benefit for humen health due to essential fatty acid contain.

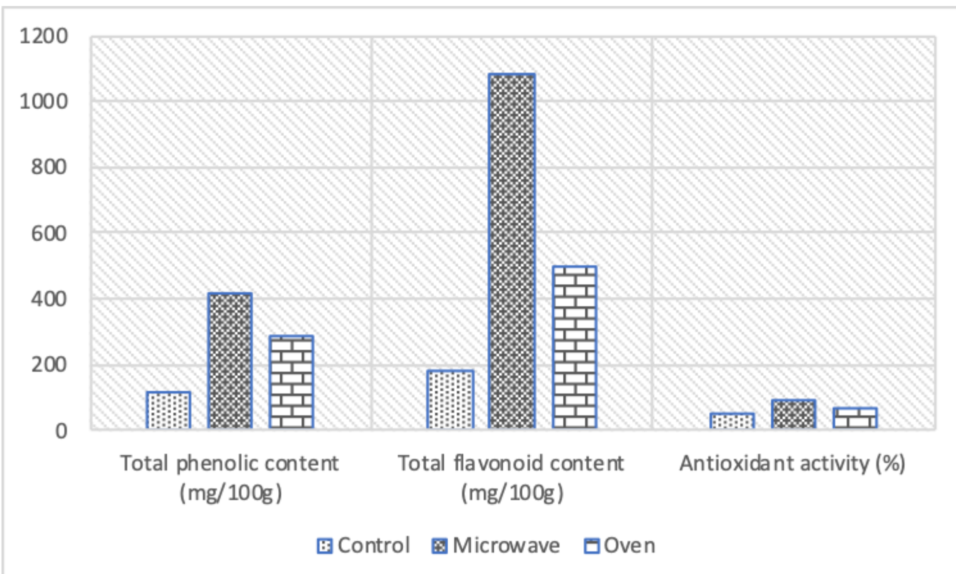
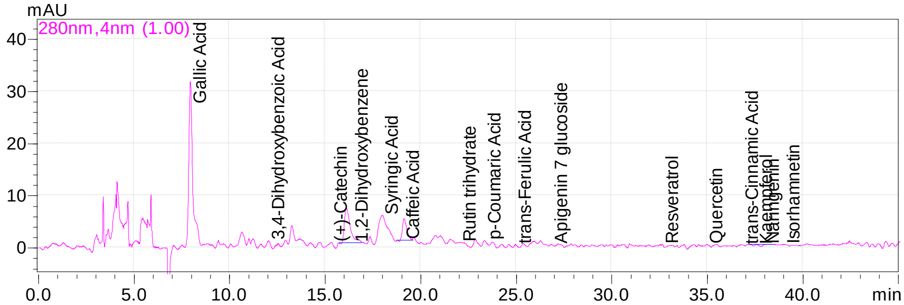
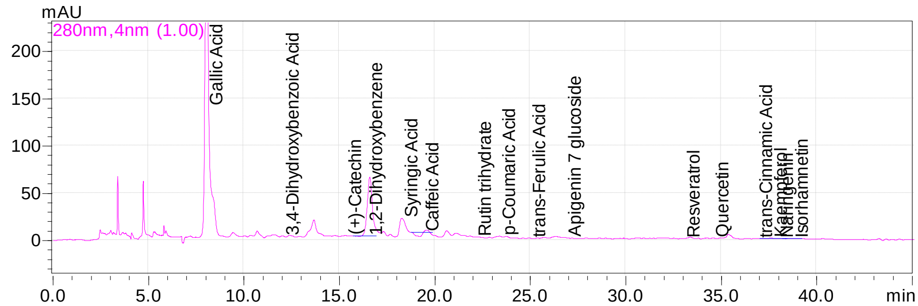


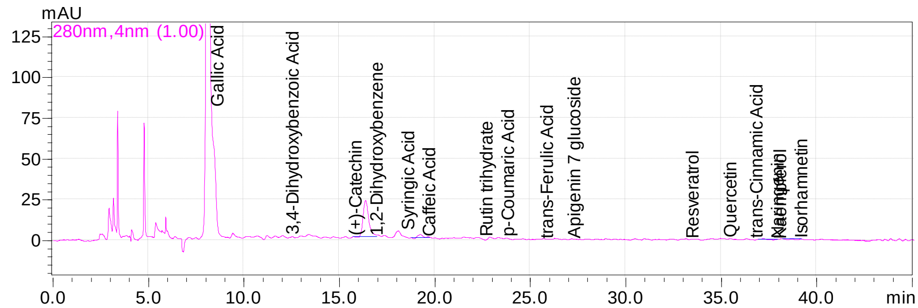
Figure 1.Bioactive compounds of freash and dehydrated mulberry fruits



Control

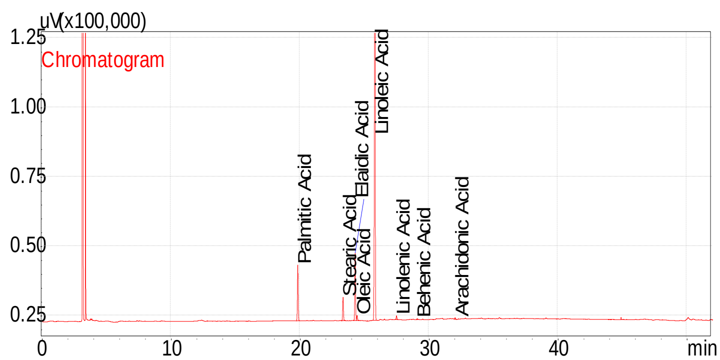


Microwave

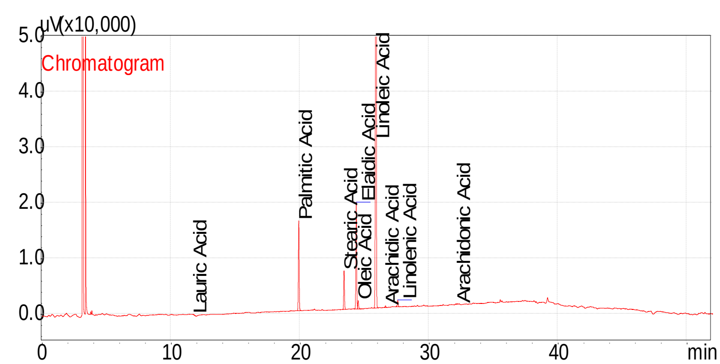


Oven

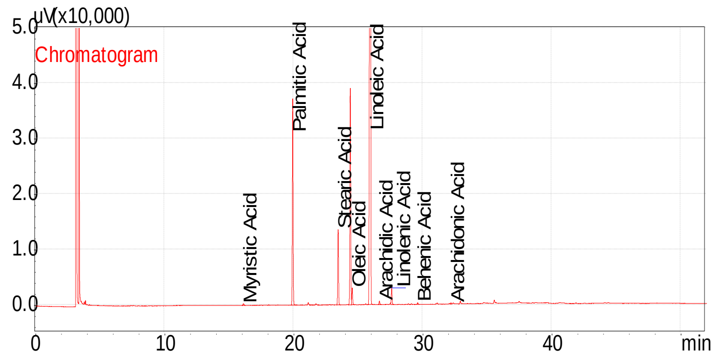
Figure 2. Phenolic chromatograms of Bioactive compounds of freash and dehydrated mulberry fruits



Control



Oven



Microwave

Figure 3. Fatty acid chromatiggrams of freash and dehydrated mulberry fruits

Conclusion

In this study, it was investigated the effect of dehydration processes on oil content, total phenol, total flavonoid, antioxidant activity, fatty acid compositions and phenolic compounds of White mulberry fruits. The maximum total phenol, total flavonoid content and antioxidant activity value were determined in sample dehydrated in microwave oven. Generally, oven drying caused a reduction in total flavonoid amounts of mulberry fruit. The drying process did not significantly affect the carotenoid amounts of mulberry samples. Gallic acid, rutin trihydrate, quercetin, and trans-cinnamic acid contents of mulberry fruits increased by the microwave and oven drying. Differences among fatty acid amounts depending on drying systems compared to control group were observed. Palmitic, linoleic, linolenic and arachidic acid contents of mulberry fruit oils imncreased together with both drying process compared to control (fresh) group. Results showed significant differences depending on dehydration systems compared to control group.

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BLACK RASBERRY (*Rubus occidentalis* L), A NEGLECTED MINOR FRUIT CROP

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Abstract

Wild black raspberries are native to North America. Their commercial cultivars were first introduced to our region in the 1960s, but their production practice did not accept them, for certain reasons. Today, there are three cultivars on the official List of Cultivars of Bosnia and Herzegovina (B&H), of which the *Rubus occidentalis* L. `Cumberland` was observed. The aim of this work is to analyze the chemical composition of its fruits, together with some of the pomological properties and to compare these parameters with the cultivated and wild berries. Samples for analysis come from a private organic plantation at the Šnjegotina Donja site, 45 km from the city of Banja Luka, B&H. The diameter and weight of the fruit were measured on a representative sample. The average fruit diameter was 11.6 +/- 1.31 mm (Δ 8-13 mm) and the average fruit weight was 0.857 +/- 0.176 g (Δ 0.55-1.62 g). These values are slightly smaller than those of wild (red) raspberry (*Rubus idaeus* L.), and are inferior to the size of commercial raspberry cultivars. In terms of basic chemical composition, it was determined that black raspberry `Cumberland` has the highest content of dry matter (19.57%), ash (0.96%) and vitamin C (19.09 mg/100$g_{F.W.}$) among fruits investigated. In terms of total sugars, `Cumberland' (7.41%) is significantly above blackberries (2.71-5.38%), and only slightly below raspberry `Willamette' (7.85%). Elemental concentrations (mg/100g) ranged from 480.76±1.90 (K) to 0.51±0.01(Mn). `Cumberland` contains the most K, Ca, Mg and Cu among the observed taxa. The concentrations of K, Ca and Mg, which are twice as high as in other species, stand out. Regarding the content of Fe, Mn and Zn, `Cumberland' is not significantly behind the maximum values of other observed taxa. Cadmium, chromium and lead were not detected at all, while copper (0.22 ± 0.04 mg/100g) and zinc (0.46 ± 0.02 mg/100g) had no illicit concentrations. Regarding phytochemicals and antioxidant activity of fresh berry fruits, `Cumberland' contains: 9.67 µgGAE/$mg_{F.W}$ of phenols, 2.41 µgQcE/$mg_{F.W}$ of flavonoids, 36.53 µg/$mL_{F.W..}$ of anthocyanins Also, in both tests (ABTS & DPPH), the highest antioxidant activity, i.e. the strongest ability to act as radicals scavenger was performed by black raspberry `Cumberland`.

Key words: *black raspberry, variety `Cumberland`, pomological properties, chemical composition, antioxidant activity.*

Introduction

Raspberry is a cosmopolitan species. It inhabits all continents, except Antarctica. The domestication of raspberry began approximately 450 years ago, and the development of the first cultivars had appeared approximately 350 years ago (Funt et Hall, 2013). One of the practical divisions of today's commercial varieties distinguishes four groups of raspberries: red-fruited varieties, black-fruited varieties, purple-fruited varieties, and yellow-fruited varieties.

There are no wild black-fruited raspberry species both in the territory of B&H and more widely, in the whole of Europe, if the statements of Kosiński et al. (2013) are excluded, that in certain localities in the Czech Republic, Slovakia, Poland and Russia, individual specimens of black-fruited raspberries can be found, considered as a locally established, non-invasive neophyte. In the eastern parts of North America grows *R. occidentalis* L. and in the western areas *R. leucodermis* Douglas ex. Torr. and A. Gray, also known as “black caps” (Weber, 2007). A large number of cultivars were developed from this starting material, some of which reached our regions. In the area of eastern Asia (China, Japan and Korea) grows bokbunja (*Rubus coreanus* Miquel), (Lee et al., 2014). Along a wide area of Asia, from Afghanistan to Taiwan, Mysore or Ceylon black raspberry (*Rubus niveus* Thunb.) is present, which has spread to eastern and southern Africa, eastern Australia, Central America including the Caribbean and South America (Starr et al., 2003). The species *Rubus racemosus* Roxb. is endemic to tropical thorn forest in southeastern India (Anon., 2016), but is also naturalized in similar forests in Jamaica (Adams, 1974; Acevedo-Rodriguez et Strong, 2012).

The production of raspberries (*Rubus idaeus* L.) in B&H has a constant growth, and in 2020 it reached 19,000 t (Anon., 2021). The most common cultivar is `Willamette` (about 90%) and then `Meeker` (about 5-6%), while the rest of the assortment is made up of everbearing cultivars `Heritage`, `Otom Blis` and `Polana` (Anon., 2020, 2021). In addition to cultivated red raspberries, cultivated black raspberries can also be found in B&H. On the official List of Cultivars of B&H, since 2010, there are three cultivars of *Rubus occidentalis* L.: `Black Hawk', `Bristol' and `Cumberland' (Anon., 2022). There is no official data when cultivated black raspberries were first introduced to B&H. According to Mišić (1974) and Šoškić (1988), the first cultivars of black raspberries were introduced from the USA to the former SFR Yugoslavia around 1963-1964. According to the same sources, they were not accepted due to sensitivity to winter frosts and temperature fluctuations, due to low yield, due to poor quality of the fruit, due to difficulty in implementing care measures regarding strong spines. However, the stated views were not supported by more detailed argumentation, especially when it comes to the quality of the fruit. Nowadays, growers in the leading production region of black raspberries in the USA recognize several key reasons why its production does not match the production of red raspberries: a) a gradual decline in yield after a few years from the establishment of the plantation due to necrosis virus (Halgren et al. 2007); b) limited possibilities for breeding progress due to the lack of genetic diversity in elite germplasm (Dossett et al., 2012); c) fruit tend to be small and seedy, whereas fresh fruit consumers prefer large fruit with a small seed fraction (Willman et al., 2022). At the same time, the demand for black-fruited raspberries increased strongly after the publication of a number of studies highlighting the potential health benefits of black raspberry consumption, thanks to their nutritional and pharmacochemical content (Chen et al. 2006; Dossett et al., 2010; Funt 2002, 2003; Hall et al., 2009; Han et al. 2005; Kresty et al. 2001, 2006, 2016; Seeram et al. 2006; Seeram 2008; Stoner 2000; Stoner et al. 2005, 2007, 2008; Wagner 2001, 2002).

The main objective of this study is to analyze the chemical composition of black raspberry fruit grown in B&H, including phytochemicals and their antioxidant properties. Taking into account the previous considerations, an additional objective is to analyze certain fruit quality parameters, such as: size (diameter), weight (mass) and characteristics of the collar region, which is associated with resistance to premature decline of berries.

Material and method

Material

The material for the analysis comes from a private organic plantation of black raspberry (*Rubus occidentalis* L.) cultivar ``Cumberland'' in the locality of Šnjegotina Donja, 45 km from the city of Banja Luka. The altitude of the locality is 420 m and the culture is five years old. The planting material was produced in the same area, in a private nursery in Stara Dubrava (Ukrina), at an altitude of 250 m. Ripe fruits were harvested by hand in 2021 and 2022.

Black raspberry `Cumberland` is a shrub growing up to 2-3 meters. Shoots (stems) are milky blueish-green, overgrown with curved prickles (fig. 1). Flowers are white to soft pink, arranged in clusters on the top of the stems (fig 2). They are rich in nectar and as such attract many pollinators. Fruits are coated in white film (fig 3), called bloom, otherwise known as epicuticular wax. At first glance, this can be considered a negative feature, because, unlike blueberries, it does not appear on a smooth but on a wrinkled surface, so it can be associated with moldiness of the fruits. However, the function of this subtle covering is protective. It stops bacterial and fungal spores from spoiling fruit. At the same time, the coating seals in moisture and preserves freshness of fruits.

Method

Fruits were harvested on June 26, 2021. and on June 22, 2022. in the stage of full ripeness. The material from the first harvest was used to measure the fruits and to determine their nutritional properties and the content of elements in them. The material from the second harvest was also used to measure the fruits and to evaluate their phytochemicals and antioxidant activity. From each harvest were taken 100 fruits and weighed on a digital scale with a reading of 0.01 g. Using calipers, the maximum diameter of the fruit was measured in the position when the collar with cavity is on the lower (basic) side. The obtained results were compared to ripe fruits of raspberry and blackberry cultivars and wild blackberries (*Rubus fruticosus* agg.). Among the 200 fruits, 40 were systematically selected (i.e. every fifth fruit). Drupelets around the collar were counted on each fruit and then the midriff of the fruit was visually assessed on the cross section.

Figure 1: Stem with curved pickles. Figure 2: Flowers. Figure 3: Fruits (photo: S. Ljubojević)

Moisture, dry weight, total ash, acidity, total sugar and vitamin C was determined by the standard AOAC methods (Horwitz & AOAC, 2000). Mineral content was analyzed in the manner described in detail by Vučić et al. (2018). For the purposes of determining total and monomeric anthocyanins, 20g of the sample was extracted with 20 mL of anthocyanin extraction solution (85 mL of 95% ethanol solution and 15 mL of 1.5 mol/l HCl solution) at 24°C for 24 hours (Kukrić et al. 2018), after standing, the resulting mixture was filtered through a paper filter and the filtrate was used for further analysis. The content of total phenols was determined by the modified Folin-Ciocalte method (Wolfe et al., 2003). As a standard compound, gallic acid was used and the results were expressed as equivalents of gallic acid (GAE), i.e. mg GAE/$g_{F.W}$. Total flavonoids were determined by Kumaran method (Kumaran & Karunakaran, 2007), and the total flavonols by Ordonez method (Ordonez et al., 2006). Quercetin (Qc) was used as the standard compound, and the results were expressed as mg Qc/g $_{F.W.}$ Total and monomeric anthocyanins were determined by spectrophotometrically modified "single" pH and pH differential method (Sun et al., 2002). The antioxidant activity in relation to the DPPH radical was determined by the method of Liyana-Pathirana and Shahidi (2005). The modified method of Re et al. (1999) was used for the ABTS radical. The results were presented with the TEAC (Trolox equivalent of antioxidant activity) value, and expressed as mgTrolox/mL. IC_{50} (inhibitory concentration) value, i.e. efficient concentration of antioxidant necessary to decrease the initial radicals concentration by 50 %, was determined from the plotted graph of scavenging activity against various concentrations of extracts. The lowest EC_{50} indicates the strongest ability of the extracts to act as a radical scavenger. Experiments were made in three parallel repetitions, and the results are expressed as the mean ± standard deviation.

Results and discussion

The weight of the black-fruited raspberry \`Cumberland\` ranges from 0.55 - 1.62 g, and is 0.86 +/- 0.176 g on average. The weight of the wild (red) raspberry (*Rubus idaeus*) is slightly larger and ranges from 0.9 - 1.7 g (Mišić, 1974). The weight of the fruits of noble raspberry cultivars is significantly higher than the weight of the black-fruited raspberry and amounts to 1.7-8.4 g, where individual fruits can weigh up to 12 g (Mišić, 1974). For now, the Russian raspberry cultivar 'Generalissimo' has the largest fruits, weights up to 23 g (Hall et al., 2009, referring to Kichina, V.V, 2005). The average weight of the black raspberry fruit in its homeland (USA) is between 1.4 - 1.9 g, and it depends a lot on the cultivar and the growing season. In an intensive plantation of nine commercial cultivars at Cornell University's New York State Agricultural Experiment Station, the \`Black Hawk\` cultivar had the lowest average fruit weight - 0.7 g, while the \`Jewel\` had the highest average fruit weight - 2.7 g (Weber, 2007).
The diameter of the black-fruited raspberry \`Cumberland\` ranges from 9.0 – 15.0 mm, and is 11.6 mm on average (tab. 1). The fruits have a fairly strong, regular collar that contributes to good resistance of collapsing. The collar region consists of 15-18 drupelets (fig 3). The following frequency distribution was determined on a small sample of 40 fruits: collar with 15 drupelets – 20%, with 16 drupelets – 46.7%, with 17 drupelets – 20% and with 18 drupelets – 13.3%. The midriff of the fruit is well-filled, which contributes to overall fruit integrity.

Table 1: Expected fruit weight and diameter of the black raspberry \`Cumberland\` grown in B&H

Parameters	Unit	X +/- s	Minimum	Maximum
Fruit weight	g	0.857 +/- 0.176	0.55	1.62
Fruit diameter	mm	11.6 +/- 1.31	9.0	15.0

The results of chemical analyzes are given in tables 2-4. Nutritional properties of fresh fruits were compared with two red raspberry cultivars - `Meeker` and `Willamette`, with the noble blackberry cultivar `Thornfree' and with wild blackberries (table 2). It was found that black raspberry `Cumberland` has the highest content of dry matter, ash and vitamin C among fruits investigated. In terms of total sugars, `Cumberland` is significantly above blackberries, and only slightly below raspberry `Willamette`. Also, `Cumberland' contain over 20% more vitamin C than fruits of the `Bristol' cultivar grown in Lithuania (Bobinaite et al., 2012). When it comes to the content of vitamin C in wild raspberry and blackberry fruits from the eastern parts of B&H, Marjanović-Balaban el al. (2012) report slightly higher average values. It is worth noting that the wild raspberry leaf is very rich in vitamin C - 137 mg/100 g (Vračarić et al., 1990). This can partially explain the fact that in ancient sources leaves and stems of rasperries were prized for their medicinal properties, but not the nutritional characteristics of their fruits (Hummer, 2010).

Table 2. Nutritional properties of fresh berry fruits

Parameters	Unit	Black raspberry `Cumberland`	Raspberries `Meeker` / `Willamette`	Blackberry 'Thornfree' /3/	Wild blackberries /4/
Dry matter	%	19.57	19.36 / 16.56 /1/	11.94	14.8-15.7
Ash content	%	0.96	0.54 / 0.54 /1/	0.34	0.45
Total acidity	%	1.02	1.28 / 1.65 /1/	1.50	0.73-1.2
Total sugars	%	7.41	10.64 / 7.85 /1/	2.71	4.19-5.38
Vitamin C	mg/100g	19.09	18.8 /2/	3.11	7.11-8.53
Coefficient of sweetness	(sugars/acidity)	7.3	8.31 / 4.76 /1/	1.8	4.48-5.73

*Sources: 1 – Stajčić et al., 2012; 2 – Bobinaite et al., 2012; 3 - Ljubojević et al. 2020; 4 - Jazić, 2019

`Cumberland' contains the most K, Ca, Mg and Cu among the observed species (tab. 3). The concentrations of K, Ca and Mg, which are twice as high as in other species, stand out. Regarding the content of Fe, Mn and Zn, `Cumberland' lags significantly behind the maximum values. The presence of Cd, Cr or Pb was not detected. Compared to wild raspberry fruits from B&H (Marjanović-Balaban et al., 2012), `Cumberland contains more iron and calcium, approximately the same amount of zinc and less phosphorus. In addition to the nutritional properties of fresh fruits, their various protective effects are equally, if not more, valued. These effects in berry fruits have been attributed to various classes of phenolic compounds, such as flavonoids and anthocyanins (Stajčić et al., 2012). The phenolic content in `Cumberland' is higher than in blackberry `Thornfree' and almost identical to the upper values of wild raspberries (tab. 4), with a note that the analyzed material was taken from the same area - the vicinity of the city of Banja Luka in B&H. Compared to the commercial cultivars `Meeker` and `Willamette` grown in Serbia (Stajčić et al., 2012), the phenol content in `Cumberland` is significantly higher. Similar conclusions were reached by Gansch et al. (2009) and Bobinaitė et al. (2012), who determined that the total phenol content in black raspberries is about 1.2-2.5 times higher than in red raspberries.

Table 3. Elements in fresh fruits

Element	Unit	Black raspberry `Cumberland`	Cultivars of red raspberries /1/	Blackberry 'Thornfree' //2/	Wild blackberries /3/
P		16.92	29	24.43	27.30
K		480.76	151	157.31	172.05
Ca		60.02	25	24.33	38.18

Mg	mg/100 g	50.08	22	20.76	28.15
Fe		0.89	0.69	1.09	0.61
Mn		0.51	0.67	0.14	0.64
Zn		0.46	0.42	0.84	0.44
Cu		0.22	0.09	0.22	0.17
Na		0.0	1.0	2.32	3.30
Al		N.A.	N.A.	1.85	N.A.
Se	µg/100 g	N.A.	0.20	0.18	0.38
Cd		0.0	0.0	0.0	0.0
Cr		0.0	0.0	0.0	N.A.
Pb		0.0	0.0	0.0	0.01

Sources: 1 – Rao et Snyder, 2010; 2 - Ljubojević et al. 2020; 3 - Jazić, 2019

The content of flavonoids is also higher in black raspberry than in blackberry fruits (tab. 4). Likewise, the content of flavonoids is higher than in the fruits of the noble raspberry `Himbo Top' grown in Croatia (Maslov Bandić et al., 2020). The results showed that black raspberry `Cumberland` accumulate considerably higher amounts of anthocyanins than blackberries, which is in accordance with the views of Bobinaitė et al. (2012, 2016) and other relevant sources. When it comes to the stability of the performed analyses, Koponen et al. (2007), Lugasi et al. (2011) and some other authors noted significant year-to-year variations in the content of anthocyanins. We have not registered such occurrences with the `Cumberland' variety in B&H. The index of anthocyanin degradation (ID) points to the conclusion that there are not significant differences regarding the degree of anthocyanin degradation, i.e. the preservation of anthocyanin monomers in black raspberries compared to the observed species (tab. 4). In both tests (ABTS and DPPH), the highest antioxidant activity, *i.e.* the strongest ability to act as radicals scavenger was performed by black raspberry `Cumberland` (tab. 4). The presented results affirm black raspberries as valuable and healthy fruit crops. Black raspberry successfully compensates for the smaller fruit weight and expected lower yield with its chemical composition and fruit quality.

Table 4. Phytochemicals and antioxidant activity of fresh berry fruits

Parameters	Unit	Black raspberry `Cumberland`	Blackberry ‘Thornfree’ /1/	Wild blackberries /2/
Phenols	µgGAE/mg $_{F..W.}$	9.67	5.47	7.91-9.98
Nonflavonoids	µgQcE/mg $_{F..W.}$	7.26	4.59	6.19-8.67
Flavonoids	µgQcE/mg $_{F..W.}$	2.41	0.89	1.72-1.31
Anthocyanins	µg/mL $_{F..W..}$	36.53	15.35	3.56 – 3.81
Monomeric anthocyanins	µg/mL $_{F..W.}$	30.73	13.80	3.05 – 3.13
ID		1.19	1.11	1.17-1.22
ABTS	IC_{50} mg Trolox/mL	0.19	0.37	6.86-10.32
DPPH	IC_{50} mg Trolox/mL	1.03	1.45	29.69-38.17

Sources: 1 – Ljubojević et al. 2020; 2 - Jazić, 2019

Conclusions

The production of red raspberries in B&H has experienced a strong expansion in recent decades, and a large number of unemployed have found employment and an additional source of income in this way. This is not the case with black raspberry. Its fresh fruits cannot be bought in the markets, nor can the products obtained from them be found in our stores. The production of black raspberries in B&H is limited to a small estate in terms of production that meets the needs of one family household. The results of our research affirm black raspberries as valuable and healthy fruit crops. Black raspberry successfully compensates for the smaller fruit weight and expected lower yield with its chemical composition and fruit quality.

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GINKGO LEAVES AND YEW ARILS – USABLE PARTS OF OLD PLANT SPECIES (*Ginkgo biloba* L. *et Taxus baccata* L.)

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Abstract

The aim of this paper is to analyze the chemical composition, the contents of selected elements and sets of fatty acids in *Ginkgo* leaves and yew arils. Although *Ginkgo* (*Ginkgo biloba* L) is d eciduous and yew (*Taxus baccata* L.) is an evergreen, these species share several common characteristics: both are gymnosperms, both are very old species, both species are *dioecious* and both species are unusually resistant to disease and pollution. Also, today there are more of their individuals in cities than in forests. *Ginkgo* and yew have undoubted aesthetic properties, which is why they are welcome in our parks, alleys, gardens and other green places. In addition to their aesthetic merits, parts of both species have certain useful values. In this paper, we focused on two products - *Ginkgo* leaf and yew aril. *Ginkgo* leaf is considered one of the most commonly used herbal medicine in the world. On the other hand, the yew aril does not appear on the market as a commodity and only a relatively small number of people are familiar with its medicinal and edible potentials, especially since all other parts of the yew are poisonous to humans and most other mammals. The material for the analysis was collected in the area of the city of Banja Luka, B&H. In terms of chemical composition, *Ginkgo* leaf contains an average of 36% dry matter and yew arils 21.5%. We found that magnesium is the most abundant element in the *Ginkgo* leaf (Mg – 176.8 mg/100 g), while in the yew aril it is sodium (Na – 64.4 mg/100 g). Related to elemental composition, no increased level of toxic elements due to urban pollution was found in the analyzed material. A significant concentration of vitamin C has been determined in the *Ginkgo* leaf (18.7 mg/100g), which is not at all behind some well-known types of fruit. The same applies to total sugars in yew aril (15.61%). Also, in *Ginkgo* leaves 21 fatty acids (FAs) were detected, of which 11 were saturated (SFAs) and 10 unsaturated (USFAs). SFAs participate with 41.98% and USFAs with 58.02%. The most common among SFAs is stearic FA with a share of 29.53%. The most common USFA is γ-linolenic with a share of 13.31%. This study also showed that the average weight of fresh yew fruit is 0.65 g, of which 86.7% is the aril and 13.3% is the seed.

Key words: *Ginkgo leaves, yew arils, chemical composition, fatty acids.*

Introduction

Ginkgo and yew are old species, whose single individuals can live to a very old age. Both species are *dioecious* (species with separate male and female individuals), both belong to gymnosperms and both are unusually resistant to disease and pollution. Also, today there are more of their individuals (trees) in cities than in forests. Yew is an evergreen species and *Ginkgo* is deciduous. Yew belongs to the *Conifer* division and *Ginkgo* to the *Ginkgophyta* division.

The *Ginkgoaceae* appeared for the first time in the Permian, 200-225 million years ago and with its representative *Ginkgo biloba* is still present on planet Earth. From this point of view, it is quite justified that Darwin called this species a "living fossil". The first ancestor of the yew, *Marskea jurassica* (formerly *Taxus jurassica*), appeared a little later, about 140 million years ago, and has most of the characteristics of today's yew. The age of these species is considered the main factor for their perception as (cit.): *a storehouse of biologically active compounds, whose complex and unique molecular frameworks give us a glimpse of the biochemical virtuosity of early plant chemistry* (Farina, 1995). The first *Ginkgo* trees arrived in B&H at the end of the 19th century, with the arrival of the Austro-Hungarian Monarchy. They were planted in the parks of Sarajevo and some other cities (Stefanović, 1955). Most of these trees still exist today. In the meantime, there was no intensive introduction of *Ginkgo* to B&H, with the difference that today a significant number of individuals trees are found in private gardens. In B&H, for now, there is no *Ginkgo* plantation intended for the production of useful biomass (leaf, fruit, wood, bark).Unlike *Ginkgo*, yew is an autochthonous species, which in our country is almost always found on limestone substrate and shallow limestone soils. As a forest species, it is most often found in the lower layer of beech-fir forests, in the *Abieto-Fagetum* community, and exceptionally also in mountain beech forests and their derivatives (Fukarek, 1957). In these stands, yew appears as a low tree or shrub, a few meters high. These are mostly shoots from the stumps of previously cut trees. Only its ability to reproduce vegetatively and to develop in the shade of dense canopies of forest trees, helped it to be maintained in our forests. Yew trees grown in solitude (as a solitary tree) or found in parks and gardens take the form of a real tree.

Ginkgo and yew have undoubted aesthetic properties, which is why they are popular species in parks, alleys, gardens and other green places. In addition to aesthetic merits, both species, or their parts, have certain useful values, which, for certain reasons, are significantly more in *Ginkgo* than in yew. Practically all parts of *Ginkgo* can be used: wood, bark, leaf, fruits with seeds. According to our pilot analysis, in 2010, around 150 *Ginkgo*-based products could be found on the world market. The following can be used from yew: wood, needles (greenery), bark and the fleshy covering of the fruit, the so-called arils.

In this paper, we focused on two products, which according to the international forestry nomenclature can be classified in the "non-wood forest products" category. These are *Ginkgo* leaf and yew aril. *Ginkgo* leaf is one of the most commonly used herbal medicine in the world (Heinonen et Gaus, 2015) and the global *Ginkgo* industry value is estimated at seven billion USD in 2022. *Ginkgo* leaf comes to the market unprocessed, as a herbal drug, and processed in the form of an extract. The extract is marketed in the form of tablets, capsules and liquid extracts (Anon., 2022). There is an enviable number of literary sources regarding *Ginkgo*, with a large number of clinical trials being reported, (Dziwenka et Coppock, 2016). More important among current uses in medical applications include: improving brain function, strengthening the cerebrovascular and cardiovascular systems, increasing blood flow and oxygen supply, suppressing allergies, asthma, hemorrhoids, inflammation, migraines (Chan et al., 2007; DeFeudis, 1998; Juretzek , 1997). The very extensive literature on *Ginkgo* leaf mainly focuses on the quantitative chemical analysis of the main secondary metabolites i.e.: terpene trilactones, flavonol glycosides, biflavones, etc. At the same time, fatty acids were covered on a much more modest scale.

The main goal of our work is to shed more light on the set of fatty acids in the *Ginkgo* leaf, and to compare it with the set of fatty acids in the yew aril, bearing in mind the common characteristics of these species. Regarding yew, many works have also been published on the subject of its chemical composition, but primarily in needles, bark and wood. All these parts are toxic to humans and most other mammals, because they contain the alkaloid taxol and other toxoids. However, taxol is considered the most successful anticancer agent developed so far (Farina, 1995; Isah, 2015). Very few papers deal with the chemistry of arils. This was exactly the motive to analyze the arils of yew from B&H. An additional objective is to analyze structural characteristics of the yew fruit.

Material and method

Material

The material for analysis originates from individual *Ginkgo* and yew trees from private urban gardens in the city of Banja Luka.

The *Ginkgo* leaf was collected at the end of October 2021, from a 47-year-old tree (Fig. 1). The tree was planted in a permanent place in the fall of 1977, as a 3-year-old seedling brought from a nursery in Italy. The optimal harvest time for *Ginkgo* leaves is not known in B&H. In China, the homeland of *Ginkgo*, the leaves are traditionally collected at the same time as the fruits, which is early autumn. In that period, the leaves are already yellow, but they are still on the tree

Figure 1: *Ginkgo* leaves
(photo: S. Ljubojević)

Figure 2: Yew fruits
(photo: S. Ljubojević)

(Del Tredici, 2000). According to Lin et al. (2020) the optimum harvest time of *Ginkgo* leaf should be before turning yellow in October because the most effective constituents then reach their maximum. This attitude is in agreement with the previous reports of Shi et al. (2012) and Zhou et al. (2017). However, when landscape application values are primarily expected with *Ginkgo*, the golden-yellow leaf color and prolonged defoliation period are indispensable characteristics of selected cultivars (Zhang et al., 2021). By the way, one of the special characteristics of *Ginkgo* is that all its leaves fall at the same time, i.e. all at once. The yew fruit (fig. 2) was collected at the end of August 2021 from a 16-year-old tree. The tree was purchased at the Garden Center Flora s.r. Busovača, B&H and planted in a permanent place in March 2013. At this point, it is necessary to specify the terminology related to the fruit itself. In spite of it`s botanical name (*baccata* = berry bearing), yew, as a member of gymnosperms, does not produce "fruits", and therefore neither berries. At the same time, although it belongs to gymnosperms, yew does not even have cones. So, we will say that the yew fruit is a seed attached to the plant, around which the aril is wrapped. The seed is a dark green woody shell, in which, in addition to the embryo, there are reserve substances, mainly fats and proteins (fig, 4), thanks to which the seed can remain fertile for up to four years. As we have already said, the whole seed is highly toxic to humans and most other mammals. The aril is bright scarlet red (fig 3). Contains a juicy, sweet-tasting and somewhat slimy pulp that is edible. The main function of the aril is to attract birds, which are the main agents of seed distribution (Hageneder, 2013). Various medicinal properties are attributed to yew aril, of which its diuretic and laxative effects are most often mentioned (Orwa et al., 2009).

Figure 3: Yew arils freed from seeds (photo: S. Ljubojević)

Figure 4: Yew seed is filled with reserve substances (photo: S. Ljubojević)

<u>Method</u>

Moisture, dry matter, ash, proteins, total acidity, vitamin C and mineral substances were determined in *Ginkgo* leaf and yew arils. Fats and cellulose are also found in the *Ginkgo* leaf and pectins in the yew aril. Futhermore, fatty acids were analyzed in the *Ginkgo* leaf, while the content of fatty acids in the yew aril was analyzed based on the work of Tabaszewska et al. (2021).

Moisture, dry weight, total ash, acidity, total sugar and vitamin C were determined by the standard AOAC methods (Horwitz & AOAC, 2000). The levels of total nitrogen were determined by the Kjeldahl method (960.52, AOAC, 2016), and percent protein was calculated as % N x 6.25. Fats content was determined by the Soxhlet method (920.39, AOAC, 2016). Pectic substances were determined using the colorimetric method according to the procedure described by Ranganna (1986). Cellulose content was estimated as described by Updegroff (1969). The analysis of elements included: Ca, Na, K, Mg, Zn, Cu, Al, P, Mo, Se, Pb, Cd, Cr, Fe and Mn. Mineral content was analyzed in the manner described in detail by Vučić et al. (2018). Sample preparation for determination of mineral substances was done by "wet" incineration with perchloric and nitric acids according to Trajković et al. (1983). Determination of mineral matter in prepared samples was done by analytical technique - inductively coupled plasma optical emission spectrometry on the instrument Optima 8000 ICP-OES Spectrometer, "Perkin Elmer". Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Ljubojević et al., 2021). For the analysis of fatty acids, *Ginkgo* leaves were air dried and samples were powdered using mesh screen in Willey mill. Fatty acids were extracted from the powder of leaves according to the following procedure. Fatty acid methyl esters were prepared by direct esterification with a saturated solution of methanol with KOH, according to Majors (2013). Obtained fatty acid methyl esters were separated in a gas chromatography from Perkin Elmer, model Clarus 680,

equiped with flame ionization detector and a capillary column Elite-WAX, 60 m long. Initially, the column temperature was set at 60 ºC for 2 min, than raised to 200 ºC at a rate of 10 ºC/min; again, it was raised at 240 ºC at a rate of 5 ºC/min and maintained at 240 ºC for 30 min. All in all, the total chromatographic run time was 54 min. The flow rate for the carrier (H_2), auxiliary (N_2) and detector flame gases (H_2 and synthetic air) was 1.5 mL/min. The composition of fatty acid methyl esters and the resulting acids is shown as a percentage of individual fatty acids in the total amount of identified fatty acids. All samples were studied four times and averaged.
From the harvest were taken 200 fruits and weighed on a digital scale with a reading of 0.01 g. Using tweezers, the seeds were carefully separated from the arils and weighed again, on the basis of which the weight (mass) ratio between the arils and the seeds were arrived at. All statistical data processing was done using free statistical software "jamovi 2.2.5".

Results and discussion

The average weight of fresh yew fruits was 0.653 +/- 0.122 g. Among the 200 weighed fruits, the lightest fruit weighed 0.40 g and the heaviest 1.07 g. The average weight of fresh arils was 0.566 g, and made up 86.7% of the total weight of the fruit, with a range variations of 84.1% - 90.5%. In principle, with lighter fruits, the relative share of seeds was higher and *vice versa*.
There are obvious differences in the moisture content of arils from different localities in Poland compared to the moisture content of the material from B&H, most likely due to different environmental conditions, such as water availability, sunlight, and wind exposure (Tabaszewska et al., 2021). Probably, for the same reasons, the ash content in the material from B&H is significantly higher than in the material from Poland, as well as the total sugars content (tab.1).

Table1. Chemical content of the observed material

Component	Unit	*Ginkgo* leaf B&H	Yew aril	
			B&H	Poland[/1/]
Moisture	%	64.07 ± 0.16	78.47 ± 0.0	73.63 - 77.90 ± 0.08 ± 0.09
Dry matter	%	35.93 ± 0.16	21.53 ± 0.0	26.37 - 22.10 ± 0.05 ±0.06
Ash	%	3.25 ± 0.02	0.65 ± 0.02	0.37 - 0.49 ± 0.02 ± 0.01
Fat	%	3.69 ± 0.08	N.A.	N.A
Cellulose	%	4.08 ± 0.29	N.A.	N.A.
Proteins	%	2.42 ± 0.04	N.A.	N.A.
Pectin	%	N.A.	0.58 ± 0.09	N.A.
Total acidity	%	1.03 ± 0.01	0.16 ± 0.05	N.A.
Total sugars	%	N.A.	15.61 ± 0.27	8.70 - 11.11[/2/] ± 0.24 ± 0.19
Vitamin C	mg/100g	18.68 ± 0.66	1.75[/] ± 0.25	N.A.

Notices: 1- Tabaszewska et al.(2021); 2 - Total sugars were calculated as the sum of glucose, fructose and sucrose; N.A. - not analyzed

In the absence of an adequate basis for comparing the chemical content of *Ginkgo* leaves, we highlight the enviable concentration of vitamin C (18.7 mg/100g), which generally lags behind, say, the values in elite raspberry varieties (Stajčić et al., 2012). In the *Ginkgo* leaf, the most abundant element is magnesium (Mg – 176.8 mg/100 g), while in the yew aril it is sodium (Na – 64.4 mg/100 g), (tab. 2).

Table 2. Average element concentration (mg/kg$_{D.W.}$) in Ginkgo leaf and yew aril

	Ca	Na	K	Mg	Zn	Cu	Al	P	Se	Cr	Fe	Mn	Cd
Ginkgo leaf	N.D.	N.D.	N.D.	176.8	N.D.	2.37	1.28	12.87	N.D.	0.005	1.01	0.26	0.005
Yew aril	5.89	64.4	15.8	7.5	2.71	0.39	0.21	22.80	0.04	N.D.	0.64	0.12	N.D.

Notice: N.D. – analyzed but not detected

In *Ginkgo* leaves from B&H, 21 FAs were detected, of which 11 were saturated (tab. 3) and 10 unsaturated (tab. 4). The ratio of saturated to unsaturated FAs is 41.98% : 58.02%. The most common among SFAs is stearic acid with a share of 29.53%, while the most common USFAs is γ-linolenic acid with a share of 13.31%. Stearic acid is an inexpensive raw material with a wide range of uses. On the other hand, α- and γ-linolenic acids, the most abundant USFAs (more precisely, polyunsaturated FAs), are much more valued, especially in terms of improving human health (Gonzales-Fernandes et al., 2020, and many other sources).

In general, significant differences were found between raw materials from different localities, both in the structure of FAs and in their relative representation. In the material from France, which comes from Bordeaux, 20 FAs were recorded, and in the material from Talence 17, that is, three less. The ratio of saturated to unsaturated FAs was almost identical in both localities. In the material from Portugal, 20 FAs were recorded, among which 14 were saturated and 6 were unsaturated. The ratio of SFAs to USFAs was 59.14% : 40.86%, which means that there are more SFAs than USFAs in the leaves of *Ginkgo* from Portugal; this is not the case with our material and raw material from France.

Table 3. Assortment of saturated fatty acids in *Ginkgo* leaves from different locations

No.	Common name	Lipid numbers	B&H Banja Luka	France[1]		Portugal[2] Bragança
				Bordeaux	Talence	
			Relative percentage (%)			
1	Butyrix acid	C4:0	0.29			
2	Caproic acid	C6:0	0.23			0.24
3	Capriylic acid	C8:0	0.72			0.27
4	Capric acid	C10:0	2.29			0.24
5	Lauric acid	C12:0	3.20	0.80	0.50	0.61
6	Tridecylic acid	C13:0	1.34			
7	Myristic acid	C14:0	0.36	0.20	1.20	6.13
8	Pentadecylic acid	C15:0	1.37	2.30	1.00	0.68
9	Palmitic acid	C16:0	0.73	24.80	23.60	35.90
10	Margaric acid	C17:0	1.92			1.28
11	Stearic acid	C18:0	29.53	1.40	1.50	4.17
12	Arachidic acid	C20:0		1.00	1.60	2.70
13	Behenic acid	C22:0		1.10	2.60	2.19
14	Tricosylic acid	C23:0				0.92
15	Lignoceric acid	C24:0				3.81
	Σ		**41.98**	**31.60**	**32.0**	**59.14**

References: 1- Mongrand et al. (2001); 2- Pereira et al. (2013)

Table 4. Assortment of unsaturated fatty acids in *Ginkgo* leaves from different locations

No.	Common name	Lipid numbers	B&H Banja Luka	France[1]		Portugal[2] Bragança
				Bordeaux	Talence	
			Relative percentage (%)			
1	Myristoleic acid	C14:1	0.44			
2	Pentadecenoic acid	C15:1	0.58			
3	Palmitoleic acid	C16:1	1.08	3.2	2.6	0.82
4	Hexadecadienoic acid	C16:2		0.3		
5	Hexadecatrienoic acid	C16:3		4.4	4.5	
6	Heptadecenoic acid	C17:1	3.58			
7	Oleic acid	C18:1		5.2	5.8	11.18
8	Taxoleic acid	C18:2		2.1	2.4	
9	Linoleic acid	C18:2	5.41	6.8	5.5	10.53
10	α-Linolenic acid	C18:3	12.94	39.3	41.4	18.03
11	γ-Linolenic acid	C18:3	13.31			
12	Eicosadienoic acid	C20:2	1.40	1.6	1.5	
13	Bishomo – α - linolenic	C20:2		1.1		
14	Dihomo - γ - linolenic	C20:3				0.11
15	Eicosatrienoic acid + Heneicosylic acid	C20:3 + C21:0		1.9	2.0	0.19
16	Juniperonic acid	C20:4		2.1	2.3	
17	Erucic acid	C22:1	6.49			
18	Docosadienoic acid	C22:2	12.79			
19	Undetermined acid with very long chain	C..		0.4		
	Σ		**58.02**	**68.4**	**68.0**	**40.86**

References: 1- Mongrand et al. (2001); 2- Pereira et al. (2013)

If we were to compare our results with the analysis of *Ginkgo* leaves from other localities, the heterogeneity of the composition of FAs would be even greater. Namely, in the material coming from the plantation of the Poznan University of Life Sciences, only nine FAs were detected, four SFAs and five USFAs, with a ratio of 7.06% : 92.94% and the largest individual relative share of oleic acid – 60.69% (Kobus-Cisowska et al., 2020).

In arils taken from yew trees at four wider locations in Poland, 25 FAs were detected, of which eight are SFAs and 17 are USFAs (Tabaszewska et al., 2021). SFAs participate by mass with 35.84% and USFAs with 61.65%, while 2.51% of the mass parts are not identified. Individually, the most abundant SFAs are palmitic (22.5% on average) and myristic (9.44% on average). The most abundant USFAs are α-linolenic acid (23.4% on average) and linoleic (23.2% on average). The yew aril is also find to contain one mini-set of the polymethylene-interupted FA (also called Δ5-olefinic acids), composed of pilolenic, sciadonic and juniperonic FA (Tabaszewska et al., 2021). This set is known as unique for seeds of gymnosperms, especially seeds of *Ginkgo* (Wolff et al., 1999).

Conclusions

Ginkgo leaf is one of the oldest preparations of traditional Chinese medicine, which is still used worldwide both in official and alternative medicine. Our research has shown that G*inkgo* leaves harvested from a tree in B&H have a respectable chemical composition, especially when it comes to fatty acids. Until the conditions for mass production of *Ginkgo* leaves, based on plantation cultivation, are achieved in our country, the current modest available quantities of this raw material can mostly be directed towards meeting individual needs.

Yaw aril is essentially a bright scarlet, slightly sweet "berry", juicy and somewhat slimy in consistency. It can be considered delicious, which has no counterpart in any other type of fruit. The fact that there is a poisonous seed inside the aril does not reduce its value in any way, primarily because the seed is separated very simply, easily and safely. Our analysis of the main macronutrients and selected elements did not indicate a deficit of any of the investigated constituents, while analyzes in other countries determined a good range of fatty acids in yew arils.

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BIOACTIVITY OF SILYLATED LIGNOCELLULOSIC BIOMASS OF SEA BUCKTHORN

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Abstract

Currently, the search for ecologically friendly products that promote plant productivity, quality, and resistance to biological and abiotic diseases without harming the environment is a very actual task. More care is needed to grow organic products, but the investment pays off with healthier and better-quality produce. The high potential of lignin-containing biomass for application in agriculture as fertilizers has been well documented and further improvements of lignin-based products efficiency by modification are under development. In this study, the residue of sea buckthorn biomass after the isolation of biologically active substances was used as a lignin-containing raw material for the preparation of the organo-mineral fertilizer. The organo-mineral complex (SBT-Si) was obtained by modifying SBT biomass with silicon (Si)-containing inorganic oligomer. The content of Si was 2 and 5 % on SBT biomass dry matter. The aim of this study was to evaluate the SBT-Si complex as an activator for the growth and development of plants. It was shown that SBT-Si at a rate of 2 g/L soil has a favourable influence on plant development, with the best results for SBT-Si with a content of silica 5% on SBT biomass dry matter. Significant development of the plant root system is confirmed by its increased volume (36-76%) and the volume of earth clod that plant roots are able to keep. The results confirmed that SBT biomass after extraction is a prospective basis for the development of organo-mineral fertilizers.

Keywords: *lignocellulosic biomass, silicon, organo-mineral complex, fertilizer, sea buckthorn.*

Introduction

The soil is a living organism and in recent decades it has been experiencing huge stress due to intensive agriculture. If previously only mechanical stress was considered the main problem, now experts note the mineralization of humus, which inevitably leads to a decrease in soil fertility. Soil also loses its ability to absorb and store nutrients; the general physical properties of the soil are getting worse as well. One of the ways to increase soil fertility is to increase the amount of organic matter by introducing organic fertilizers into it. Natural organic fertilizers nourish the soil, the soil becomes loose and saturated with air, absorbs more moisture and nutrients, promotes the growth of soil microorganisms, and as a result, the soil microbiome nourishes the plants, contributing to the development of a healthy plant root system. Traditional organic fertilizers, such as manure, have certain limitations in their use, such as heating during decomposition, high nitrate content in fresh feces, the presence of pathogens, and others. Sea buckthorn (SBT) lignocellulosic biomass is a valuable source of organic matter. There are two reasons why a significant volume of SBT biomass forms in the industrial SBT berries production: after harvesting in the late summer and during autumn, since the berries are collected by cutting the whole branch; and after regenerative pruning in the late winter. SBT bush gives the largest harvest of berries in the 3rd and 4th years and after four years, the big part of SBT bush is cut off, since it is difficult for farmers to deal with the trees that are overgrown (Figure 1). These two types of SBT biomass are a large-tonnage valuable waste.

The development of organo-mineral fertilizers based on lignocellulosic biomass makes it possible to return to nature the organic part that is taken from it, which is necessary for the normal operation of the soil-biotic complex.

Figure 1. Four years old sea buckthorn bushes (lignocellulosic biomass waste)

It is known that wood and bark contain a fairly large amount of water-soluble polyphenolic compounds, that are a valuable source for obtaining polyfunctional additives. But they could negatively affect plant growth when introduced to the soil. Therefore, preliminary removal of polyphenolic compounds from lignocellulosic biomass before its application as a soil additive would serve two purposes: (1) obtaining high value-added polyphenols-based products for their application as antioxidants, antimicrobial agents, and components of adhesive systems [Janceva, 2022], etc., and (2) improvement of the biomass properties as a soil additive. In addition, preliminary bark extraction removes spores of fungi, bacteria, and other harmful microorganisms from it. Therefore, the residue after biomass extraction is a valuable raw material for obtaining fertilizers. This approach is promising from a circular bioeconomy point of view, for the cascading use of biomass, obtaining the maximum number of value-added products from the production cycle. The literature data, as well as the authors’ own experience, showed that polyphenolic compounds can be successfully used in pharmacological, food, and cosmetic industries (Balasundram, 2006; Janceva, 2017; Pandey, 2009). Lignocellulosic biomass contains organic matter, macro and microelements (boron, manganese, copper, iron, etc.) for successful plant growth and development, as well as substances that stimulate plant growth processes (e.g., phytohormones, carbohydrates in the form of glucose). But recent research and our experience show that the functionality of such soil additives can be improved by adding inorganic components, which work in synergy and increase their effect. It is well-known how important a role nitrogen, phosphorus, and potassium, play in plant’s life and how unfavorable the deficiency of these basic elements affects plant development. Si is one of the most abundant micronutrients (the eighth-most abundant element in nature and the second most abundant element in soil after oxygen), which plays an important role in plant resistance to environmental stress, diseases, and pathogens. In addition, Si can improve the health of soils that contain toxic levels of heavy

metals. It is mentioned that silicon minimizes the toxicity of Fe, and Al, and increases plant resistance to drought, salt, high temperatures, and frost due to the formation of silylated tissues in plants (Kadlecová, 2020, Lebedeva, 2007, Sahebi, 2015, Telisheva 2009). The role of silicon can be compared to the role of organic secondary metabolites, which play a protective role in plants. Silicon deficiency causes growth, developmental and reproductive disorders in many plants. Silylation of technical lignins, formed as by-products of the chemical processing of wood raw material, made it possible to obtain high-efficient plant growth and development activator ‘Lignosilicon’ (Telysheva, 2004). Testing of silicon-containing organo-mineral fertilizer ‘Lignosilicon’ in our lab has shown that it promotes enhanced resistance of plants to unfavourable environmental conditions and diseases, contributes to the improvement of market-oriented plant quality, and facilitates the increase of the harvest volume, at a low rate of application (40 kg/ha and lower) (Telysheva, 2004).
The aim of this study was to synthesize silica-modified fertilizer on the basis of SBT lignocellulosic biomass and to evaluate the synthesized product in the laboratory as an activator of the growth and development of plants.

Materials and Methods

The organo-mineral complex obtained on the basis of the residue of SBT biomass after water-ethanol extraction (further in the text – SBT biomass) enriched with Si was tested as a biologically active soil additive for plant growth and development. The chemical characterization of SBT biomass was carried out according to the following European standards: LVS EN 13040:2008 (humidity and dry matter, %); LVS EN 13039:2012 (organic matter content, %); LVS EN 13654-1:2003/NAC:2004 (total nitrogen, %); LVS 398:2002 (total phosphorus, %), LVS ISO 11466:1995 (total potassium, %), LVS 346:2005; LVS ISO 11047:1998A; LVS ISO 110466:1995 (heavy metals, mg/100g). The content of lignin was determined by the Klason method. Siliceous organo-mineral complex (SBT-Si) was prepared similarly to the method applied for siliceous lignin production (Telysheva, 2004), and it contained about 2 and 5 % of silica. ‘Lignosilicon’ used as a reference sample contained 5 % of silica.
Express biotests were performed in a climatic chamber at 24 ºC, for four days without light. Seeds of the cucumber, and Scarlett runner bean were placed on the Petri dishes (0-20 seeds on each), dusted by the SBT-Si and ‘Lignosilicon’, and 5-10 mL of water (depending on seeds size) were added.
Vegetation tests were performed in a glass house (beginning of June, average temperature in a glass house 20-25ºC), in 0,5 L film pots for seedlings filled with a mix of soil and sand in the ratio 1:1 (v/v). The agrochemical parameters of the soil were as follows: pH 6.0, organic matter content 2.7 %, amount of phosphorus available to plants 51 mg kg^{-1} (low), and potassium 67 mg kg^{-1} (low). The rate of SBT-Si and ‘Lignosilicon’ applications was 2 g/L of soil. The amount of cucumber (*Cucumis sativus* L.) and Scarlet runner bean (*Phaseolus coccineus* L.) seeds was 50 for each plant. The root system of plants was characterized using a calibration scanner STD-1600 and program Win Rhizo 2002 C. Confidence intervals (CI) for a mean using a Student’s T-distribution were calculated at a significance level of $\alpha = 0.05$.

Results and Discussion

The porous structure and the presence of active functional groups of different nature make the remains of the chemical processing of lignocellulosic biomass a valuable material for creating organo-mineral complex with prolonged action. The chemical characterization of SBT biomass shows that it is a great source of organic matter (97%). Based on the results of analytical pyrolysis, the content of lignin in the lignocellulosic complex was 12.3 %. SBT biomass in small quantities contains all the elements (N – 400 mg/100g, K – 12 mg/kg; P – 600 mg/kg) necessary for the successful growth and development of plants, as well as substances that stimulate the processes of plant growth. The ash content of the SBT biomass was up to 3%. Heavy metal content was, Hg: 0.0037 mg/kg; Pb: 0.084 mg/ kg; Cd: 0.021 mg/kg). The content of heavy metals in the SBT biomass does not exceed the maximum permissible concentrations and complies with Cabinet Regulation No. 350 requirements (permissible norms of heavy metals: Hg 2 mg/kg, Pb 10 mg/kg; Cd 1 mg/kg).

The introduction of 2 – 5% of Si into the lignocellulosic biomass compensates for the low content of elements responsible for plant growth and development. In addition, the SBT-Si complex has a basic environment (pH 7.6 - 8.7) and can serve as a neutralizer of soil acidity.

Express biotests in Petri dishes have shown that the activity of the SBT biomass new soil additives was significantly increased by Si-modification. In four days’ time, the additive of SBT-Si, compared with the control (seeds without treatment) and the additive of SBT, favoured the development of plant seedlings: an increase in the dry mass of coleoptiles was, correspondingly, 10% and 50%, but a dry mass of roots 20 % and 60%, in comparison with the control.

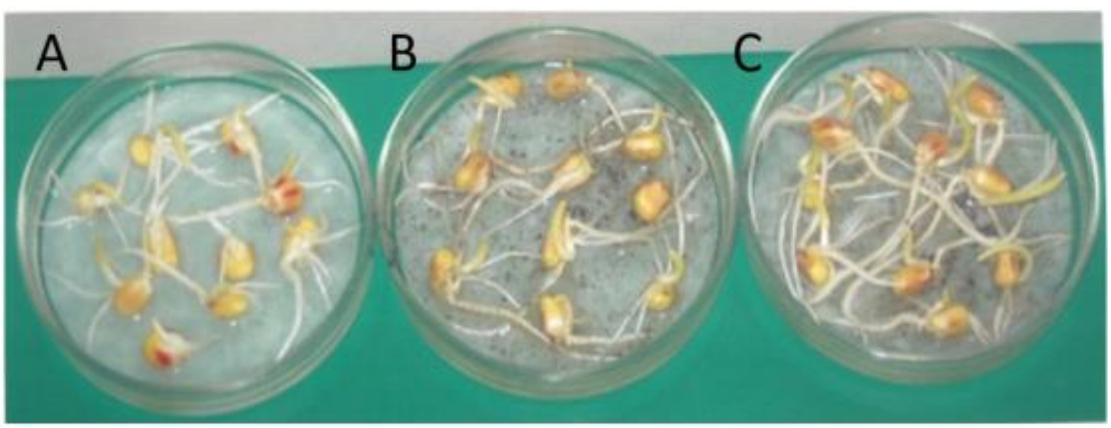

Figure 2. Root samples (*Cucumis sativus L.*): A- control; B - SBT-Si (2% Si in SBT biomass); C- SBT-Si (5% Si in SBT biomass)

Results of the vegetation tests were exemplified by the development of cucumber and Scarlett runner bean plants. The results for cucumber showed that in 12 days after sowing, all plants grown on the basis of tested additives (SBT-Si (2%), SBT-Si (5%), ‘Lignosilicon’) had more branched root systems keeping earth clod of larger volume than control plants.

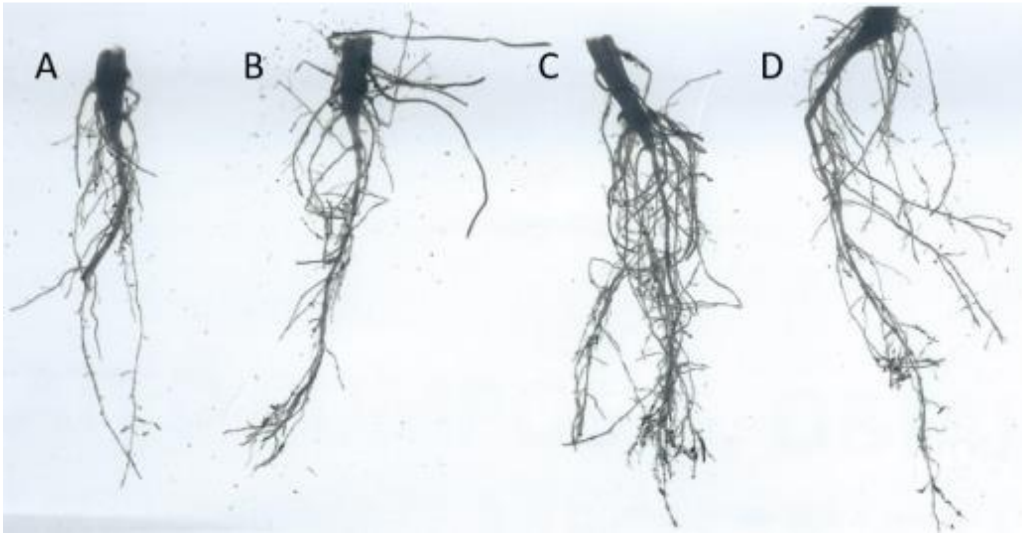

Figure 3. Root samples scans (*Cucumis sativus L.*): A – control; B – SBT-Si (2% of Si); C – SBT-Si (5% of Si); D – ‘Lignosilicon’.

In 20 days after sowing all tested additives had a positive influence on the development of cucumbers’ root system volume. The application of SBT-Si (5% Si in SBT dry biomass) and ‘Lignosilicon’ (LSi), increased the cucumber’s root system volume by 67 and 76%, and the dry mass of the cucumber’s roots increased by 36 and 44%, correspondingly, in comparison with the control (Table 1). At the same time, the application of SBT-Si (2% Si in SBT dry biomass) increased the cucumber root system volume by only 7%, which is within the confidence interval level, and the dry mass of the cucumber’s roots increased insignificantly. In the experiments with Scarlett runner bean, it was observed that on the background of SBT-Si (5% Si in SBT dry biomass) and ‘Lignosilicon’, the total volume of plant root system increased, in comparison with the control, by 36% and 39%, correspondingly, and dry root mass increased by 38% for both (Table 1). The addition of non-modified SBT biomass in the substrate at the same rate did not influence these plant parameters.

Table 1. Effect of organic soil additives (rate 2g/L) on plant development in 20 days after sowing (Whin-Rhizo data).

Variant	Average dry mass of above-ground part (g/1 plant)*	Average dry mass of root, g/10 plant**	Average volume of the plant root system, cm^3 ***	Total plant root length, cm ****
Cucumber (*Cucumis sativus L.)*				
Control	0.27	0.09	0.067	52.12
SBT-Si (2% Si in SBT dry biomass)	0.28	0.10	0.072	76.22
SBT-Si (5% Si in SBT dry biomass)	0.37	0.13	0.112	113.16
Lignosilicon	0.39	0.14	0.114	124.87
Scarlett runner bean (*Phaseolus coccineus*)				

Control	0.34	0.56	0.352	114.18
SBT-Si (2% Si in SBT dry biomass)	0.36	0.58	0.342	114.53
SBT-Si (5% Si in SBT dry biomass)	0.47	0.64	0.479	156.27
Lignosilicon	0.47	0.71	0.488	161.61

*CI ≤0.03 at α = 0.05; **CI ≤ 0.03 at α = 0.05; ***CI ≤ 0,05 at α = 0.05; ***CI ≤ 18 at α = 0.05

Significant development of Scarlett runner bean root system was confirmed by the value of earth clod that plant roots are able to keep.

Conclusions

The results confirmed that SBT waste biomass after extraction is a perspective organic part for the development of organo-mineral fertilizers. Modification of the SBT biomass by Si component was achieved in the laboratory conditions, and the soil additives obtained by Si-modification have demonstrated properties of plant development activator. The positive effect of SBT-Si at the early stage of plant vegetation, which is very important for the growth of seedlings, was revealed, presumably through the improvement of the parameters of the root system. Further research is planned on the effect of SBT-Si on soil microbiota.

In addition, the SBT-Si complex has a basic environment (pH 7.6 - 8.7) and can serve as a neutralizer of soil acidity.

Acknowledgments

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SOIL FUNCTIONAL-ECOLOGICAL ASSESSMENT IN TRADITIONAL AND ORGANIC VINEYARDS ACCORDING TO SOIL RESPIRATION AND MICROBIAL BIOMASS

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Abstract

Compared to other agroecosystems, vineyards are more susceptible to environmental risks such as erosion, biodiversity loss, soil and plant pollution due to high pesticide load. Early detection and prevention of viticulture environmental impacts are of great relevance. This paper examines the ecological state of brown mountain gravelly loamy soils under vineyards cultivated using traditional and organic technologies in 15 farms of the Northern Black Sea region. Assessment of soils ecological functions and their microbiota ecophysiological state is based on various parameters of soil respiration and their correlation with soil agrochemical state. The indicators of substrate-induced respiration and the carbon content of microbial biomass were 3.4 times higher in the soil of organic farms than traditional ones, but their microbial metabolic coefficients, on the contrary, were 1.5 times lower indicating a more stable state of the soil-biotic system in organic farms. At the same time, the low coefficient of microbial respiration in the soils of organic farms compared to traditional ones (0.02 – 0.08 vs. 0.10 - 0.27) connected with their insufficient supply of mobile phosphorus and exchangeable potassium. So, analyzing the ecophysiological state of the soil microbiota is important not only for assessing the current state of ampelocenosis soils, but also for making timely and informed decisions on making changes to vineyard management elements in order to support the ecosystem services of the soil and obtain high-quality wine products that is very valuable for agroecological DSS.

Keywords: *Vineyard, Organic farming, Soil quality, Soil respiration, Environmentally friendly agrotechnologies.*

Introduction

According to the FAO classification, vineyards are a multifunctional type of plantation-garden land use, in which the properties of the soil directly affect the quantity and quality of the products obtained. Considering that soil is one of the most important components of agroecosystems, the study of its ability to function sustainably under conditions of agrogenic loads on ampelocenoses is of particular importance. The successful performance of their ecological functions by soils largely depends on the state of the soil microbiome (Dobrovol’skaya et al., 2015). It is shown that soil microorganisms are responsible for many functions in the biosphere, which provide a wide range of food, regulatory and supporting ecosystem services (Adhikari and Hartemink, 1995; Ivashchenko et al., 2014; Saccá et al., 2017). In this regard, the use of microbial indicators for monitoring the state of ampelocenosis soils is very important. From a wide range of determined microbiological indicators, for example, such as taxonomic diversity and structure, functional diversity, the presence of pathogenic organisms, enzymatic activity, etc., the determination of the ecophysiological status of the soil microbial community deserves special attention. Microbial ecophysiological indices characterizing the specific activity of the microbiome (respiratory and enzymatic activity per unit of biomass) are informative indicators for assessing the effectiveness of the destruction of organic matter. It is noted that these indicators reflect a wide range of ecosystem services, are characterized by a certain reliability (recognition by the scientific community), the methods of their determination are standardized and relatively easy to implement (Ananyeva et al., 2021)

Due to the high sensitivity of microorganisms even to low concentrations of pollutants, the analysis of soil microbiome ecophysiological status helps to identify early the negative effects of agrogenic impact on soil. Wine-growing areas are susceptible to a serious environmental risks of soil pollution due to high pesticide load (Viers et al., 2015). In particular, the accumulation of excessive concentrations of copper in the soil is a consequence of the repeated use of various copper–containing pesticides to control pests and diseases in orchards and vineyards (Ninkova et al., 2012) where the accumulation of copper in the soil increases as the age of plantings increases (Veliksar et al., 2014). Seasonal processing of vineyards with copper compounds (copper sulfate, bordeaux liquid, copper chloride, etc.) is included in the list of measures to protect the vine from fungal and some bacterial infections that are allowed to be used not only in farms implementing the traditional farming system, but also in organic ones. In this regard, early identification of risks caused by anthropogenic causes is of great relevance in order to timely adjust the elements of vineyard management technologies to obtain high-quality wine products.

In this paper we analyze the ecophysiological state of soil microbiota based on soil respiration parameters and their correlation with soil agrochemical characteristics in case of brown mountain gravelly loamy soils under vineyards cultivated using traditional and organic technologies in fifteen farms of the Northern Black Sea region.

Material and Methods

The objects of the study were brown mountain gravelly loamy soils under vineyards in fifteen farms of the Northern Black Sea region of the Russian Federation implementing organic and traditional (including a chemical plant protection system) land use systems (farms 1-6 and 11-15 in the table, respectively), as well as the fallow soils on which agricultural production was previously carried out according to traditional agricultural technologies (farms 7-10). The farms are located in a zone of moderately warm Mediterranean climate type with moderately hot dry summers, predominance of autumn-winter precipitation and mild winters with frequent thaws. Monthly precipitation amounts of the winter period were significantly higher than their average long-term values, and summer ones – significantly lower than average values. In this regard, the amount of precipitation acts as a limiting ecological factor for most field crops, but the agro-climatic and soil conditions of the region are favorable for the viticulture and winemaking.

Soil samples of 0-10 and 10-20 cm were taken with a soil drill. After incubation, they were sifted through a sieve of 1 mm. Plant roots, stones and other inclusions were removed.

The *pH (H_2O)* was measured by the potentiometric method using the Mettler Toledo Seven Compact s220 pH meter. The *total organic C* – by the Tyurin method using the Leki UV2107 spectrophotometer. *Basal respiration* (BR) – by the ISO 16072:2002. After incubation in vials for 24 hours of soil moistened with distilled water at a temperature of 22 ± 0.5°C, 10 ml of air was taken with a syringe and CO_2 content in it was analyzed using a gas chromatograph “Chromatek – Crystal 5000.1”. The BR rate was expressed in μg CO_2-C g^{-1} soil per hour. The repetition is fivefold. *Substrate-induced respiration* (SIR) was determined in a similar way, but instead of distilled water, the soil was moistened with an aqueous glucose solution (10 mg/g of soil) and incubated for 3.5 hours at a temperature of 22 ± 0.5°C. The SIR was expressed in µL CO_2 g^{-1} soil per hour. The repetition is fivefold. *Carbon of microbial biomass* (Cmic) was recalculated from the SIR according to the formula (1) by Anderson and Domsch (1978:

SIR (µL CO_2 g^{-1} soil·h^{-1}) × 40,04 + 0,37 (1)

The microbial metabolic coefficient (qCO_2) was calculated as the ratio of basal respiration and microbial biomass carbon and expressed in µg CO_2-C mg^{-1} Cmic·h^{-1} (2):

qCO_2 = BR/Cmic (2)

Statistical data processing was carried out using the R-studio software.

Results and Discussion

The pH values of the water extract from the soils of all the studied farms varied slightly and ranged from 7.18 to 8.38 (Table 1), which corresponds to a very slightly alkaline to moderately alkaline reaction. In most cases, the pH values of the soil increased with depth, which is obviously due to the carbonate bedrocks. The organic C content in the topsoil varied in a wide range from 1.48 to 4.34%, which reflects the high spatial diversity of soil fertility without obvious correlation to the land use system.

Table. Hydrogen index (pH), total organic C (%), basal respiration (BR, µg CO_2-C g^{-1} soil·h^{-1}), substrate induced respiration (SIR, µL CO_2 g^{-1} soil·h^{-1}), microbial C (Cmic, µg C g^{-1} soil) and metabolic coefficient (qCO_2, µg CO_2-C mg^{-1} Cmic·h^{-1}) in the soils of viticultural farms

Farm number	Land use system	Depth (cm)	pH_{H2O}	Total organic C	BR	SIR	Cmic	qCO_2
1	Organic	0-10	7.52	1.48	0.59	9.05	363	1.63
		10-20	7.78	2.47	0.67	8.57	344	1.94
2		0-10	7.94	3.53	0.48	6.27	251	1.90
		10-20	7.78	2.48	0.24	8.47	340	0.69
3		0-10	6.52	2.28	0.74	22.65	907	0.82
		10-20	6.65	2.21	0.53	21.38	856	0.61
4		0-10	7.85	2.78	0.74	15.58	624	1.20
		10-20	7.86	2.48	0.20	2.13	86	2.16
5		0-10	7.18	3.48	1.50	19.89	797	1.90
		10-20	7.26	3.84	0.92	7.18	288	3.21
6		0-10	7.21	1.93	0.25	8.31	333	0.73
		10-20	7.42	1.74	0.50	8.95	359	1.41
7	Fallow after traditional	0-10	8.26	3.31	0.45	2.03	82	5.57
		10-20	8.38	3.09	0.11	3.19	128	0.84
8		0-10	8.19	3.64	0.21	3.10	125	1.70
		10-20	8.22	3.83	0.06	4.46	179	0.33
9		0-10	8.25	3.17	0.29	1.98	80	3.61
		10-20	8.27	3.08	0.31	2.50	100	3.14
10		0-10	7.24	3.49	1.20	1.98	80	15.15
		10-20	7.24	1.74	0.35	2.57	103	3.40

11	Traditional	0-10	7.94	3.53	0.48	6.27	251	1.90
		10-20	7.85	3.41	0.10	3.87	155	0.63
12		0-10	7.93	3.49	0.30	3.52	141	2.15
		10-20	8.06	3.28	0.18	7.52	302	0.58
13		0-10	8.06	4.34	0.21	2.11	85	2.44
		10-20	8.15	4.13	0.13	3.04	122	1.11
14		0-10	7.47	2.04	0.54	5.89	236	2.31
		10-20	7.56	1.96	0.09	4.85	194	0.45
15		0-10	8.13	3.24	0.11	2.28	92	1.23
		10-20	8.19	3.19	0.33	2.13	86	4.01

Soil basal respiration (BR) is widely used to determine the physiological status of the soil microbiome (Alef, 1975). The correlation analysis between the BR indicators and the land use system applied in the farm is shown a tendency to decrease BR in farms with a chemical plant protection system compared to organic ones, although the differences were not statistically significant (Fig. 1). Fallow soils occupied an intermediate position.

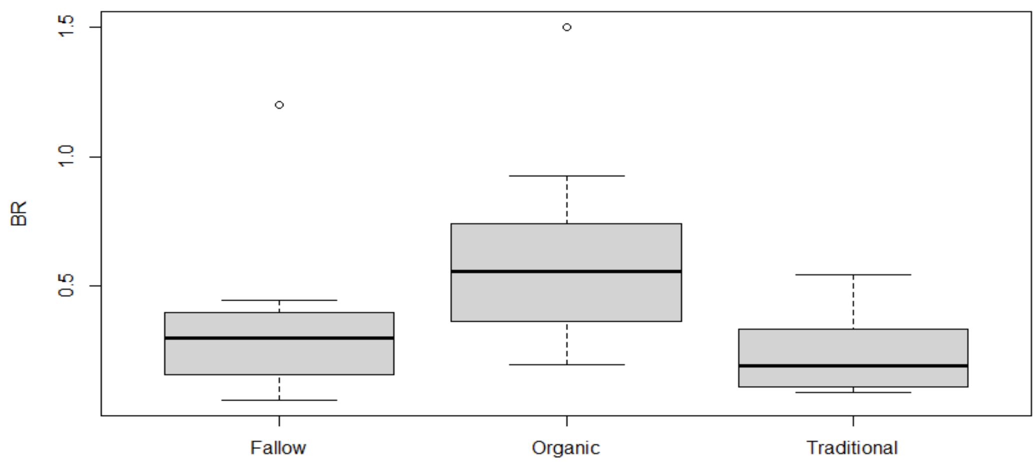

Fig. 1. Basal respiration (BR, µg CO_2-C g^{-1} soil·h^{-1}) in the topsoil of ampelocenoses with different land use systems according to the Kruskal-Wallis Test (p-value = 0.01341).

The value of substrate-induced respiration (SIR), which characterizes the potential activity of the soil microbial community, varied widely in the topsoil of organic farms – from 6.27 to 22.65 µL CO_2 g^{-1} soil·h^{-1} (Fig. 2). In the fallow soils, this indicator was low and varied in a narrow range from 1.98 to 3.10 µL CO_2 g^{-1} soil·h^{-1}, obviously due to the absence of agrogenic impact and phytocenosis stability. It is believed that high values of the SIR indicate a greater ability of the system to maintain balance under the external influence. Thus, the microbiota in the soils of organic farms was characterized by a more stability and resistance compared to the soils of farms practicing traditional system of land use.

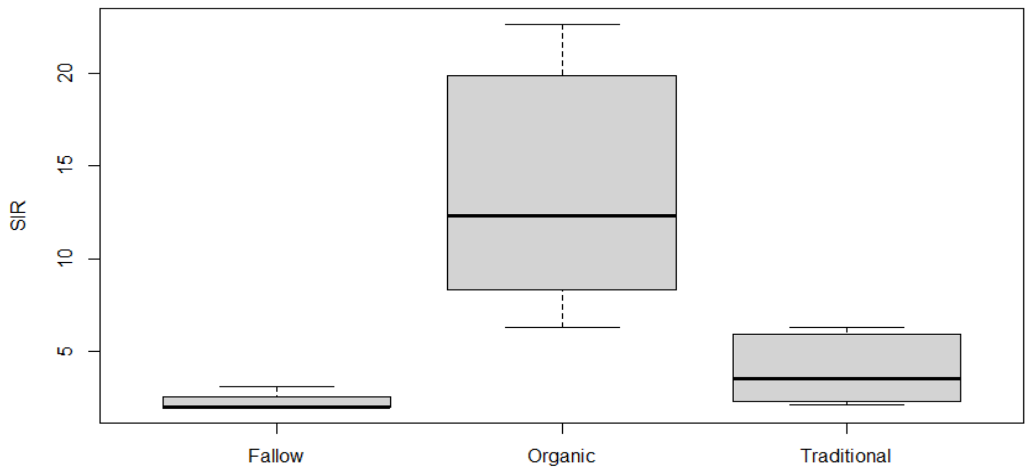

Fig. 2. Substrate-induced respiration (SIR, μL CO_2 g^{-1} soil·h^{-1}) in the topsoil of ampelocenoses with different land use systems according to the Kruskal-Wallis Test (p-value = 0.003491).

The undoubted advantage of the method for determining substrate-induced respiration is the possibility of simple and rapid determination of carbon of microbial biomass in soils (Cmic). It is based on the fact that the initial rate of CO_2 production by microorganisms in response to the introduction of an easily accessible energy substrate into the soil is proportional to their mass. Glucose was used as an easily accessible substrate, the main source of which in the soil is plant litter, consisting of 70-80% cellulose. Therefore, glucose consumption can be considered as a model of mineralization of organic litter by soil microorganisms. Similarly, we identified a significant relationship between Cmic and land-use system used in the farms. The highest microbial C in the 0-10 cm topsoil was observed in organic farms: 545.8 μg C g^{-1} soil versus 161.1 μg C g^{-1} soil in topsoil of the farms with traditional land use.

The microbial metabolic coefficient (qCO_2) is one of the most important indicators in the environmental monitoring of soils and represents specific respiration, calculated as the ratio of basal respiration and carbon of microbial biomass Cmic. It is known that the qCO_2 is a "sensitive" to any changes in land use (Bastida et al., 2006; Blagodatskaya et al., 2003) and can quantify the ecophysiological state of the microbial community. The microbial metabolic coefficient is called an indicator of microbial stress. High qCO_2 values are observed under various anthropogenic loads, in degraded and polluted soils (Fliessbach et al., 2009). including heavy metals (Papa et al., 2010). Our studies have shown that there are no significant differences in the microbial metabolic coefficient in case of traditional and organic farms, with a tendency to decrease qCO_2 in the 0 – 10 cm topsoil of organic farms compared with traditional ones. In the fallow soils, the qCO_2 values were significantly higher (Fig. 3).

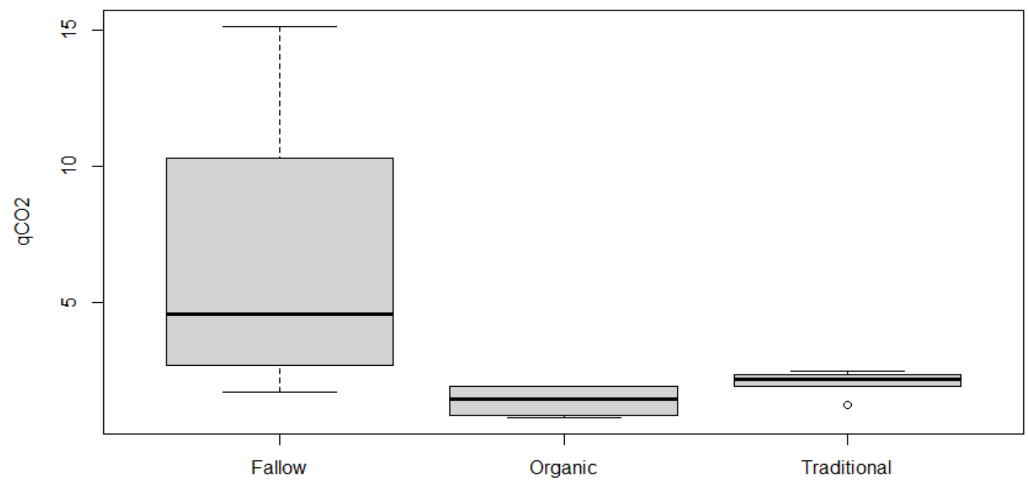

Fig. 3. Microbial metabolic coefficient (qCO_2, µg CO_2-C mg^{-1} Cmic·h^{-}1) in the topsoil of ampelocenoses with different land use systems according to the Kruskal-Wallis Test (p-value = 0.02513).

Apparently, this can be explained by the fact that fallow soils have been involved in agricultural land use for about 40-60 years, while the vineyards of the studied organic and traditional farms are relatively young, aged 5-20 years, therefore differences in the microbial metabolic coefficient in the soils of these farms were not expressed.

Among the parameters of soil respiration, the microbial respiration coefficient (QR) is quite informative. It represents the ratio of the BR value to the SIR. The coefficient of microbial respiration is an integral indicator that allows assessing the ecological state of the soil and its microbial pool. It is generally assumed that the QR value in the range of 0.1-0.2 indicates a positive state of the soil microbial community (Blagodatskaya et al., 2003). In our study, the values of the microbial respiration coefficient in the upper 10-centimeter soil layer of traditional farms and fallows were in the range of 0.09 – 0.27, whereas in the soil of all organic farms studied, the average QR value was less than 0.1. On the one hand, these data indicate the absence of expressed negative effects on the soil microbiome of the ampelocenoses studied, and on the other hand, low QR values in organic farms are a sign of a nutrients' deficiency in the soil, which is confirmed by the results of our studies on the content of available phosphorus and potassium. The supply of mobile phosphorus in the soils of all studied farms ranged from 5.22 to 84.77 mg P_2O_5/kg soil and was estimated as very low and low. On the contrary, the studied soils were well supplied with exchangeable potassium, its content varied in the range of 428-709 mg K_2O/kg soil, which corresponds to increased, high and very high availability. At the same time, the topsoil of traditional farms is significantly better provided with exchangeable potassium compared to organic farms and fallows (Fig. 4).

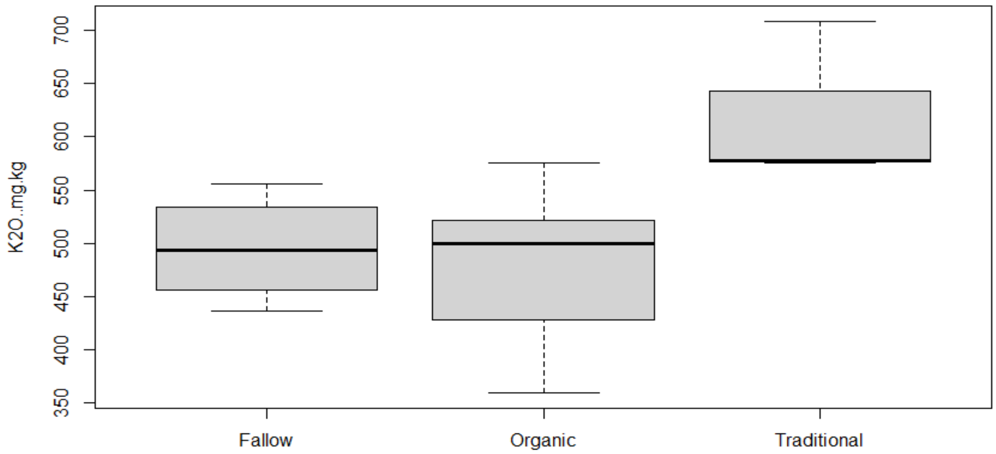

Fig. 4. Exchangeable potassium (K_2O, mg/kg) in the topsoil of ampelocenoses with different land use systems according to the Kruskal-Wallis Test (p-value = 0.04877).

Conclusions

Determinated microbial ecophysiological parameters in brown mountain gravelly soil of 15 viticultural farms located in the Northern Black Sea region hasn't shown signs of the soil microbiome's disturbance under the influence of natural or anthropogenic factors. However, the indicators of soil respiration in organic farms significantly differed from traditional ones practicing a chemical plant protection system. So, the substrate-induced respiration and the carbon of microbial biomass were 3.4 times higher in the soil of organic farms than traditional ones, but their microbial metabolic coefficients, on the contrary, were 1.5 times lower indicating a more stable state of the soil-biotic system in organic farms. At the same time, the low coefficient of microbial respiration in the soils of organic farms compared to traditional ones (0.02 – 0.08 vs. 0.10 - 0.27) indicated their insufficient supply of nutrients, especially in the content of available phosphorus in the topsoil. Thus, analyzing the ecophysiological state of the soil microbiome is important not only for assessing the current state of ampelocenosis soils, but also for making timely and informed decisions on making changes to vineyard management elements in order to support the ecosystem services of the soil and obtain high-quality wine products that is very valuable for agroecological DSS.

Acknowledgements

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CHITOSAN AND OTHER ANTITRANSPIRANTS – THEIR INFLUENCE ON ASPECTS OF ORGANIC AND CONVENTIONAL PLANT PRODUCTION

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Abstract

The work presents main findings from investigations of our and other different researcher carried out on different cultivars using chitosan, its derivatives, nanoparticles and other antritranspirant compounds (like Di-1-p-menthene). The work demonstrates that this chitosan (and other antitranspirants) is highly effective against the most dangerous diseases and pathogens in different cultures. Also, natural origin of chitosan (and also, other antitranspirants) makes it suitable for use in organic agriculture. Furthermore, it also contributes to improving yield and different plant physiological and growth parameters. Additionally, it induces excellent resistance to some abiotic stresses (drought, salt, and low temperature) and reduces their negative impact on different cultivars.

Key words: *antritranspirants, phytopathology, organic agriculture, yield, yield components, quality of crop yield.*

Introduction

This article presents the some findings from investigations carried out on different cultivars using chitosan, as antritranspirant compounds. Research into the use of that substances in agriculture is growing in popularity. Since 2000, more than 200 original scientific articles indexed in different databases have been published on this topic. Many researcher of the topic focused mainly on main cereals, but application in other cultures is not neglected. Also, natural origin of chitosan makes it suitable for use in organic agriculture. Chitosan is a derivative of chitin and is considered the second most common polymer in the world after cellulose and it is classified as polysaccharides containing randomly distributed β-(1-4)-linked D-glucosamine and N-acetylglucosamine units. Chitosan is mainly obtained from different sea invertebrates obtained from seafood processing. Furthermore, the potential to receive it from waste fungal mycelium is also indicated. In agriculture, chitosan is used through foliar application to plants, seed treatment, or as a direct soil fertilizer. The work demonstrates that this chitosan is highly effective against the some vicious diseases and pathogens in a crops. Furthermore, it also contributes to improving yield, yield components, as well as some chemical and biochemical parameters, as indicators of crop yield quality. Additionally, it induces excellent resistance to some abiotic stresses (drought, salt, and low temperature) and reduces their negative impact on different cultivars. However, further studies are needed to demonstrate the full field efficacy of chitosan (and also, other antitranspirants, such as Di-1-p-menthene).

Material and methods

The conditions for growing crops are stated in the works Waisi et al. (2014) and Dragičević et al. (2016). A micro-trial (plot size $1m^2$) was set up in spring barley, on degraded chernozem (Zemun Polje location, Serbia), where the first treatment was carried out at the end of the vegetative phase of this crop's development. The experiment for apples was set up in an orchard in Padinska Skela (Serbia) on alluvial soil. The elementary plot consisted of 5 apple trees, and the first treatment was carried out at the beginning of flowering. The conditions of standard chemical (ICP-OAS), biochemical (different methods) and microbiological analyzes are stated in the papers Waisi et al. (2014), Dragičević et al. (2016) and Živković et al. (2018).

Results and disscusions

In Table 1 we see that chitosan-treated barley plants give the highest average yield over two years (2013 and 2014), despite unfavorable agrometeorological conditions. However, this is not due to the increase in the mass of 1000 grains of barley (Table 1). In Table 2, we show the availability of different nutrients (inorganic P, β-carotene, Mg, Ca, Fe, Zn and Mn) in barley seed, expressing them relative to the phytate, unavailable form of phosphorus, so that the lower the values of this ratio, the increased availability of these nutrients, both for germination of barley seeds and in the diet of domestic animals and humans. Chitosan has a particularly beneficial effect on the availability of inorganic phosphorus, beta carotene, while moderately increasing the availability of trace elements (Table 2). Chitosan is known to have a beneficial effect on the resistance of crops and other plants to abiotic stresses (Ludwig et al., 2010; Iriti et al., 2010; Safaei et al., 2014; Hidangmayum et al., 2019; Kocięcka and Liberacki, 2021), but from the attached we can conclude that it also increases their nutritional value (Table 2; Dragičević et al., 2016).

Table 1. Grain yield and 1,000 grains weight of barley (cv. Apolon) influenced by the different foliar fertilizers (according: Dragičević et al., 2016).

Treatment	Grain yield (kg ha^{-1})			1000 grain weight(g)		
	2013	2014	Average	2013	2014	Average
Control	3231.7	922.3	2077.0	37.80	29.09	33.44
Epin extra	3113.0	1043.1	2078.0	39.30	36.64	37.97
Zircon	3752.0	623.7	2187.9	38.69	32.84	35.77
Chitosan	3856.3	1098.8	2477.6	39.14	31.55	35.34
Benzyladenine	3244.3	1107.5	2175.9	40.01	30.49	35.25
Siliplant	3194.3	933.3	2063.8	39.40	32.63	36.01
Propikonazole	3328.7	653.1	1990.9	40.67	33.78	37.23
Average	3388.6	911.7		39.29	32.43	
LSD 0.05*	Treatment	Year	T X Y	Treatment	Year	T X Y
	1462.0	532.5	569.4	4.03	1.95	1.05

*Least significant difference, P = 0.05 (n = 4)

Table 2. The effect of different foliar fertilizers on relations between phytic and inorganic P, phytate, *β*-carotene, Mg, Ca, Fe, Zn and Mn in barley (cv. Apolon) grain (according: Dragičević et al., 2016).

Treatment	Pphy/Pi	Phy/β-carot.	Phy/Mg	Phy/Ca	Phy/Fe	Phy/Zn	Phy/Mn
Control	5.10	5356.60	2.15	2.86	107.34	40.22	74.1
Epin extra	4.58	5242.48	2.11	2.68	100.90	27.52	60.0
Zircon	4.62	5411.22	2.14	3.11	103.15	37.21	71.0
Chitosan	4.60	5088.97	2.14	4.21	72.38	34.10	70.1
Benzyladenine	4.60	5349.25	2.03	2.36	62.91	31.81	69.1
Siliplant	4.47	5610.72	2.05	2.96	51.13	28.70	76.1
Propikonazole	4.74	5828.46	2.16	2.96	55.46	35.80	80.3
LSD 0.05*	0.8	2397.6	0.11	0.58	262.7	15.66	104.3

*Least significant difference, P = 0.05 (n = 4)

Chitosan also has a beneficial effect on the yield and yield components of apples (Tables 3 and 4). Since chitosan has so far been mainly tested on cereals, with rarer examples of its beneficial effects on vegetables and herbs, (Ludwig et al., 2010; Iriti et al., 2010; Safaei et al., 2014), this preliminary finding seems encouraging (Table 3 and 4), particularly because the season (2014) in which we tested influence of chitosan on apple yield and quality of yield was very humid.

Table 3. The effect of different foliar fertilizers on average and relative apple yield and different parameters of quantity of apple fruits (according: Waisi et al., 2014).

Type of fertilizer	Average (kg/ t) and relative (%) apple yield per trunk and per elementary area (1 ha)				different quantity (g/ %) parameters of apple fruit	
	Average yield per trunk (kg)	Relative yield per trunk (%)	Assesed yield per ha (t/ha)	Relative yield per ha (%)	Averaged weight of fruit (g)	Relative weight of fruit (%)
Control	15.984±5.78	100	20.779	100	217.44±31.338	100
Vegard (plant extract)	27.789±9.476	173.86	33.959	163.43	222.61±39.46	102.38
Eko-Fus (plant extract)	37.568±4.854	235.04	48.839	235.04	228.96±26.05	105.30
Calbit-C (plant extract)	20.222±2.235	128.93	26.289	126.52	220.24±42.82	101.29
Chitosan (plant& schell extract)	56.465±13.161	353.26	73.405	353.27	211.81±32.93	97.41
Cirkon (plant extract)	34.833±6.363	217.92	45.391	218.45	202.81±42.37	93.27
Cropmax (aminoacid fertilizer)	30.527±9.813	190.98	39.685	190.99	217.91±37.98	100.22

Table 4. The effect of different foliar fertilizers on average and relative apple yield and different parameters of quantity and quality of apple fruits (according: Waisi et al., 2014).

Type of fertilizer	different quantity (g/ %) parameters of apple fruit		different parameters of quality of apple fruit (corrected (at 25^{0}C) values of refraction coefficients (% Brix) extracts of apple fruit picking in different days of 2014 season)			
	Numbers (n) of apple fruits per trunk	Relative (%) numbers of apple fruits per trunk	19.05.	03.06.	02.07.	09.09.
Control	15.984±5.78	100	5.01	4.75	4.42	6.94
Vegard (plant extract)	27.789±9.476	173.86	-	5.07	5.02	5.34
Eko-Fus (plant extract)	37.568±4.854	235.04	-	4.88	5.02	7.94
Calbit-C (plant extract)	20.222±2.235	128.93	-	5.07	5.02	6.54
Chitosan (plant& schell extract)	56.465±13.161	353.26	-	5.00	4.55	5.34
Cirkon (plant extract)	34.833±6.363	217.92	-	5.07	4.35	6.74
Cropmax (aminoacid fertilizer)	30.527±9.813	190.98		4.87	4.55	6.54

It should be mentioned that chitosan induces increased resistance of crops (Iriti et al., 2010; Sanchez-Vallet et al., 2014; Trouvelot et al., 2014; Ghule et al. 2021) to biotic factors (fungi, bacteria, etc.), while the mechanisms of action of chitosan on these processes are still being investigated. These conclusions we proved by our preliminary work (Živković et al., 2018).

Table 5. Effects of chitosan on *A. alternata* and *C. gloeosporioides* decay on apple fruits (according: Živković et al., 2018).

Treatment	*A. alternata* lesion diameter (mm)	*C. gloeosporioides* lesion diameter (mm)
Control +	26.33 ± 0.57 a	33.50 ± 1.32 a
Chitosan 1 mg/ml	21.50 ± 0.50 b	26.00 ± 1.00 b
Chitosan 2 mg/ml	17.50 ± 1.32 c	17.67 ± 1.55 c
Chitosan 3 mg/ml	8.33 ± 1.15 d	11.00 ± 1.00 d
Control -	0.00 ± 0.00 e	0.00 ± 0.00 e

Conclusion

In this brief review, we have shown that chitosan affects the yield and yield components of arable (Tables 1-2) and fruit (Tables 3-4) crops, and also the resistance of stored fruit (Table 5) to some of the important diseases that can reduce the quality of fruit yield. Further directions of research will concern different formulations of chitosan and other antitranspirants, whereby the emphasis will be on monitoring a number of quality indicators of crop yields.

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ENVIRONMENT PROTECTION AND NATURAL RESOURCES MANAGEMENT

RIVER NETWORK DENSITY BY LARGE NATURAL REGIONS IN BOSNIA AND HERZEGOVINA

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Abstract

The paper analyze EU-Hydro database on river network density within the Bosnia and Herzegovina (B&H). EU-Hydro is a dataset for all European Environment Agency 39 countries (EEA39), including B&H, providing a photo-interpreted river network, consistent of surface interpretation of water bodies (lakes and wide rivers), and a drainage model (also called Drainage Network), derived from EU DEM, with catchments and drainage lines and nodes. It is based on remote sensing imagery from the years 2006, 2009, and 2012. The density of the river network depends on several abiotic factors: climate, relief, soil type, geological layers, vegetation, etc. These factors differ in B&H according to the large natural regions that have a significant impact: the Pannonian Plain in the north, the Dinaric Mountains in the central part and the Mediterranean zone in the south. Research results show that the average density of the river network in B&H is 0.438 km/km^2. However, according to biogeographical zones that mostly matching with large natural areas, the density of the river network differs: it is densest in the Continental region with 0.477 km/km^2 followed by the largest central region with 0.433 km/km^2, and the rarest in the Mediterranean region with 0.372 km/km^2. The aim of this paper is to figure out which abiotic factors by natural regions crucially affect the density of the river network in B&H. In addition, it will be analyzed which regions have the highest risk of floods and whether the density of the river network affects that.

Keywords: *river, network, density, B&H, EU-Hydro.*

Introduction

B&H is a country in the Western Balkans, with an area of 51.209 km^2, and is composed of two autonomous political entities roughly equal in size: The Federation of Bosnia and Herzegovina (FB&H) and Republic of Srpska (RS), including a third unit as well, Brčko District (BDB&H) governed by the local government. The population of B&H is around 3.5 million (AS B&H, 2016). It is located at the contact zone of three major natural and geographic units: the Pannonian Plain, the Adriatic Sea, and the Dinarides Mountains, which effect on three climate types: temperate-continental, mountainous and Adriatic (Mediterranean) (Milosavljević, 1973). Average annual air temperatures ranges from 5 °C in the central mountain region to over 14 °C in the south of the country. The annual rainfall varies from 700 mm in the north along the Sava River, to 2000 mm in the central and southern regions. Above the rest of the country, precipitation ranges from 850 to 1500 mm (Bajić & Trbić, 2016). The main types of soil are: brown (about 50% out of which 27% are brown and 23% sour brown), black soil on limestone (16%), hydromorphic soil (20%), ilimerised (7%) and red soil (1.17%). Two large floral areas—

Eurosiberian and Mediterranean—meet in B&H (Musa, 2007). As a consequence of different nature and landscape, there are three biogeographical regions, according to the EEA (2016): Continental, Alpine and Mediterranean. Continental region is located on the north at Peripanonian lowland (33.3% of the country territory). Alpine region cover the largest central part with mountain-hilly terrain (50.6%). Mediterranean region occupy karstic zone at the south, with intensive influence of Adriatic Sea (16.1%). Total internal renewable water resources in B&H amount to 10.680 m^3/per capita/per year for period 2018-2022, which ranks it among the countries rich in water (countries with amount of 10.000-20.000 m^3/per capita/year). In comparison with Germany total internal renewable water resources per capita is 1.287 m^3/per capita/per year (FAO, 2022). The average runoff from the territory of B&H is 1200 m^3/s and the average runoff coefficient is 0.57. Such a high runoff coefficient indicates some circumstances: river runoff regimes are torrential with fast flows, so losses are reduced; hydrogeological boundaries of some basins are larger than orographic ones, so that in some basins underground inflows from the territories of other countries also participate (MAFWM RS, 2015).
The runoff is towards the Danube river basin from an area of 38.719 km^2 (75.7% B&H) and towards the Adriatic Sea from an area of 12.410 km^2 (24.3%) with the total amount of water towards the Danube river basin of 722 m^3/s, and in the direction of the Adriatic Sea 433 m^3/s (MAWMF FB&H, 2010). The large river basins in B&H belongs to Black Sea via Danube basin are: Drina, Bosna, Sava, Vrbas, Una and Sana. The basins of Neretva, Trebišnjica and Cetina belongs to the Adriatic Sea basin. The watershed is a mountain massif of the Dinarides, over 300 km long and 80-200 km wide. The highest peak at the mountain Maglić with 2367 m. Densely populated agricultural regions of Posavina and Semberija in the north and along the middle course of Bosna river valley in the central part of the country have less water per capita than the average. The purpose of the article is to show the influence of environmental factors of large natural regions to river network density. The most important factors influencing river regimes and flows are climate, vegetation, relief and geology. Bearing in mind that the new biogeographical regionalization of Europe was carried out in 2016 by the European Environment Agency (EEA, 2016) the influence of these regions on the density of the river network in B&H has not been analyzed so far. In addition, the first EU-Hydro data are released during the last decade, so this type of the research, calculation of river network density by biogeographic region, is not yet implemented in B&H.

Materials and methods

The feature of EU-Hydro data (CLMS, 2020) extraction has been performed mainly by photo interpretation of Very High Resolution (VHR) Image based on remote sensing imagery from the period 2011 to 2013, with a spatial resolution of 2.5 metres. EU hydro is not only based on photo interpretation of VHR imagery, but also includes river segments obtained from Digital Elevation Model (EU DEM v1.1): stream polylines were generated through drainage modelling from and integrated to EU hydro river network in order to create a more complete coverage of the existing river network, enhancing the usability of the dataset (CLMS, 2019).
The river network has been performed as a naturally flowing watercourse wider than 50 m and narrower than 50 m, showed by two types of shapefiles: polygons (wider than 50 m) and polylines (narrower than 50 m). The DEM (CLMS, 2016) with a resolution of 25 m has been used for showing the altitude and slope. Country territory has been divided by four altitude zones of 0-500 m (39.47%), 500-1000 m (35.53%), 1000-1500 m (21.9%) and over 1500 m (3.1%).

Attitude average by biogeographical regions has been calculated: Continental 281 m, Alpine 924.3 m and Mediterranean 720.9 m. The high terrain slopes, especially in Alpine region, contribute to the rapid runoff. All the data has been processed by Geographic Information System (GIS) tools, exported to Excel and calculated by regions. River network density has been performed using the Neumann's equation $D = \Sigma L/F$ (D-density, ΣL-sum of river length, F-area) (Dukić & Gavrilović, 2006). According to this pattern, the average length of watercourses per 1 km^2 has been obtained. It depends on many factors and the most important are the climate, geological structure, relief and vegetation. The Aqueduct Water Risk Atlas, developed by the World Resources Institute (WRI), is a global water risk mapping tool that helps users to understand where and how water risks and opportunities are emerging worldwide. The Atlas uses peer reviewed methodology and the best-available data to create high-resolution global maps of water risk. Riverine flood risk measures the percentage of population expected to be affected by Riverine flooding in an average year, accounting for existing flood-protection standards. Flood risk is assessed using hazard (inundation caused by river overflow), exposure (population in flood zone), and vulnerability. It is important to note that this indicator represents flood risk not in terms of maximum possible impact but rather as average annual impact. The impacts from infrequent, extreme flood years are averaged with more common, less newsworthy flood years to produce the "expected annual affected population." (WRI, 2019).

Results and discussion

According to the EU-Hydro database the total river length in B&H is 22.418 km. Total river network density is 0.438 km/km^2. When it comes to large natural (biogeographical) regions, river network is the densest at Continental region at the north with 0.477 km/km^2, followed by Alpine (central) and Mediterranean regions on the south with 0.433 km/km^2 and 0.372 km/km^2 (Table 1).

Table 1: River density by biogeographical regions in B&H

	Area		Rivers narrower than 50 m		Rivers wider than 50 m		Rivers (Total)
Biogeoregion	km^2	%	L (km)	D (km/km^2)	L (km)	D (km/km^2)	D (km/km^2)
Continental	17028.1	33.3	6689.2	0.393	1425	0.084	0.477
Alpine	25932.1	50.6	10693.6	0.412	540.3	0.021	0.433
Mediterranian	8248.8	16.1	2846.7	0.345	224	0.027	0.372
B&H	51209	100.0	20229.5	0.395	2189.3	0.043	0.438

*Source: Author's calculation based on the EU-Hydro data.

Total length of the rivers wider than 50 m is 2189.3 km, with the highest density of the river network in the Continental region with 0.084 km/km^2, followed by the Mediterranean and Alpine with 0.027 km/km^2 and 0.021 km/km^2 (Figure 1). Total length of the rivers narrower than 50 m is 20229.5 km. The highest density is recorded at Alpine region with 0.412 km/km^2, followed by Continental with 0.393 km/km^2 and Mediterranian with 0.345 km/km^2.

It is expected because the Continental region belongs to the lowland area watercourses flows into from the mountainous area of the central region. Due to the low terrain slopes, the flows are slowly and sediment deposit discharge is intensive. On the other hand, the mountainous region is characterized by a denser network of short rivers, high slopes, vertical erosion and rapid surface runoff. The vast majority of the Mediterranean region is represented by mezozic limstone and

dolomite. Subterranean rivers cross the fields, whereas surface runoff is extremely weak and almost does not exist. With a total length of 187 km above and under the ground, Trebišnjica is one of the longest sinking rivers in the world.
Also, as the distance from the Adriatic Sea increases, precipitation decreases, so the density of the river network should be the highest in the Mediterranean and the lowest in the Continental region. But, considering that within the Mediterranean region the predominantly karst relief prevails with the majority of underground runoff, the river density in this region is lower than expected. Two factors contribute to the alpine region having the highest rivers narrower than 50 m density, relatively high amount of precipitation and mauntain's relief with high slope of the terrain. It means that there are more small, short streams.

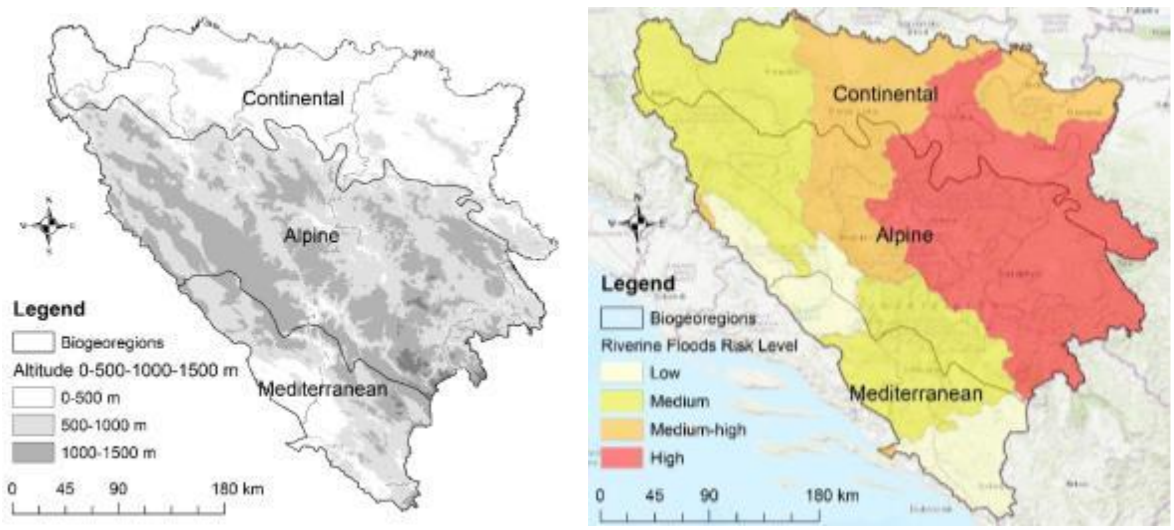

Figure 1-2. River wider than 50 m by biogeographical regions and Riverine floods risk level in B&H

Analyzes show that in over 50% of the time during the year the flows are lower than 80% of the average flow. This means that most of the water flows in short-term floods, followed by long periods with low flows (MAFWM RS, 2015). The floods had on several occasions, including the last large-scale floods in May of 2014, caused great material damage to the economy, infrastructure facilities, environment, citizens’ health, including losses of human lives.
According to WRI data (2019), 34.7% of the territory in B&H has a high Riverine flood risk, mostly in densely populated areas in the Drina and Bosnia river basins. 21.3% of the territory belongs to medium-high flood risk, at the Vrbas and Sava basins, and 32% to low-medium risk, mainly Una and Sana basins in the northwest and Neretva in the south. 12% of the territory belongs to low risk, in the Cetina and Trebišnjica river basins (Figure 2). Higher values indicate that a greater proportion of the population is expected to be impacted by Riverine floods on average.

Conclusions

The river network density in B&H is relatively high and amounts to 0.438 km/km^2. It is densest in the Continental region in the north with 0.477 km/km^2, followed by the central Alpine area with 0.433 km/km^2 and the Mediterranean karst region in the south with 0.372 km/km^2. If we talk about rivers narrower than 50 m, the densest river network is in the Alpine region with 0.412

km/km^2 followed by Continental and Mediterranean region with 0.393 km/km^2 and 0.345 km/km^2 respectively. Due to the slope of the terrain, there are more alpine rivers, but they are shorter. The mountainous-hilly Alpine region is exposed to the risk of torrential floods, due to combination of intensive rainfall and high slope The lowland continental region is exposed to the risk of overflow from the bed of the rivers wider than 50 m. When it comes to river basins, most affected of floods are river Bosnia basin in central part of the country and river Drina basin on east.

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MONITORING OF SENSORY DYNAMICS OF *AMBROSIA ARTEMISIIFOLIA L.* POLLEN AS AN AEROPOLUTANT IN THE UNA-SANA CANTON DURING 2012-2021

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Abstract

Ambrosia artemisiifolia L. is known as an invasive roof. In addition to the fact that this plant suppresses the indigenous plant species, the pollen of this invasive plant spreads very quickly in the environment. It is both a weed and an allergen, but also a medicinal plant. It came to Europe and our region from America as bird food (pheasant).

Nowadays, ragweed pollen grains in particular are of great interest to scientists and researchers in terms of its spread in the environment, but also the impact of high concentrations of pollen on human health. This plant is considered one of the harmful allergens for humans and is therefore present as a health problem. Due to its easy adaptation in the environment and suppression of indigenous plant species, ragweed is also an environmental problem. Monitoring of ragweed pollen is a very important segment of general environmental monitoring. High concentrations of pollen grains of this plant are especially present in the summer months until autumn. Especially very high concentrations are expressed in the period of pollen maturation. Since 2008, the concentration of ragweed pollen has been regularly monitored in the Una-Sana Canton. Equipment used research included a Hirst type pollen trap (sampler) Burkad, placed in Bihac.

In general, the aim of this paper was based on the sensory representation of the dynamics of ragweed pollen for many years. In this research, the period of monitoring the dynamics of ragweed pollen was from 2012 to 2021. Research has shown that the highest annual concentrations of ragweed pollen was present from the second half of July, in August and the first half of September. In the observed period, the prevalence of ragweed pollen was about 50% compared to grasses about 15%, trees about 25% and other weeds about 10%.

Keywords: *Ambrose, Monitoring, Health problem, Ecological problem, Agricultural problem.*

Introduction

Pollen grains of the genus *Ambrosia artemisiifolia L.*, among others, are considered one of the significant allergens for humans today. This plant is considered a very problematic weed known under different names: ragweed, pheasant, bitteweed, etc. In addition to the production of consideralble amounts of pollen, it also belongs into the group of invasive weeds, suppresses indigenous species and cause considerable damages to various ecosystems. Ambrosia is very easy to adapt to the environment, and becomes the dominant weed. It is an annual herbaceous plant whose vegetation period lasts from 150-170 days. It is also a heliophil and termophill species, a short-day plant. *Ambrosia artemisiifolia L.* is represented in the wider area of Europe. According to Bašić *et al.*, 2017, it continues to expand intensively in Bosnia and Herzegovina. Laaidi *et al.*, (2003) emphasizes that pollen grains have good aerodynamic properties and that one plant can have a daily production of up to 2.5 billion pollen grains, which means that 250 pollen grains in 10.000 m^3 of air. According to Trkulja *et al.*, (2010) spreading rate of ragweed averages 6-20 km per year. According to the official data from 2020 and 2021, Una-Sana Canton is in the zone of the highest concentration of ambrosia pollen in Europe, together with Croatia and Hungary[1]. Various measures (agricultural, health, environmental, educational and legal) are taken to prevent the spread, control and destruction of ragweed. This plant causes multiple problems that have been previously described in the research of Muhamedagić *et al.*, (2012), which occurred in both rural ecosystems and urban ecosystems. From an ecological point of view, ambrosia as an aeroallergen with favorable wind flow as an abiotic ecological factor can spread tought the environment at speed reaching up to 300 km/h traveling distances from 10 to 100 km. It also suppresses and threatens the original plant diversity. (Ya.Maryushkina, 1991; Comtois, 1998; Genton *et al.*, 2005, etc.) Taking into account the research of Wayne *et al.* (2002) who point out that in the future an increase in CO_2 from 350 to 700 mmol/mol may result in increased ragweed pollen production of about 60-90%. Also taking into account Ziska *et al.* (2003) research on temperature and CO_2 gradients as well as other research about effects of climatic changes and urban air polution on the rising trends of respiratory allergy and astma. (D'Amato *et al.*, 2011). In general, when vieved from the ecological aspect, helt status of human population being one of the indicators of living, it could be stated that alergy is also one of the sirious modern time ennvironmental problems as it has a serios impact on it (Muhamedagić *et al.*, 2012). From a health point of view, ragweed pollen grains (*Ambrosia artemisiifolia L.*) are aeroallergens responsible for allergic diseases in humans, which manifest themselves in the form of rickets, rhinchoconjunctivitis and bronchial asthma (Gioulekas *et al.*, 2004; D'Amato *et al.*, 2007; Trkulja *et al.*, 2010). In general, the fact is that with a high concentration of ragweed in the air in people of different ages and ages, with long and long-term exposure, in addition to allergies, there is a decline in immunity. In agriculture, ragweed as a weed causes great damage in the production of soybean, maize, true cereals, medicinal plants, clover, as well as fruit plantations. To summarize, according to Trkulja *et al.*, (2010) damages to agriculture caused by ragweed are as follows: it mehanically kill (suffocate) crops, reduces yields of cultivated plants, causes deterioration of gyality in cultivated crops, reduces soil temperature, makes soil cultivation more difficult, comlicates implementation of various agrotechnical practices, indirectly conduces to the incidence of plant diseases, etc.

Bearing in mind the mentioned multiple issues, and with the fact that *Ambrosia artemisiifolia L.* is widespread in the Una-Sana Canton, the primary objective of this papaer was to make an analysis of the annual seasonal dynamics of common ragweed in this area, i.e. analyze the monitoring of aeroallergenic ragweed pollen in the period 2012-2021.

Material and Methods

Ragweed pollen sampling methodology as defined by the International Aerobiology Association – IAA was used. The Concentration of pollen was measured by standard Hirst's (1952) method. Sampling of aeroallergenic ragweed pollen was performed during the pollination in the period of

[1] Source: Official report of the Cantonal Agricultural Institute from 2020 and 2021.

2012 – 2021. Equipment used for research included a Hirst type pollen trap (sampler) Burkad (Picture 1.), placed in the town of Bihać, 15 meters above the ground, at the locality of „Borići“ (*Burkard Manufacturing Co., Uxbridge, Middlesex, UK*).

Picture 1-3.) Burkad type pollen sampler at the Borići location for Una-Sana Canton

The results obtained were interpreted by means of thr so called „pollen traffic light“ scale (color – based rating scale) with relevant percentage of individuals that are likely to develop allergy symptoms (Table 1). Allergenic plants are classifield into three types: trees, grasses and weeds.

Table 1. Criteria for rating the concentration of airborne pollen

Level of pollen (color)	**Count of pollen grains/m^3 of air**			**Occurrence of allergic reaction symtoms**
	Trees	Grass	Weeds	
Absent (white)	0	0	0	No symptoms
Low (green)	1-15	1-5	1-10	Only in extremly sensitive persons
Moderate (yellow)	16-90	6-20	11-50	In 50% of sensitive persons
High (red)	91-1500	21-200	51-500	In almost all allergic persons
Very high (purple)	> 1500	> 200	> 500	In all allergic person

Counting and visual identification of ragweed polen was performed using a light microscope at magnification of x400. After having established the count of pollen grains, relevant, mathematical formulas were used to calculate the concentration of airborne pollen grains.

For determining the concentration the following parameters were into account:

r – diameter of the microscope field (r=0,5 mm);

P_t – area of strip (total area);

C_{sum} – number of strips that are observed (three strips);

P_p – area of the microscope field (observed area);

CF – correlation factor (CF = 9,3).

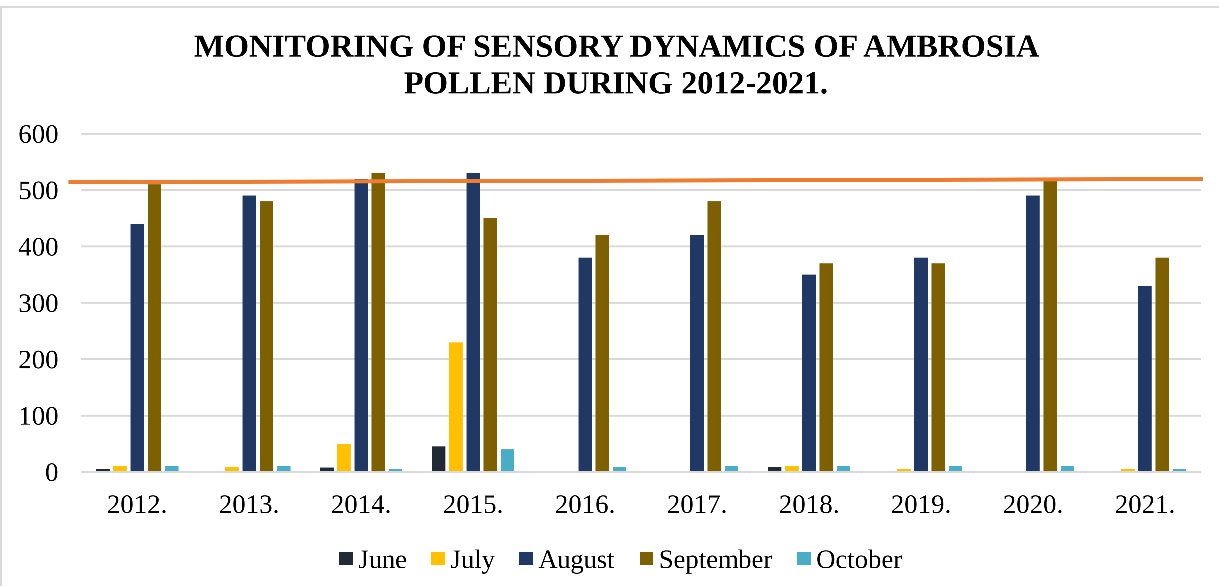
$$P_{observed} = r \quad x b \quad x \quad c_{sum} \; ; \qquad CF = \frac{P_t}{P_p}$$

X – concentration of airborne pollen grains (p/m^3)

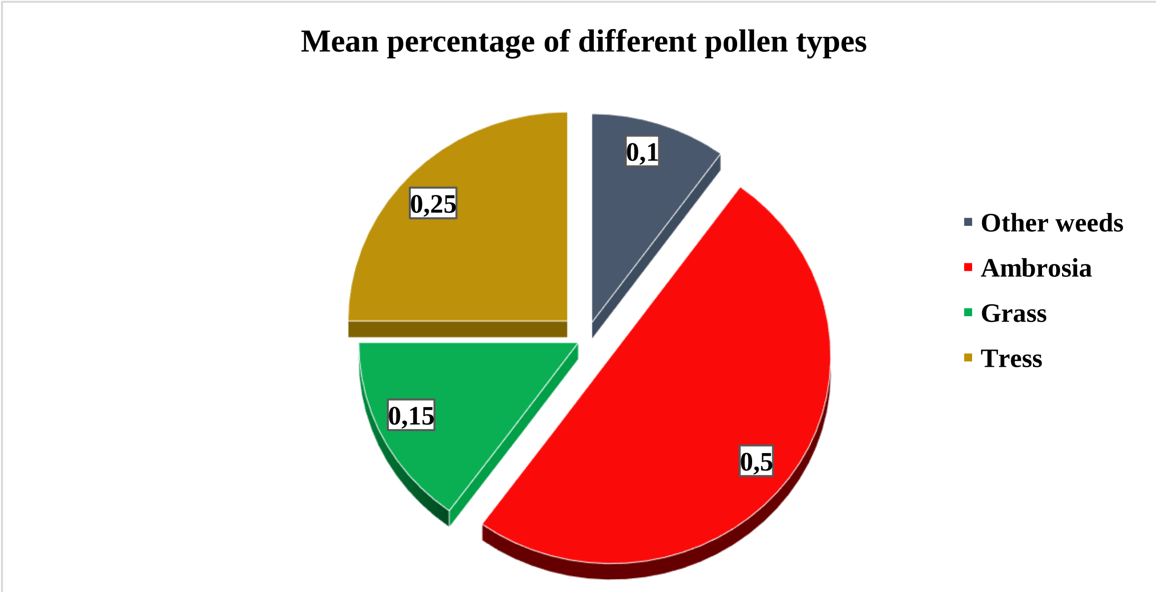
$$X = \frac{CF \quad x \quad pollen \quad grein}{14{,}4 m^3}$$

Results and Discussion

Results of monitoring the aeroallergenic ragweed pollen in the period from 2012 to 2021 in the area of the town of Bihać in the Una-Sana Canton are depicted ih Graph 1.



Percentage share of different types of pollen in the air in the Una-Sana Canton in the observed period 2012-2021. on graph no. 2.



Graph 2. Mean percentage of different pollen types in the Una-Sana Canton from 2012 to 2022.

Pollen grains of ragweed during the nine-year follow-up were recorded in July (second half of the month), August (full month) and September (first half of the month). Very high concentrations of ragweed pollen >500 p/m^3 were registered mainly in August and the second half of July. In August, very high concentrations of ragweed pollen were recorded in 2014, 2015 and 2020, while in September very high concentrations of ragweed pollen were recorded in 2012, 2014 and 2020. High concentrations of ragweed pollen (51-500 p/m^3) in the observed period were registered mainly in August (2012, 2013, 2016-2019 and 2021) and September (2013, 2015-2019 and 2021), but also in the second half of July 2015. In June and October, ragweed pollen concentrations were mostly low (1-10 p/m^3) or not evident at all (2016, 2017 and 2020). In other months, the presence of ragweed was not registered. Measurements in the Una-Sana Canton from 2008 to 2011 also indicated that the highest concentration of Ambrosia pollen was in the pollution period (second half of July and August) when favorable climatic conditions dominate (high temperatures, low relative humidity, high atmospheric pressure, warm wind and etc.). Authors Wayne (2002), Ziska (2003), Trkulja (2010), Muhamedagić (2012) and others state that in the summer months, the most common factors of ragweed pollen grain transfer are humans (anthropogenic factor) in addition to suitable climatic conditions (natural factor). Also, it is evident that during the period of Ambrosia pollution, the demand for anti-allergic drugs increased.

In general, the average representation of different types of pollen in the observed period from 2012 to 2021 was the highest in Ambrosia, about 50%. In the observed period, the prevalence of ragweed pollen was about 50% compared to grasses about 15%, trees about 25% and other weeds about 10%. In research in the Una-Sana Canton from 2012 for the period from 2010 to 2011, the percentage ratio was from 31% to 64% (Muhamedagić *et al.*, 2012), which indicates that even today *Ambrosia artemisiifolia L.* is still at a high percentage level.

Conclusions

In the observed nine-year period, ragweed pollen grains were registered from the second half of July (moderate concentrations), August (high and very high concentrations) and September (high and very high concentrations), which coincided with the climatic conditions in BiH when ragweed blooms.
In the Una-Sana Canton, high pollen concentrations were recorded in late August and in the first half of September, which confirms that ragweed pollen is most widespread at a time when very favorable climatic conditions (high temperatures, low relative humidity, high atmospheric pressure).
In the Una-Sana Canton, 2014 was characterized by drought and high temperatures, especially in August, the second half of July and the first half of September, which affected the spread of ragweed pollen in the environment when very high concentrations >500 p/m^3 were recorded. Based on the monitoring and the obtained results of ragweed pollen measurement, a direct dependence between the level of ragweed pollen concentration and the favorable abiotic environmental factors (climatic) can be determined.
In the observed period, the prevalence of ragweed pollen was about 50% compared to grasses about 15%, trees about 25% and other weeds about 10%.
Due to the seriousness of the problem with *Ambrosia artemisiifolia L*. as an air pollutant, it is necessary to carry out field protection measures in addition to monitoring. Every individual, as well as society in general, should participate in the protection measures.The most common field protection measures are: uprooting the plant in its habitats, preventing its spread in the environment (mowing), arranging neglected areas (embankments, routes along roads, abandoned households), other potential habitats, etc. Also, in addition to field protection measures, it is necessary to implement other protection measures (legal, scientific-educational, amelioration, etc.), which relate to the way of suppressing the spread of non-native weed plants.

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MELISOPALINOLOGICAL ANALYSIS OF HONEY SAMPLES FROM BOSNIA AND HERZEGOVINA

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Abstract

Melisopalinological research of honey samples from different localities from the territory of Bosnia and Herzegovina identified 21 species of pollen grains. Honey samples, five of them, can be divided into three groups: rapeseed honey, acacia honey and chestnut honey.
Three families dominate, while the rest are present in a smaller percentage. Dominant pollen belongs to the families *Brassicaceae* with the highest representation, *Fagaceae* with a lower percentage and the family *Fabaceae*. In samples number 1 and 2, rapeseed pollen (*Brassica napus* L.) predominates and in the first sample it is 68%, and in the second sample rapeseed pollen accounts for 78%. Sample number 3 from Derventa has the highest representation of acacia pollen grains (*Robinia pseudoacacia* L.) with 44%. Samples numbers 4 and 5 according to pollen analysis show the dominance of chestnut pollen grains (*Castanea sativa* Mill.) in values of 86% for sample number 4 and 90% for sample number 5. Plant species of rapeseed (*Brassica napus* L.), acacia (*Robinia pseudoacacia* L.) and chestnut (*Castanea sativa* Mill.) proved to be the most important, despite a very short flowering period of only one month.

Keywords: *Honey, Melisopalinological analysis, Bosnia and Herzegovina.*

Introduction

Palynology is a branch of botany that deals with the analysis of pollen and plant spores. Melisopalinology is a branch of palynology that studies pollen and other microscopic elements found in honey.
Pollen or melisopalinological analysis is a widely used method of determining the botanical origin of honey, and consists of counting pollen grains in honey sediment. It is used as an indicator of the botanical and geographical origin of honey and to determine the quality of honey (Louveaux et al., 1978). Pollen analysis is also part of the legislation ("Official Gazette of BiH" No. 37/09; Regulation on honey and other bee products).
The chemical composition of honey is variable due to the wide range of plant sources from which nectar is obtained (Ball, 2007). By pollen analysis of honey, it is possible to obtain information on the geographical and botanical origin and quality of honey, and the share of pollen grains of individual plant species (Valencia et al., 2000) of honey. Microscopic analysis of honey is a widely applicable method and consists of counting pollen grains in insoluble honey sediment (Louveaux et al. 1978).
According to international standards, honey can be labeled according to flower or plant species, if it comes entirely or mostly from the specified source, and if its organoleptic, physicochemical properties correspond to the origin (Council of the European Union, 2002). Geographical and

botanical properties are important for the quality of honey, and the taste, smell and color of honey changes according to the nectar from the flower (Kaya et al. 2005; Sabo et al., 2011).
The type of honey is monofloral, if honey in insoluble sediment contains at least 45% of pollen grains of the same plant species. Exceptionally, honey is classified in the group of monofloral honeys, if the share of pollen grains in insoluble sediment is at least 45% of pollen of the same plant species, however, there are deviations for certain types of honey. Tame chestnut (*Castanea sativa* Mill.) 85%, alfalfa (*Maedicago sativa* L.) 30%, rosemary (*Rosmarius officinale* L.) 30%, linden (*Tilia spp*.) 25%, sage (*Salvia spp*.) 20%, acacia (*Robinia pseudoacacia* L.) 20%, lavender (*Lavender spp*.) 20%. Polyfloral honey is a mixture of monofloral types of honey from different plants ("Official Gazette of BiH" No. 37/09; Regulation on honey and other bee products). The goal of this work was to determine the botanical origin of honey from different locations in Bosnia and Herzegovina with the help of pollen analysis.

Material and Methods

Honey samples, five of them, were collected from different localities in Bosnia and Herzegovina (Table 1). The analysis of honey was performed in the laboratory for honey, medicinal and aromatic plants of the Faculty of Agriculture and Food Technology, University of Mostar.

Table 1. List of sites from which honey samples were collected

Ordinal number of the sample	**Location**
1.	Orašje
2.	Orašje
3.	Derventa
4.	Konjic
5.	Bihać

Preparations for pollen analysis were made by the method according to Louveaux et al. (1978). The percentage (%) of pollen grains of a particular plant species in honey was obtained by counting and identifying at least 500 pollen grains. Microscopy was performed with a light microscope (Olympus Bx41) at 400x magnification. The dimensions of the pollen grains were measured using the Olympus DP-Soft program. Determination of pollen grains was performed according to the relevant literature (Von der Ohe and Von der Ohe, 2003; Von der Ohe et al., 2004) and based on the dimensions of pollen grains. Pollen grains were counted on two preparations for each honey, and each type of pollen was expressed as a percentage of the total number of pollen grains. Based on the number and frequency of pollen grains in honey samples, they were ranked by groups according to Louveaux et al. (1978), in the dominant (> 45%), and secondary groups (16-45%), then in the group where pollen grains are present in small quantities (3-15%) and pollen grains that are found in traces or rare group (<3%). All analyzes were performed by methods according to national and international legislation in an accredited laboratory.

Results and Discussion

Melisopalinological research of honey samples from four localities from the territory of Bosnia and Herzegovina identified 21 species of pollen grains and classified them into 14 families. Honey samples are dominated by three families, while the others are present in a smaller percentage (Graph 1). Dominant pollen belongs to the families *Brassicaceae* with the highest representation of 35%, *Fagaceae* with a lower percentage of 28% and families *Fabaceae* with 20%.

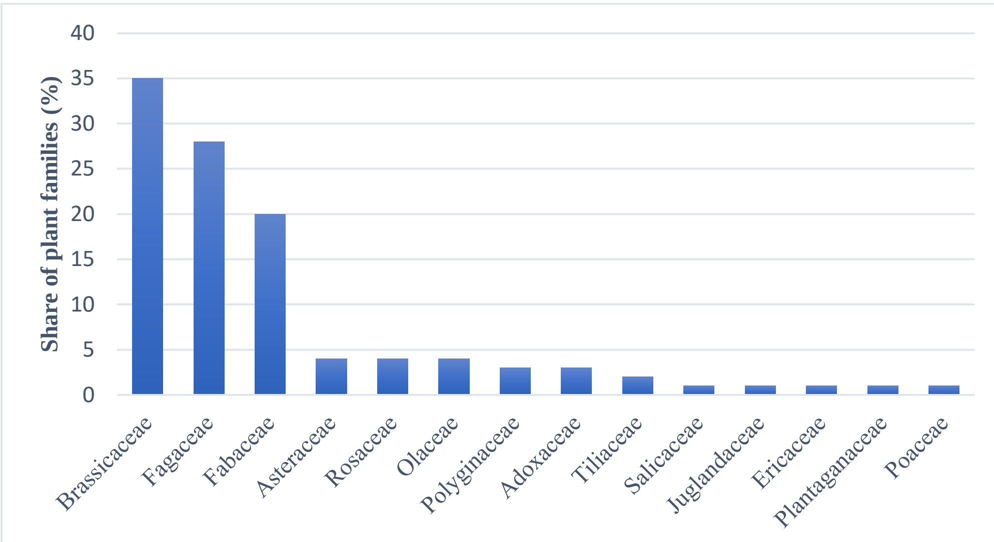

Figure 1. Percentage share of plant families in the studied honey samples

According to the number, the pollen of oilseed rape (*Brassica napus* L.), acacia (*Robinia pseudoacacia* L.) and chestnut (*Castanea sativa* Mill.) dominates, although they have a very short flowering lasting only one month. Sabo et al. (2011) for Varaždin County (Republic of Croatia) state the dominance of pollen grains from the families *Brassicaceae*, *Fagaceae* and *Fabaceae*. They also state that the number of pollen of rapeseed (*Brassica napus* L.) and chestnut (*Castanea sativa* Mill.) dominates. The results of botanical origin of the investigated honey samples are shown in Table 2. Melisopalinological analysis determined that two honeys belong to monofloral oil of rapeseed, one is acacia honey and two are chestnut honey.

Table 2. Pollen analysis in the studied honey samples

Ordinal number of the sample	**Type of honey**
1.	Rapeseed
2.	Rapeseed
3.	Acacia
4.	Chestnut
5.	Chestnut

Monofloral honey of rapeseed (*Brassica napus* L.) was determined in samples number 1 and 2 (Orašje). In sample number 1, 6 species of pollen grains were identified. Of this, rape pollen (*Brassica napus* L.) dominates with 65% and chestnut pollen (*Castanea sativa* Mill.) with 15% in a smaller presence. Pollen grains of acacia (*Robinia pseudoacacia* L.) and fruit trees of the genus *Prunus* with 6%, red clover (*Trifolium pratesnse* L.) with 5%. Dandelion (*Taraxacum officinale* Weber.) with 3% were present in traces (Table 3).

Table 3. Pollen analysis of sample number 1 (Orašje)

Plant species	**Pollen (%)**
Brassica napus L.	65
Castanea sativa Mill.	15
Prunus spp.	6
Robinia pseudoacacia L.	6
Trifolium pratense L.	5
Taraxacum officinale Weber	3
Total	100

Pollen analysis of sample number 2 from Orašje showed a higher percentage of pollen grains of oilseed rape (*Brassica napus* L.) in the amount of 78% (Table 4). In a smaller presence, pollen grains of chestnut (*Castanea sativa* Mill.) With 7%, sunflower (*Helianthus anus* L.) with 6%, acacia (*Robinia pseudoacacia* L.) with 5%, red clover (*Trifolium pratense* L.) with 3% and dandelion (*Taraxacum officinale* Weber.) with 1%.

Table 4. Pollen analysis of sample number 2 (Orašje)

Plant species	**Pollen (%)**
Brassica napus L.	78
Castanea sativa Mill.	7
Helianthus anus L.	6
Robinia pseudoacacia L.	5
Trifolium pratense L.	3
Taraxacum officinale Weber.	1
Total	100

In sample number 3 from Derventa, pollen of 6 plant species was found. The most common is acacia pollen (*Robinia pseudoacacia* L.) with 44%. In addition to acacia, the secondary pollen is from amorphous (*Amorfa fruticosa* L.) with 29%. Minor pollen includes linden (*Tilia cordata* L.) with 8 % and fruit trees of the genus *Prunus* with 11%. Species of the genus *Aster* and pollen grains of olive (*Olea europaea* L.) are sporadically represented.

Table 5. Pollen analysis of sample number 3 (Derventa)

Plant species	**Pollen (%)**
Robinia pseudoacacia L.	44
Amorpha fruticosa L.	29
Prunus spp.	11
Tilia cordata L.	8
Aster spp.	4
Olea europaea L.	4
Total	100

Pollen analysis of sample number 4 from Konjic determined pollen grains of chestnut (*Castanea sativa* Mill.) With a percentage of 86%. the sample contains pollen grains of acacia (*Robinia pseudoacacia* L.) with 5% and amorphous (*Amorfa fruticosa* L.) with 3%, while pollen grains of elderberry (*Sambucus nigra* L.) have a share of 2% and dandelion pollen grains (*Taraxacum officinale* Weber) 2%. Pollen grains of linden (*Tilia cordata* L.) and walnut (*Juglans regia* L.) with 1% each are present in traces (Table 6).

Table 6. Pollen analysis of sample number 4 (Konjic)

Plant species	**Pollen (%)**
Castanea sativa Mill.	86
Robinia pseudoacacia L.	5
Amorpha fruticosa L.	3
Sambucus nigra L.	2
Taraxacum officinale Weber	2
Juglans regia L.	1
Tilia cordata L.	1
Total	100

Table 7 shows the results of pollen analysis for sample number 5 in which the predominant pollen is chestnut (*Castanea sativa* Mill.) with a share of 90%. In a smaller presence in equal proportions, acacia pollen grains (*Robinia pseudoacacia* L.) and *Salix* species with 3% each are present. Olive pollen grains (*Olea europeae* L.) are present in 2%, while elderberry (*Sambucus nigra* L.) and linden (*Tilia cordata* L.) are found in traces (Table 7).

Table 7. Pollen analysis of sample number 5 (Bihać)

Plant species	**Pollen (%)**
Castanea sativa Mill.	90
Robinia pseudoacacia L.	3
Salix spp.	3
Olea europeae L.	2
Sambucus nigra L.	1
Tilia cordata L.	1
Total	100

Conclusion

Pollen analysis revealed two rapeseed and chestnut honeys and one acacia honey. The species of rapeseed (*Brassica napus* L.), acacia (*Robinia pseudoacacia* L.) and chestnut (*Castanea sativa Mill*.) which proved to be the most dominant in the study, bloom only one month, which shows the importance of flowering of these plant species for bee grazing. Spring and summer are very important periods for beekeepers because then about 60% of honey plants bloom. The mentioned period is considered to be the most honey-bearing for bee pasture. Of the dominant pollen grains in the first sample, rapeseed predominates with 65% and in the second sample with 78%. In the third sample, acacia pollen grains were represented by 44%, and samples number 4 and 5 have a high proportion of chestnut pollen grains in proportions of 86% and 90%. Pollen grains from the families *Asteraceae, Rosaceae, Oleaceae, Polygonaceae, Ericaceae, Tiliaceae, Salicaceae, Poaceae* have a significantly lower percentage in the tested samples. Based on pollen analysis, number and frequency of pollen grains and according to the Regulation on honey and other bee products ("Official Gazette of BiH" No. 37/09), the investigated honey samples can be determined as monofloral honeys.

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GREEN ALTERNATIVES IN ENVIRONMENTAL PROTECTION- PLANTS AS HYPERACCUMULATORS OF HEAVY METALS IN PHYTOREMEDIATION

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Abstract

Throughout the substantial increase in the anthropogenic activities, industrialization, urbanization and infrastructure works, intensive and incompetent use of fertilizers, along with rapid human population growth, heavy metals or potentially toxic elements such as cadmium (Cd), arsenic (As), nickel (Ni), chromium (Cr), lead (Pb), and mercury (Hg) have entered the soil and contaminated it. This represents a serious and significant threat to the environment that leads to further damage to the overall ecosystem structure and functioning. Ecotoxicological effects of metals on agricultural, forest, field and aquatic ecosystems, as well as toxicological effects to humans, have been well documented in literature. The most suitable methods for overcoming this problem of environmental pollution are biological remediation technologies, in which green plants are used. The utilization of higher plants to treat pollution is known as phytoremediation. Phytoremediation is the most promising, cost-effective, eco-friendly and sustainable technology, and nowadays it represents commercial and sustainable phyto management. This paper provides an overview of the most important plant species that are hyperaccumulators of heavy metals in the soil, contamination of soil by these pollutants, their distribution and ways of absorbing by plants. The aim of the paper is to emphasize the importance of using green alternative and more economical phytoremediation technology, with special reference to future perspectives and the new advances in remediation technologies. A comparative review of the literature so far has in detail discussed the applicability of higher plants in the method of phytoremediation and led to the conclusion that this technology is more acceptable, modern and economical tool than the other conventional technologies.

Keywords: *Heavy metals, Pollution, Higher plants, Phytoremediation.*

Introduction

The enormous and substantial increase in the anthropogenic activities, industrialization, urbanization and infrastructure works, intensive and incompetent use of fertilizers, along with rapid human population growth, represent a serious and significant threat to the environment that leads to further damage to the overall ecosystem structure and its functioning. Through these activities heavy metals or potentially toxic elements such as cadmium (Cd), arsenic (As), nickel (Ni), chromium (Cr), lead (Pb), and mercury (Hg) have entered the soil and contaminated it.
Heavy metals are natural group of metals and metaloids, with none physiological and metabolical function, that are found at various levels in the bedrock, natural water, soil and sediment (Majeti, 2004). Thus, for example, Ni, Cr and Co exist abundantly in serpentine soils, whereas Zn, Pb and Cd are present in calamine soils. Heavy metals are persistent and cannot be

destroyed in the environment. If metals are found in the environment, they are exposed to chemical and microbial changes with metal solubility ultimately approaching thermodynamic equilibrium with native soil minerals and organic matter. The soil and sediment colloids are negatively charged (Mengel and Kirkby, 1982), and positive metal ions are attracted to these charges. The rate and extent of solubilization depend on the physical and chemical properties of the deposited material, soil processes, and soil properties (Cataldo and Windung, 1987). Generally speaking, acidic and reducing conditions were most favorable for metal solubilization, and the effect of pH was more significant than that of redox potential (Chuan *et al*., 1996). The most discussed factors influencing the bioavailability of metals in soil are pH, clay content and organic matter content (Majeti, 2004). Heavy metals from the soil accumulated in plants enter the food chains and their excessive amount can be toxic for humans and animals. Toxicological effects of metals to humans, particularly those of Cd, As, Hg, and Pb, have been well documented, as well as their ecotoxicological effects on agricultural, forest, field and water ecosystems (Adriano, 2001). Researchers globally are working to find an inexpensive new technique of cleaning the rhizosphere. Traditional approaches of remediation are not cost effective and suitable, but also they sometimes cause additional environmental health hazards rather than offering solution (Adeoye Azeez, 2022). The problem of environmental pollution could be overcome in many ways, but the most suitable methods are biological remediation technologies, in which green plants are used. The utilization of plants to treat pollution is known as phytoremediation. Phytoremediation is a green, and economical bioremediation approach by which the harmful heavy metals in the contaminated ecosystem can be detoxified and accumulated in the plant (Yaashikaa *et al*., 2022). Nowadays it is the most promising, cost-effective, eco-friendly and sustainable technology. The objective of this paper is to provide an overview of the most important plant species that are hyperaccumulators of heavy metals in the soil, ways of absorbing these pollutants, emphasizes the importance of using green alternative and more economical phytoremediation technology, with special reference to future perspectives.

Materials and methods

A comparative review of the literature so far has in detail discussed the applicability of higher plants in the method of phytoremediation and led to the conclusion that this technology is a more acceptable, modern and economical tool than the other conventional technologies.

The plant uptake of chemical substances in soil solution is dependent on a number of plant factors. These include: physical processes such as root intrusion, water, ion fluxes and their relationship with the kinetics of metal solubilization in soils; biological parameters, including the way of membrane transport, ion interactions, and metabolic fate of absorbed ions; and the ability of plants to adapt metabolically to changing metal stresses in the environment (Majeti, 2004). Chang et al. (1987) found soil temperature to be one of the major factors accounting for variations in metal accumulation by crops. Metal ions are probably generally taken up into cells by membrane transport proteins designed for acquisition of nutrient metals (Majeti, 2004).

During their transportation through the plant, metals get bound largely on the cell walls, which explains why most of the metal taken up is commonly found in the roots (about 75- 90 %) and smaller amounts are distributed in the shoot. Jarvis et al. (1976) examined the Cd distribution in 23 species and found that the higher up in the plant, the lower the Cd concentration, which decreased in the following order: fibrous roots >storage roots >stems >leaves (Majeti, 2004).

Different species accumulate different concentrations of a specific metal. Hyperaccumulator plants are the metallophytes that have capability to accumulate extreme amount of trace metals usually >10,000 mg kg^{-1} Mn and Zn >100 mg kg^{-1} in their aerial parts from the heavy metal enriched soil. A common strategy to grow on metal-contaminated soils is to accumulate the metals in the roots. The reason for very high accumulation in some genotypes or species is not yet known. High biomass production was given as a cause of high uptake and accumulation (Majeti, 2004). There was, however, no correlation between biomass production rate and metal accumulation when different metal-accumulating *Salix* clones were investigated (Greger and Landberg, 2001).

Hyperaccumulators have a low biomass production since they have to use their energy in the mechanisms they have evolved to cope with the high metal concentrations in the tissues. The mechanisms behind the high accumulation as well as the tolerance are today poorly understood, and more information can be found in the review written by Baker et al. (Baker et al., 1998).

Nearly 500 plant species of 45 plant families are reported to have hyperaccumulation characteristics, and most of them are Ni hyperaccumulator (Van der Ent *et al*., 2013). Hyperaccumulators have been found in areas of New Caledonia, Australia, southern Europe/Mediterranean, southeast Asia, Cuba, the Dominican Republic, California in the USA, Zimbabwe, Transvaal in South Africa, Goias in Brazil, Hokkaido in Japan, and Newfoundland in Canada (Baker et al., 1998). In the Balkans, known types of hyperaccumulators are *Halacsya sendtneri, Helianthus marantae, Asplenium cuneifolium, Silene paradoxa, Echium ruburum, Alussum murale,* etc. (Baker and Brooks, 1989). *Viola arsenic* is a species that is an indicator of soils that are rich in arsenic. Probably the most known and important heavy metal hyperaccumulator is the species *Thlaspi caerulescens*. While most plants show symptoms of toxicity at a zinc concentration of about 100 ppm, this plant accumulates Zn at a concentration of 26,000 ppm without any damage. Many plants, including *T.caerulescens*, show the ability to colonize habitats (soils) enriched with Pb, Zn and Cd, the so-called Calm soils (Lagerwerff, 1972; Lasat, 2000). This species together with the species *Brassica juncea* represent a model for research into the physiology and biochemistry of heavy metal uptake. *Urtica dioica* and *Urtica urens* indicate an increased concentration of nitrate and nitrite in the soil and usually inhabit nitrified habitats in urban areas (Sekulić *et al*., 2018).

Table 1. Overview of plant hyperaccumulators, with shown lowest metal concentration in leaves, numbers of taxa and families which are hyperaccumulators, and examples of hyperaccumulators (Baker *et al*., 1998; Reeves and Baker, 1998)

Metal	**Concentration in leaves mg(g DW)$^{-1}$**	**Number of**		**Example of species**
		Taxa	**Families**	
Cd	>0.1	1	1	*Thlaspi caerulescens*
Pb	>1	14	6	*Minuartia verna*
Co	>1	28	11	*Aeollanthus biformifolius*
Cu	>1	37	15	*Aeollanthus biformifolius*
Ni	>1	317	37	*Berkheya coddi*
Mn	>10	9	5	*Macadamia neurophylla*
Zn	>10	11	5	*Thlaspi caerulescens*

*Source: (Majeti, 2004)

An overview of the most important types of hyperaccumulators is given in Table 1. Copper (Cu) is tolerated in large quantities by *Minuartia verna*, ecotypes of *Silena vulgaris*, *Gupsophila patrinii* in Central Asia, *Polucarpaea spirostulis* in Australia, some species of *Gladiolus* in Africa, etc. (Stoltz and Greger, 2002a). Zn is resistant to ecotypes of *Minuartia verna*, then *Silene vulgaris, Armeria maritima, Thlaspi alpestre subsp. calaminare*, *Viola calaminaria*. Cd accumulates *Minuartia verna* and *Thlaspi alpestre subsp. calaminare* (Arduini *et al.*, 1996; Haghiri, 1974).
The use of plants in remediation of soil contaminated with organic materials is appealing for a variety of reasons: (1) plants provide a remediation strategy that utilizes solar energy; (2) vegetation is aesthetically appealing; (3) plant samples can be harvested and tested to show the level of remediation; (4) rhizosphere microbial communities are able to biodegrade a wide variety of organic contaminants; and (5) many plants have mechanisms for transporting oxygen to the rhizosphere (Shimp, 1993). Phytoremediation includes a range of plant-based remediation techniques such as rhizofiltration, phytostabilization, phytoextraction, phytovolatilization, and phytodegradation focused on a different degree of remediation mechanisms (Yadav *et al*., 2022). The efficiency of phytoremediation relies on various parameters such as soil properties (pH and type of the soil), concentration of organic matter in soil, the type of heavy metal, nature of rhizosphere, characteristics of microorganisms in rhizosphere (Yaashikaa *et al*., 2022).

Results and Discussion

Phytoremediation is a relatively new removal technique of pollutants in soil, with many advantages: it is cost-effective, green, with only usage of solar energy. In addition to the distinct advantages of phytoremediation in relation to classical remediation procedures, the disadvantages of phytoremediation should be pointed out: the selection of the species to be used in phytoremediation is a critical step - knowledge of the species, their overall ecology, physiology, anatomy are vital; concentration of pollutants in nature is a limiting factor - if that concentration exceeds the capacity for a given substance, it will have a suppressive effect on the species, maybe even lethal. The availability of pollutants to the plant and its rhizosphere must not be too deep, nor too tightly tied to the particles of contaminated soil (as is the case when there is a large share of clay fraction).
However, the information regarding which plant species is ideal and appropriate for a specific type of waste and its mechanism of phytoremediation is not fully synthesized. Therefore, it is mandatory to find out suitable plant species for phytoremediation purposes based on needs and mechanisms whenever is required in a changing environment.
Among several phytoremediation strategies phytoextraction, which is also known as biomining or phytomining (Pivetz, 2001), is considered to be a promising green alternative in order to remediate heavy metal contamination of soil through plants. Plant used for phytoextraction must have attributes such as rapid growth and enormous biomass production, efficient hyperaccumulation, and translocation property along with good assimilation rate, better root system to explore large soil volume, high tolerance against metal contaminants, and better adaptability toward environment as well as easy agricultural management (Sheoran *et al*., 2016). In recent years, amalgamation involving the molecular mechanism of phytoextraction with novel bioengineering technique enhances its phytoremediation efficiency (Prasad *et al*., 2022).

Conclusion

A long time ago, in 1968, Allawy suggested the role of trace elements in human and animal health that is expanded and that the agronomists may be called upon to develop more sophisticated regulation of the concentration of an increasing number of trace elements in plants.
Phytoremediation is a suitable green approach, with potentially widening application. Nowadays, it represents commercial and sustainable phytomanagement. Environmental pollutants such as toxic heavy metals and organic pollutants are major targets of phytoremediation which are concentrated and metabolized to fewer toxic substances by plants. Plants probably can play a promising role in the development of a database for future research aspects in remediation of contaminated rhizosphere.
The most recent studies show that the new breakthroughs in remediation technologies are based on the usage of genetically modified micro-organisms, but much further research should be done.

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SUBSTITUTION ACIDITY, PHOSPHORUS AND POTASSIUM CONTENT IN THE LAND OF THE BRANIČEVO DISTRICT IN THE REPUBLIC OF SERBIA

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Abstract

During 2018, on the territory of the Braničevo district in the Republic of Serbia, the agrochemical analyzes were used to examine the substitution acidity, the content of easily accessible phosphorus and potassium for 4322 soils under fields, 152 meadows, 208 orchards and 95 vineyards. Soils under fields, meadows, orchards and vineyards showed significant variations at depths from 0 to 30 cm in terms of substitution acidity and content of easily accessible phosphorus and potassium depending on the location, purpose and application of mineral fertilizers. Substitution acidity for all surveyed soils varied in the range from 3.45 to 8.03, with the lowest average value expressed by meadows (5.14), and the highest by soils under vineyards (6.11). Acidic and strongly acidic reaction (pH_{KCl} < 5.5) had 68.2% of examined meadows, 58.8% soils under fileds, 56.3% orachards and 55.8% vineyards. Most of the examined soils showed poor phosphorus supply. The average values of easily accessible phosphoidal content of field, meadow and soil under vineyards were less than 10 mg 100 g^{-1} per soil. Among group of soils with poor phosphorus supply (P_2O_5 < 10 mg 100 g^{-1}) was 83.2% vineyards, 75.5% soils under fileds, 74.2% meadows and 62.5% orchards. The average values of easily accessible potassium from examined soils under fields, meadows, orchards and vineyards were above 20 mg 100 g^{-1} per soil indicating a good supply of this element. Value of K_2O above 20 mg 100 g^{-1} per soil was showen in 68.8% orchards, 62.5% soils under fields, 61.1% vineyards and 60.3% meadows.

Keywords: *Substitution acidity, Phosphorus, Potassium, Soils, Braničevo district.*

Introduction

Braničevo District is located in north-eastern Serbia with a total area of 3,865 km^2. It includes eight municipalities: Požerevac, Veliko Gradište, Golubac, Malo Crniće, Žabari, Petrovac, Kučevo and Žagubica. The total available area of agricultural land in the Braničevo district is 210,234 ha, of which slightly more than half (133,748 ha) is used for agricultural production (Mitić *et al*., 2021). Arable land and gardens occupy 105,186 ha, followed by meadows and pastures on 25,025 ha, orchards with 4,020 ha and vineyards with 653 ha (Republic Bureau of Statistics, 2013; Mitić *et al*., 2021). The district consists of three relief units: plain terrains in the Danube and Velika Morava valleys (Požarevac Pomoravlje, Podunavlje and Stig), hilly areas in the middle course of the rivers Mlava and Pek, and mountainous areas of North Kučaj, Homoljski mountains and Beljanica. The presence of different geomorphological units, climatic conditions and the presence of surface and groundwater had a visible impact on the formation and development of land in the Braničevo district. The complexity of the influence of these factors is reflected in the formation of a large number of soil types and subtypes: Chernozems (leached, marshy, degraded), Vertisols, Vertisol allluvial, Vertisol in podzolization, Eutric Cambisols, Eutric Cambisols in podzolazition, riparian black soil (Humis Gleysols), Colluviums (Regosols), Arenosols, Litosols, ponds and swamps (Stepanović, 2011; Mrvić *et al*., 2016).

Soil is a basic resource of agricultural production. Soil fertility is a dynamic natural property which can change under the influence of natural and human induced factors. In agriculture, depletion can be due to excessively intense cultivation and inadequate soil management (Denis *et al*., 2017). Landscaping of agricultural land can be achieved only by applying appropriate measures that include agro-technical and reclamation measures (Pivić et al., 2020). One of the measures aimed at protecting and preserving the chemical and biological properties of agricultural soil and ensuring the proper use of mineral and organic fertilizers is the systematic control of soil fertility. Soil analysis is usually carried out to check in for soil quality, nutrient content, changes in various parameters of soil and hence gives all the information that is necessary to comprehend the nutrient inputs required to make the soil better (Bhatia *et al*., 2021). The basic parameters of soil fertility include active and substitution acidity, content of carbonates, humus, easily accessible forms of phosphorus and potassium and the content of total nitrogen. Soil reaction is one of the most important properties, which affects numerous chemical characteristics, microbiological processes, as well as certain physical properties of soil (Soil Institute Belgrade, 2014). An easily accessible form of phosphorus is often in short supply. The level of this nutrient in the soil depends on the amount and composition of its reserves and conditions for inactivation and mobilization of elements, while the content of easily accessible potassium depends on the presence of clay particles and fertilization intensity (Soil Institute Belgrade, 2014).

The aim of this paper is to consider the state of soil acidity and the content of easily accessible forms of phosphorus and potassium on the basis of agrochemical analyzes, as the most common parameters that induce the need to implement land reclamation measures.

Material and Methods

The study area covers the territory of the Braničevo District from 44° 82´ to 44° 09´ north latitude, and from 21° 06´to 22° 00´ east longitude, total area of 3,865 km^2. The implementation of the systemic fertility control of agricultural land, the Agricultural Advisory and Expert Service Požarevac performed in the period from 4.09.2017. until 12.07.2018. years. During this period, a total of 4,777 samples of individual agricultural producers were examined, of which 4322 soils under fields, 152 meadows, 208 orchards and 95 vineyards.

Data on climatic parameters of the study site were collected at the meteorological station Veliko Gradište(44° 45′ 14.4″ N, 21° 30′ 29.4″ E, altitude 68 m) (source www.hidmet.gov.rs). The examined locality belongs to the area of temperate continental climate with clearly expressed seasons. The average annual air temperature based on statistical processing of available data for the period 1991 – 2020. is 11.9° C, the average minimum is 6.7 °C, and the average maximum is 17.6° C. The average frequency of frost days is 82 days. Tropical days occur from April to October and their average number for this period is 45.6. The warmest month is July (22.6 °C), and the coldest is January (0.5 °C). The average annual rainfall is 669.8 mm. The highest rainfall falls in May (73.2 mm), June (76.0 mm) and July (76.3 mm), and the lowest average in February (41.5 mm) and March (41.5 mm). During the colder part of the year, the east and southeast wind

- Košava - dominates. For the summer period, it is important to point out the appearance of southern winds when there is a great drying of the soil, especially if the average temperatures are high (Institute for Soil Belgrade, 2014).

Agrochemical analyzes soils were performed according to the following methods:

- Substitution acidity - pH in 1 M KCl - potentiometrically, method SRPS ISO 10390: 2007;
- Easily accessible phosphorus and potassium - AL method according to Egner-Riehm, ZILUH-7 and ZILUH-6.

Statistical data processing (average value, standard deviation and coefficient of variance) was performed using the 2016 Excel program.

Results and Discussion

The soil has a buffering capacity, which makes it suitable for resisting pH variations. This ability is related to the presence of colloids. The available amount of essential nutrients is influenced by its pH, with the ideal range being generally between 6.5 and 7.3 (Chaouqi *et al*., 2018). Low pH value of the soil, ranging from 2-3, is an indication of the existence of free mineral acid, most commonly sulphuric acid (H_2SO_4) (Bhatia *et al*., 2021). The soils that have higher pH values to the tunes of 8 and above, it has been observed that hydrolysis of basic cations is the main factor behind marinating a stable pH with dilution (Thomas, 1996).

Table 1. Average values, absolute minimum, absolute maximum, deviation standard and coefficient of variance for surveyed field, meadow, fruit and vineyard lands

Source of variation	N	$\bar{X}$	MIN	MAX	σ	CV (%)
			pH_{KCl}			
Fields	4322	5.35	3.45	8.03	1.08	20.14
Meadows	152	5.14	3.58	7.10	0.94	18.24
Orchards	208	5.44	3.56	7.45	0.98	18.06
Vineyards	95	6.11	3.96	7.85	1.21	19.75
			P_2O_5 (mg 100 g^{-1} per soil)			
Fields	4322	9.56	1.01	110.60	12.88	134.65
Meadows	152	8.77	1.01	82.13	11.98	136.61
Orchards	208	13.76	1.06	64.35	16.03	116.45
Vineyards	95	8.91	1.27	66.41	13.28	149.02
			K_2O (mg 100 g^{-1} per soil)			
Fields	4322	22.06	4.75	>40.00	9.90	44.90
Meadows	152	22.45	8.25	39.75	10.44	46.48
Orchards	208	23.80	8.75	99.25	13.54	56.89
Vineyards	95	22.40	7.67	39.50	8.82	39.38

Substitutional acidity is bound to adsorbed ions H^+ and Al^{3+}, which can be easily exchanged by K^+ ions from KCl solution. On average, the highest substitution acidity was shown soils by meadows (5.14), followed by fields (5.35), orchards (5.44), and the lowest vineyard soils (6.11) (Table 1). The variation of the substitution acidity of the examined soils ranged from 3.45 to

8.03, with the highest coefficient of variance recorded by fields soils (20.14%) (Table 2). More than half of the meadows soils (68.21%) was in the group of acidic (pH 4.5-5.5) and strongly acidic soils (pH <4.5) (Table 2). These acidity groups include 58.84% of fields soils, 56.25% of orchards and 35.79% of vineyards. The low pH value of the soil in the examined area is a consequence of inadequate mineral fertilization, reduced intake of organic matter, as well as poor crop rotation system. Where the previous cropping system has caused a depletion of soil organic matter, the soils are more likely to be acidic with limited capacity to hold N, P, K, Ca and some essential micronutrients (Mugo *et al*., 2020). Long term fertilization regime and manure use affects nutrient concentration and microbial life in the soil (Cui *et al*., 2020). Microbial activity that releases N from organic matter and certain fertilizers are particularly affected by low soil pH since microbial activities occur best at soil pH range of 5.5 to 7.0 (Lamb). Low soil pH affects the uptake of most macro and secondary plant nutrients by either its effects on microbial activity, and dissolution of Al / Fe ions (Mugo *et al*., 2020). For that reason, on acid soils, ameliorative measures of pH correction (calcification) are necessary.

Phosphorus has been called the “Master key to agriculture” because low crop production is attributed mainly due to lack of phosphorus than the deficiency of other elements, except nitrogen (Singh *et al*., 2016). The variation of readily available phosphorus in the test soils was in a wide range, from 1.01 to 110.60 mg 100 g^{-1} per soil. The average values of fields, meadows and vineyards soils were below 10.00 mg 100 g^{-1} per soil. The group of poorly provided soils with this macronutrient (<10.00 mg 100 g^{-1} per soil) consisted of 83.16% of vineyards, 75.47% of fields, 74.17% of meadows and 62.50% of orchards. This observation could be ascribed to the predominant clay soils (Gachene *et al*., 2003) as well as to the low soil pH (Mugo *et al*., 2020). Clay soils and acidic soils have high Al and Fe contents which besides fixing the available soil P are associated with increased soil acidity (Westerman *et a*l., 1990; Gachene *et al*., 2003). At acidic pH values, phosphate ions react with Al and Fe to form less soluble compounds (Amara *et al*., 2017). For these reasons, in accordance with agrochemical analyzes, there is a need to implement ameliorative measures to improve the content of readily available phosphorus (phosphatization measures).

Table 2. Grouping of soils according to the values of substitution acidity and content with easily accessible phosphorus and potassium

Agrochemical parameters	Group	Felds		Meadows		Orchards		Vineyards	
		n	%	n	%	n	%	n	%
pH_{KCl}	>7.2	103	2,38%	0	0.00%	8	3.85%	19	20.00%
	6.5-7.2	819	18.95%	16	10.60%	28	13.46%	34	35.79%
	5.5-6.5	857	19.83%	32	21.19%	55	26.44%	8	8.42%
	4.5-5.5	1350	31.24%	57	37.75%	78	37.50%	22	23.16%
	<4.5	1193	27.60%	46	30.46%	39	18.75%	12	12.63%
P_2O_5	>20	553	12.80%	20	13.25%	52	25.00%	12	12.63%
	10-20	507	11.73%	19	12.58%	26	12.50%	4	4.21%
	<10	3262	75.47%	112	74.17%	130	62.50%	79	83.16%
K_2O	>20	2702	62.52%	91	60.26%	143	68.75%	58	61.05%
	10-20	1525	35.28%	56	37.09%	60	28.85%	35	36.84%
	<10	95	2.20%	4	2.65%	5	2.40%	2	2.11%
	N	4322		151		208		95	

Potassium exists in K^+ form and its function appears to be catalytic in nature (Singh *et al*., 2016). Potassium is important for plant because it participates in the activation of large number of enzymes which are involved in the physiological processes of plants. It controls the water economy and provides the resistance against a number of pests, diseases and environmental stresses (Denis *et al*., 2017). The content of easily accessible potassium varied from 4.75 to over 40 mg 100 g^{-1} per soil. Over 60% of the tested samples in arable, meadow, fruit and vineyard lands were well provided with this nutrient. Low content was shown by slightly more than 2% of the examined samples (from 2.11% of vineyard to 2.65% of meadow land). High content of easily available potassium may be a consequence of its high content in the parent substrate, primarily, the predominance of mica and potassium-rich feldspar minerals. In addition, Kaolinite type of clay mineralogy may be the cause for their medium and low rating for available potassium (Pulakeshi *et al*., 2012). Further to this, low soil pH indirectly affects K uptake. At low soil pH, Al becomes soluble thus dominating CEC hence lowering the soil capacity to hold K (Havlin, 2013).

Conclusion

Soils under fields, meadows, orchards and vineyards showed significant variations at depths from 0 to 30 cm in terms of substitution acidity and content of easily accessible phosphorus and potassium depending on the location, purpose and application of mineral fertilizers. Substitution acidity for all surveyed soils varied in the range from 3.45 to 8.03, with the lowest average value expressed by meadows (5.14), and the highest by soils under vineyards (6.11). Acidic and strongly acidic reaction (pH_{KCl} < 5.5) had 68.2% of examined meadows, 58.8% soils under fileds, 56.3% orachards and 55.8% vineyards. Most of the examined soils showed poor phosphorus supply. The average values of easily accessible phosphoidal content of field, meadow and soil under vineyards were less than 10 mg 100 g^{-1} per soil. Among group of soils with poor phosphorus supply (P_2O_5 < 10 mg 100 g^{-1}) was 83.2% vineyards, 75.5% soils under fileds, 74.2% meadows and 62.5% orchards. The average values of easily accessible potassium from examined soils under fields, meadows, orchards and vineyards were above 20 mg 100 g^{-1} per soil indicating a good supply of this element. Value of K_2O above 20 mg 100 g^{-1} per soil was showen in 68.8% orchards, 62.5% soils under fields, 61.1% vineyards and 60.3% meadows.
In accordance with agrochemical analyzes, there is a need to implement reclamation measures to improve the content of readily available phosphorus (phosphatization) and pH correction measures (calcification).

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A NOVEL APPROACH TO THE ENCAPSULATION OF THYME ESSENTIAL OIL

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Abstract

Thyme essential oil appears to be an attractive natural alternative with high bioactive compounds for food preservation applications. The electrospraying technique can encapsulate bioactive food compounds to produce engineered solid particles to improve the stability and availability of bioactive compounds. This study assessed thyme essential oil (TEO) encapsulated maltodextrin (MD) particles produced by the electrospraying technique. Engineered MD-TEO particle morphology and antimicrobial activity and antioxidant potential were evaluated. The morphology of obtained particles was evaluated by scanning electron microscopy (SEM) and optical microscopy. Also, the structure of engineered particles was analyzed by Fourier-transform infrared spectroscopy (FTIR). The antioxidant activity was evaluated by the cupric ion-reducing antioxidant capacity (CUPRAC) method. The antimicrobial activity was assessed *in-vitro* against five reference bacterial strains. Engineered MD-TEO electrosprayed particles exhibited high antioxidant activity and antimicrobial activity against *Bacillus cereus*, *Staphyloccocus aureus*, *Listeria innocua*, *Escherichia coli*, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium. Engineered particles were spherical and homogeneous. The results of this study showed that electrospraying is a promising particle engineering technique for obtaining MD-TEO food-grade particles. In addition, thyme essential oil encapsulated maltodextrin particles have the potential to be utilized as a natural preservative for improving food safety and quality.

Keywords: *Electrospraying, Thyme Essential Oil, Maltodextrin, Food Safety.*

Introduction

Encapsulation is one of the most attractive methods to protect sensitive and unstable bioactive compounds from harsh processes and environmental conditions (Coelho et al., 2021). The nature of essential oils (EOs) contains a complex mixture of chemical components that can break down in the presence of oxygen, light, and heat (Dima et al., 2016). EOs cannot be directly used in food systems and nutraceutical formulations due to poor water solubility, volatility, bioavailability, and unpleasant odor (Fonseca et al., 2020; Granata et al., 2021). Therefore, encapsulation is an efficient strategy to overcome these challenges for practical applications (Majeed et al., 2015). A wide range of techniques has been carried out for the production of micro-nano particles for essential oil encapsulation, including complex coacervation (Bastos et al., 2020), spray drying (Alvarenga Botrel et al., 2012), inclusion complex formation (Rakmaia et al., 2017), and emulsion systems (Volić et al., 2018). Apart from these conventional encapsulation techniques, electrohydrodynamic processes, including electrospinning and electrospraying, have been recently used to encapsulate bioactive molecules (Asadi et al., 2021; González-Cruz et al., 2023). The main advantage of the electrospraying system is that it does not require heat, pressure, and harsh chemicals to encapsulate bioactive compounds (Niu et al., 2020). Furthermore, this technique is most suitable for heat-liable biological (Librán et al., 2017) and bioactive molecules for enhancing functional advantages such as stability of bioactive compounds and efficient encapsulation (Bhushani & Anandharamakrishnan, 2014). In addition, using suitable encapsulation techniques, and encapsulating materials play a vital role in the formulation of micro-nano particle properties (Alehosseini et al., 2018). Gelatin and sodium alginate (Bastos et al., 2020), maltodextrin, arabic gum, modified starch (Alvarenga Botrel et al., 2012), whey protein concentrate (Turasan et al., 2015), chitosan, alginate, inulin (Dima et al., 2016), and cyclodextrins (Köse et al., 2021; Siva et al., 2020) has been commonly used for essential oils encapsulation. Besides, chitosan and arabic gum (Hasani & Hasani, 2018), potato starch (Fonseca et al., 2020), β-cyclodextrin (Rezaei et al., 2021), whey protein concentrate, and alginate (Volić et al., 2022), have been recently evaluated as encapsulating agents for thyme essential oil. Among all, maltodextrin (MD) is available, cost-effective, and food-grade biopolymer as an encapsulating agent. Moreover, MD possesses favorable properties, including high solubility, low viscosity at high concentrations, film-forming capacity, and emulsifying properties which can be used as a wall material for bioactive agents encapsulation (Lekshmi et al., 2021), in electrospraying technique (Librán et al., 2017). Natural alternatives to synthetic substances, such as thyme essential oil (TEO), are ideal for food safety, alternative medicine, and health (Fonseca et al., 2020). TEO contains phenolic compounds such as thymol, carvacrol, *p*-cymene, and eugenol, which can lead to strong biological activity (Asllani & Toska, 2003; Jang et al., 2017). The present study was designed to investigate whether electrospraying is a promising particle engineering technique for obtaining maltodextrin (MD)-thyme essential oil (TEO) food-grade particles for practical applications of food and nutraceutical formulations.

Materials and methods

Maltodextrin solution was prepared using the slightly modified methodology proposed by Pérez-masiá et al. (2014). Maltodextrin solution was prepared at 100 wt.% distilled water and stirred at 400 rpm at room temperature for 6 hours to completely solubilized. Lecithin (LEC) at 0.6 wt.% concentration was added to all solutions with respect to the polymer weight. Different concentrations of thyme essential oil (10, 5, and 2,5 % (w/v)) were added to the polymer mixture. Electrospraying parameters were set to a; flow rate of 0.3 mL/h, an applied voltage of 20 kV, and a tip-to-collector distance of 10 cm with an electrospinning apparatus (OptoSense, Tekno-TIP, Turkey). Particle morphology was examined using scanning electron microscopy (SEM) (Fei Quanta 250 Feg, USA) and optical microscopy (Camera Olympus SC180, BX43F, Tokyo, Japan). ImageJ was used for particle size distribution analysis. Attenuated total reflectance infrared spectroscopy (ATR-FTIR) (PerkinElmer® Spectrum™ 100) was performed for spectra of the electrosprayed particles. All spectra were collected in a range of 550–4000 cm^{-1} wavenumber at 4 cm^{-1} resolution by averaging 64 scans. The antimicrobial activity of MD particles with TEO was performed according to the method described by Karaaslan et al. (2021). Briefly, an agar well diffusion assay was carried out to the obtained MD and MD-TEO particles antimicrobial activity against bacteria, including *Bacillus cereus NRRL-B-3711*, *Listeria innocua ATCC 33090*, *Escherichia coli ATCC 25922*, and *Salmonella enterica subsp enterica* serovar Typhimurium *ATCC 14028*. The antimicrobial activity of particles and penicillin-streptomycin solution (1%) was recorded as the zone of inhibition (mm). The antioxidant capacity of obtained MD and MD-TEO particles was determined based on the CUPRAC assay according to the method of Apak et al. (2004). One-way analysis of variance (ANOVA) and Tukey tests were performed with Minitab 17 to evaluate the significant differences between sample means. The data were recorded as mean ± standard deviation.

Results and discussion

In this study, TEO was successfully encapsulated in food-grade polymer maltodextrin in the presence of lecithin, and the obtained MD-TEO particles were characterized. Lecithin, as a surfactant, has lipophilic hydrophilic ends. The presence of lecithin in the MD-TEO electrospray polymer solution improves the spraying properties of the polymer and helps to stabilize the particles (Talón et al., 2019).

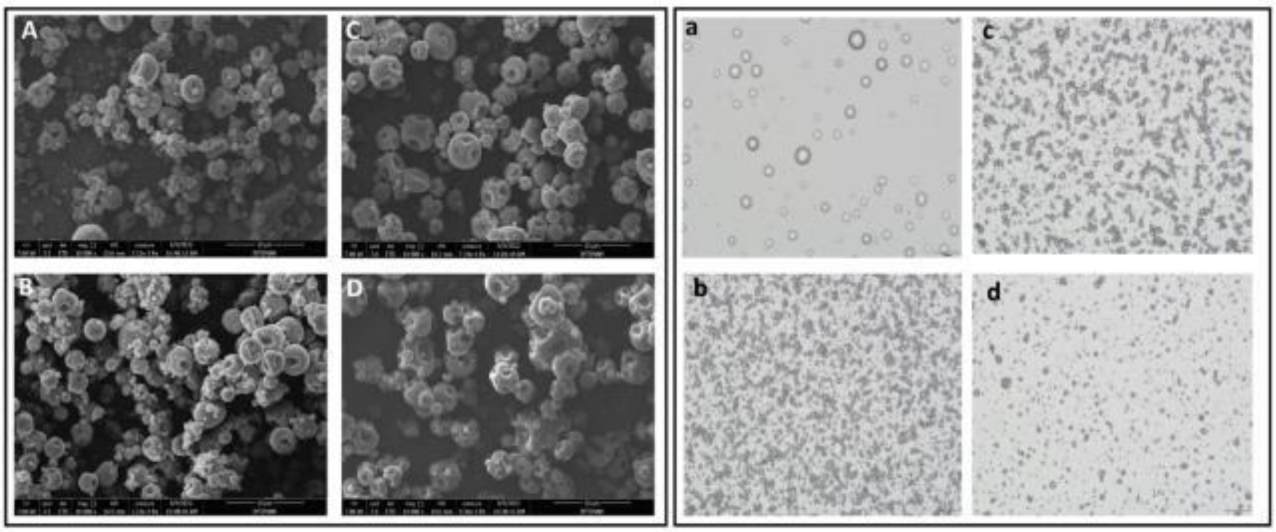

Figure 1. SEM ((A) 10%, (B) 5%, (C) 2.5 % and,(D) MD), and optical microscopy images ((a) 10%, (b) 5%, (c) 2.5 % and,(d) MD) of MD (control) and MD-TEO particles with different thyme essential oil concentrations

The SEM and optical microscopy images of MD control and MD-TEO particles at different concentrations were illustrated in Figure 1. According to the SEM images, the particles prepared with maltodextrin and maltodextrin with thyme essential oil possessed spherical particles, and MD-TEO particles resulted in dense particles compared to MD. The produced maltodextrin (MD, control) particles presented a higher particle size, with 3.63±2.86 µm, the particle size of MD-TEO 10%, 5%, 2.5 %, were 1.97±1.64 µm, 1.86±1.72 µm, and 2.62±1.56 µm, respectively.

Table 1. Antimicrobial activity of MD-TEO particles

	Zone of Inhibition (mm)				
MD-TEO Particles	*B.cereus*	*S.aureus*	*L.innocua*	*E.coli*	*S.Thyphimirium*
MD-TEO (10%)	22.80 ±0.97[a]	18.88 ±0.35[a]	15.97 ±018[a]	18.53 ±0.54[a]	12.90 ±0.19[a]
MD-TEO (5%)	13.61 ±0.07[b]	16.21 ±0.60[b]	11.51 ±0.48[b]	16.70 ±0.12[b]	11.16 ±0.23[b]
MD-TEO (2.5%)	10.65 ±0.03[c]	12.72 ±1.48[c]	n.d	13.83 ±0.09[c]	10.07 ±0.16[c]
MD(Control)	n.d	n.d	n.d	n.d	n.d
Antibiotic (Pen-Strep(1%))	35.13 ±0.75	49.24 ±1.08	33.60 ±1.13	22.58 ±0.85	22.52 ±0.73

All values are means ± SD, n=3 n.d, not detected
a-c: Means in the same column with different letters are significantly different ($p<0.05$)

The results of the antimicrobial efficacy of the MD and MD-TEO particles were presented in Table 1. In this study, engineered MD-TEO particles possessed high antimicrobial activity against all tested bacteria; among all, *B.cereus* was more sensitive to MD-TEO particles containing 10% TEO concentration. As reported in Table 1, antimicrobial activity increased with increasing the TEO concentration in the obtained particles. However, MD without TEO did not show antibacterial activity. Other researchers also reported similar results on the antimicrobial activity of encapsulated TEO particles, TEO in β-cyclodextrin nanosponge (Rezaei et al., 2021), and TEO in chitosan nanoparticles (Barrera-Ruiz et al., 2020), and zein particles (Bilenler et al., 2015).

Table 2. Antioxidant activity of MD-TEO particles

In vitro Antioxidant Activity	
CUPRAC(μgTE/mg particle)	
MD-TEO (10%)	464.77 ±0.44[a]
MD-TEO (5%)	251.62 ±4.97[b]
MD-TEO (2.5%)	163.51 ±5.36[c]
MD(Control)	n.d

All values are means ± SD, n=3 n.d, not detected
a-c: Means in the same column with different letters are significantly different ($p<0.05$)

The antioxidant properties of the obtained MD-TEO particles at different concentrations were assessed for their antioxidant activity by the CUPRAC method (Table 2). The content of phenolic compounds in TEO contributes to its antioxidant activity (Rezaei et al ., 2021). The antioxidant activity increased with increasing the TEO concentration, and therefore, the 10 %(w/v) TEO-containing particles possessed the highest antioxidant activity. MD (control) without TEO did not show antioxidant activity. This observation is consistent with other researchers, that reported the antioxidant activity of TEO in different encapsulation systems such as TEO encapsulated β- and γ-cyclodextrin complexes (Ahmed et al., 2022), zein particles (Bilenler et al., 2015) and TEO incorporated starch nanofibers (Fonseca et al., 2020).

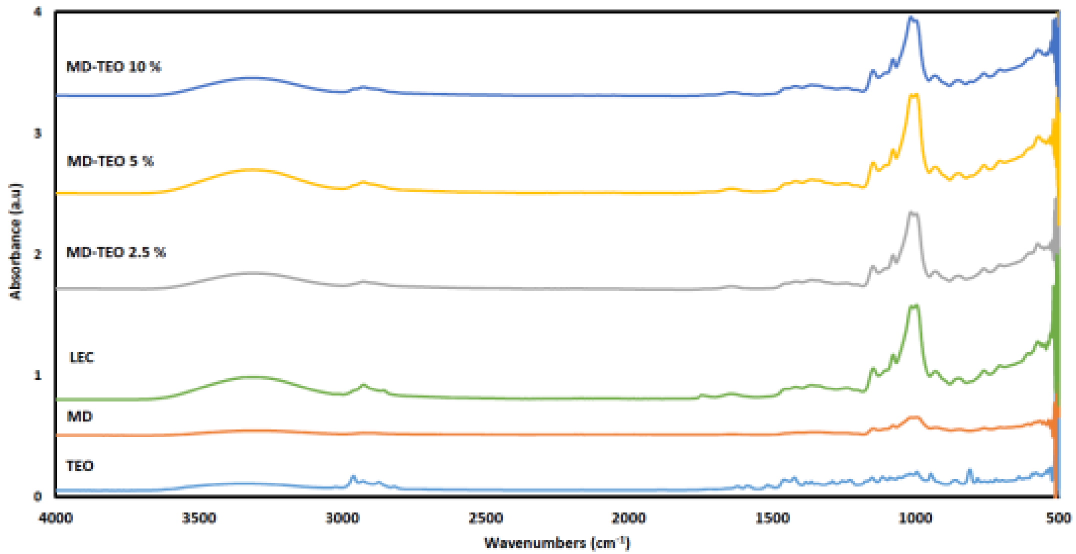

Figure 2. FTIR spectra of TEO, LEC, MD, and MD-TEO particles

The molecular interactions between TEO, lecithin, and maltodextrin were confirmed by FTIR analysis (Figure 2). The characteristic peaks of 3405 cm^{-1} (-OH stretching), 1421 cm^{-1} (−OH bending vibration), and 1228 cm^{-1} (C–O bending vibration) are associated with TEO (Rezaei et al., 2021). The characteristic peaks of 1619 cm^{-1}, and 1458 cm^{-1} correspond to the phenolic compounds of TEO (Hu et al., 2018). The MD adsorption bands at 2800 cm^{-1} and 3700 cm^{-1} belong to hydrogen bonds, 1653 cm^{-1} (C=O stretching), 1155 cm^{-1}, and 1081 cm^{-1} (C-O stretching and C-O-H bending), and the bands at 1017 cm^{-1} and 850 cm^{-1} are associated with maltodextrin structure (Kang et al., 2019; Sarabandi et al., 2019). The peak at 1737 cm^{-1} is related to the C=O stretching of the lecithin molecule (Tantipolphan et al., 2007).

Conclusions

The thyme essential oil encapsulated maltodextrin particles were successfully prepared using the simple and eco-friend electrospray method with improved biological properties. The results of FTIR and images of SEM and optical microscopy indicated the formation of MD-TEO particles. Engineered MD-TEO particles possessed high antimicrobial activity against *B.cereus*, *S.aureus*, *L.innocua*, *E.coli*, and *S.* Typhimurium, and exhibited high antioxidant activity. Engineered MD-TEO particles could open a new window for essential oil practical applications alternative to synthetic preservatives in food, medicine, and pharmaceuticals to improve bioavailability and sensory attributes.

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EVALUATION OF BEST MANAGEMENT PRACTICES FOR CONTROLLING SEDIMENT AND NUTRIENT TRANSPORT AT THE DEVELI BASIN, TURKEY

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Abstract

In this study, a watershed-scale Soil and Water Assessment Tool (SWAT) model was developed for the Develi Basin (Turkey) and the effects of best management practices to control sediment and nutrient transport were evaluated with the model. The Develi Basin is a semi-arid agricultural basin located in the Central Anatolia Region of Turkey, where irrigated agriculture has been intensified significantly in recent decades. Agricultural drainage from irrigated areas has caused reduction in the quality of surface water bodies. Water quality and sediment and nutrient transport in the basin were simulated with SWAT. Six management scenarios that can reduce sediment and nutrient transport were determined considering the feasibility of the applications and evaluated with the model. The scenarios were as follows: 1) Reduction in fertilizer use by 10% 2) Reduction in fertilizer use by 20% 3) Conservation tillage 4) No tillage 5) Vegetated filter strip (2 m) 6) Vegetated filter strip (5 m). The model performed well for simulating sediment and nutrient transport in the Develi Basin during calibration and validation. The simulation of individual scenarios revealed that vegetated filter strips showed the highest sediment and nutrient reduction, followed by no tillage and conservation tillage practices. This study showed that sustainable watershed management can be achieved by application of best management practices in agricultural areas in the Develi Basin.

Keywords: Water Quality Modelling, SWAT, Best Management Practices.

Introduction

Agricultural “diffuse source” pollution is among the most important factors threatening the quality of surface water bodies, such as lakes and wetlands (Isermann, 1990). Changes in water quality of these systems adversely affect the composition of the species and the nutrient balance. The European Union Water Framework Directive and Surface Water Quality Regulation of Turkey emphasize the importance of detecting and controlling diffuse source pollutants. However, the reduction and prevention of diffuse source pollution is challenging. In addition, due to the spatial and temporal variability, it is difficult to identify the sources and quantify the amount transported from diffuse sources. The use of modeling techniques provides an advantage in detecting, measuring and determining the effects of diffuse source pollution.

In this study, a watershed scale water quality model was developed to simulate the effects of various strategies for controlling agricultural diffuse pollution. The model was developed using Soil and Water Assessment Tool (SWAT). SWAT is used worldwide for the analysis of the effects of diffuse and point source pollution loads (Huang et al., 2009; Parajuli et al., 2008; Tripathi et al., 2003; Zhang et al., 2008). The analysis was conducted in the Develi Basin in Turkey, which is a semi-arid agricultural basin, affected significantly by agricultural diffuse source pollution (Figure 1). Improving water quality in the basin is important as it hosts a large wetland complex, called the Sultan Marshes. The basin is a closed basin and agricultural drainage waters eventually discharge into the Sultan Marshes.

Materials and Methods

The water quality model was prepared based on a hydrologic model developed by the Soil and Water Assessment Tool (SWAT) in a previous study (Jouma & Dadaser-Celik, 2022). The details about the hydrologic model including input data and model calibration and validation studies can be found at Jouma and Dadaser-Celik (2022).

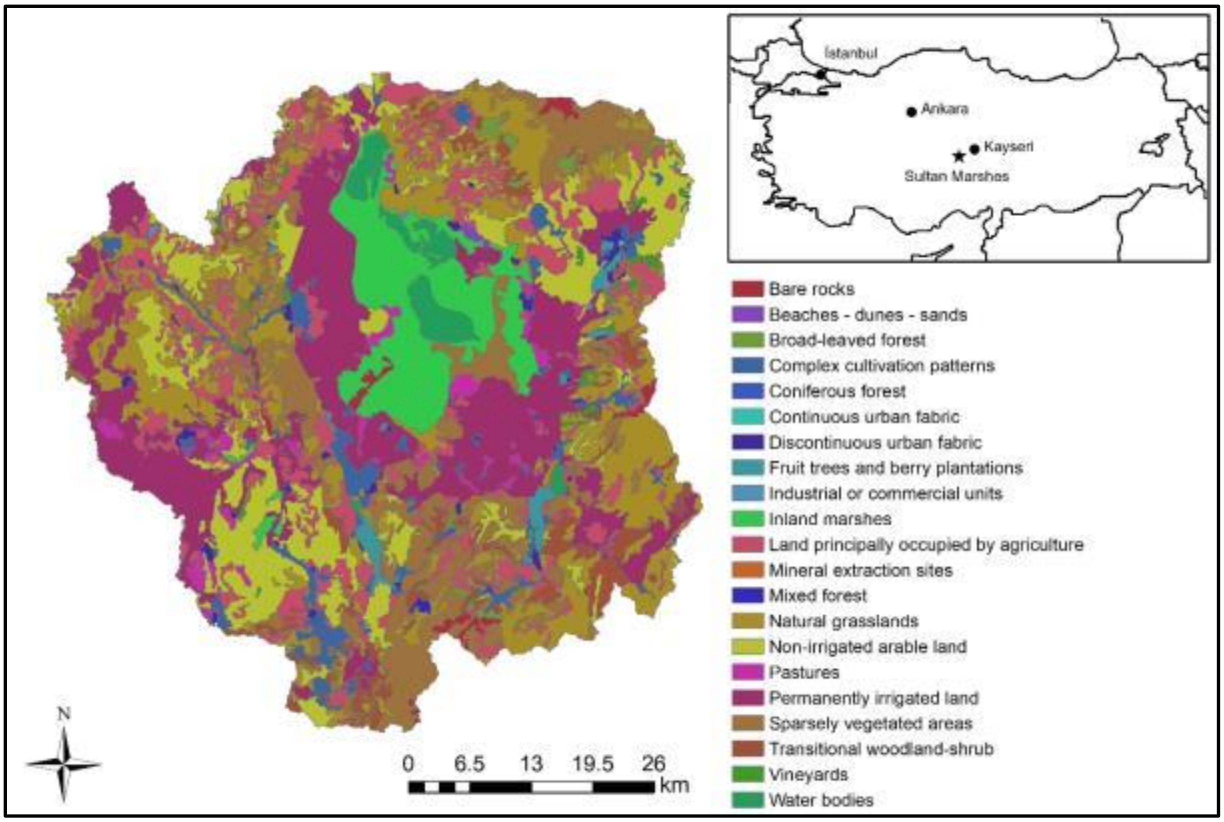

Figure 1. Location and physical characteristics of the Develi Basin

Water quality model for the Develi Basin represented pollutant loads coming from agricultural and pasture areas and by points discharges from settlements. The model was calibrated and validated for sediment, nitrate-nitrogen (Nitrate-N), total nitrogen (TN) and total phosphorus (TP). The parameters used in the water quality model calibration and the initial selected ranges for the calibration are shown in Table 1. These parameters are among the frequently used parameters in studies conducted to date (Abbaspour et al., 2015; Arnold et al., 2012). The calibration and validation of the model was performed using the SWAT-CUP program using the sequential uncertainty fitting 2 (SUFI-2) algorithm (Abbaspour et al., 2007). SUFI-2 is a semi-automated method and require prior knowledge about the range of parameters. In SUFI-2, we conducted several iterations with 500 model runs for each iteration by importing best parameter ranges from the previous iterations. The iterations continued until the algorithm provides no further improvement on the model performance. The performance of the model was evaluated by using determination coefficient (R^2), Nash-Sutcliffe efficiency (NSE), and percent bias (PBIAS). According to Moriasi et al. (2007), the performance of water quality models is “satisfactory” when R^2 and NSE are greater than 0.5, “good “when R^2 and NSE are greater than 0.65, and “very good” when R^2 and NSE are greater than 0.75. For PBIAS, the values in the range of 0-±25 denote “very good” model performance, values in the range of “±25-±40” denote “good” model performance, and values in the range of ±40-±70 denote “satisfactory” model performance.

Table 1. Parameters used in model calibration and selected values

Parameter	Min	Max	Calibration Type*	Value Selected
RCN.bsn	0	15	v	5.535902
ERORGN.hru	0	1000	v	1.960381
NPERCO.bsn	0	1	v	0.191508
BC3.swq	0.02	0.4	v	0.472352
BC1.swq	0.1	1	v	0.819880
BC2.swq	0.2	2	v	0.330731
CMN.bsn	0.001	0.003		0.002643
RDSCO.bsn	0	1	v	0.696204
FIXCO.bsn	0	1	v	14.855688
SOL_NO3.chm	0	100	v	0.567129
AI1.wwq	0.02	0.09	v	0.125622
BC4.swq	0.01	0.7	v	0.060903
ERORGP.hru	0	1000	v	0.067159
PPERCO.bsn	10	17.5	v	640.667297
ANION_EXCL_BSN.bsn	0.01	1	v	4.267746
SHALLST_N.gw	0	1000	v	11.330987
PHOSKD.bsn	100	200	v	151.718063
PSP.bsn	0.01	0.7	v	0.115037
BIOMIX.mgt	0.2	0.5	v	0.207454
AI2.wwq	0.01	0.02	v	0.012346
RS4.swq	0.001	0.1	v	0.020936
RS5.swq	0.001	0.1	v	0.012300
USLE_P.mgt	0	1	v	0.650363
USLE_K.sol	0	0.65	v	0.025478
SPCON.bsn	0.0001	0.01	v	0.000479
SPEXP.bsn	1	1.5	v	1.108766

* r means that the parameter is multiplied by 1+r, v means that the parameter is replaced by the v value, both r and v are the numbers between the lower and upper limits.

Six management scenarios were created in order to reduce the sediment and nutrient transport in the Develi Basin and the effects of these scenarios were simulated with the SWAT model. The scenarios focused on the fertilizer use, tillage operations and application of vegetated filter strips. The scenarios were as follows: 1) Reduction in fertilizer use by 10% 2) Reduction in fertilizer use by 20% 3) Conservation tillage 4) No tillage 5) Vegetated filter strip (2 m) 6) Vegetated filter strip (5 m). Reduction in fertilizer use can reduce nutrient input over the land surface, can therefore reduce nutrient transport. Tillage operations can affect sediment transport and can alter sediment and nutrient concentrations in the surface runoff. Vegetated filter strips have the potential to slow down runoff and reduce sediment and nutrient concentrations. The change in the pollutant loads caused by the scenarios was evaluated according to Equation 1 at the point where the drainage waters are discharged into the Sultan Marshes.

$$Reduction\ (\%) = \frac{100(\text{Scenario} - \text{Base Simulation})}{\text{Base Simulation}} \qquad (1)$$

The first group of scenarios focused on reducing the amount of fertilizer use in agricultural areas. The effects of 10% reduction in the amount of fertilizer use was simulated in Scenario 1 and the effects of 20% reduction in the amount of fertilizer use was simulated in Scenario 2. The second group of scenarios focused on evaluating the effects of tillage operations. In Scenario 3, the effects of conservation tillage and in Scenario 4, the effects of no tillage were simulated. In the third group of scenarios, the effect of creating filter strips was evaluated. Filter strips are vegetated areas that can purify runoff water and filter pollutants. Filter strips increase biological conversion and reduce nutrient load through plant uptake, microbial uptake, nitrification and denitrification (Lam et al., 2011). The effects of applying filter strips to agricultural fields and pastures in the Develi Basin were evaluated. In Scenario 5, 2 m of filter strip was applied and in Scenario 6, 5 m of filter strip was applied.

Results and Discussion

Calibration and validation results for sediment, Nitrate-N, TN and TP parameters are presented in Table 2. The parameter values selected as a result of the calibration are presented in Table 1. As can be seen, the model produced successful results for sediment with NSE value of 0.60 and R^2 value of 0.69 during calibration and NSE value of 0.74 and R^2 value of 0.74 during validation. PBIAS value was -21.5 during calibration and -1.3 during validation. According to Moriasi et al. (2007), the model showed “good” performance based on R^2 and NSE and "very good" performance based on PBIAS. For Nitrate-N, the NSE value was 0.67, R^2 value was 0.73 and PBIAS value was 24.7 during calibration. NSE, R^2, and PBIAS values were -0.25, 0.01 and 40.5 during validation. Although model performance was “good” or “very good” during model calibration, performance was not satisfactory based on NSE and R^2 during validation. However, the model provided a satisfactory performance based on PBIAS during model validation. Similarly, we obtained “very good” or “good” model performance during calibration for TN and TP but the performance was not very good during model validation. Among the reasons for this situation are the variations between the state in which the model is calibrated and the state in which it is validated, errors in the calibration/verification data, and uncertainties in the model inputs (EPA., 2002). The SWAT model can have high uncertainty due to some processes that are not sufficiently taken into account in the calculation of parameters such as sediment and TP (Abbaspour et al., 2007). Successful calibration of a water quality model at the basin scale is only possible with the availability of data that can reflect the heterogeneity of the basin (Abbaspour et al., 2007). Considering the expected uncertainties in water quality data, it is stated that 10% and 31% difference between measured and simulated values is typical (Harmel et al., 2006). In cases where sampling points and frequency decrease, the uncertainties arising from the data increase and the expected error in the model increases (Harmel et al., 2006). The average difference between the measured and simulated values in the model developed in this study was around 20% for sediment, Nitrate-N and TP during the calibration period. In the validation period, there is a difference of 1% for sediment and 40% for nitrate.

Table 2. Calibration and validation results for sediment, Nitrate-N, TN ve TP

Process	Parameter	R^2	NSE	PBIAS
Calibration	Sediment	0.69	0.60	-21.5
	Nitrate-N	0.73	0.67	24.7
	TN	0.99	0.21	72.5
	TP	0.94	0.89	18.9
Validation	Sediment	0.74	0.74	-1.3
	Nitrate-N	0.01	-0.35	40.5
	TN	0.13	-0.60	95.9
	TP	0.46	-0.43	99.3

Various management scenarios were created to reduce the pollutant loads created in the Develi Basin and their effects were evaluated with the SWAT model (Table 3). In scenarios 1 and 2, the amount of fertilizer use in the Develi Basin was reduced by 10% and 20%, respectively. 10% reduction in fertilizer use (Scenario 1) causes a 3.4% decrease in nitrate loads and a 3.0% decrease in TN loads. On the other hand, a 20% decrease in the amount of fertilizer use creates a 6.5% decrease in nitrate loads and a 5.5% decrease in TN loads. The decrease in the amount of fertilization did not make a significant difference in the sediment and TP loads. It is thought that this situation arises from the adaptation of plants to changes in the amount of phosphorus, the periodicity of fertilization activities and the application of plant needs in the period. Some other studies showed that reduction in fertilization levels reduce pollutant loads (Lamba et al., 2016). However, it is also possible that this reduction has negative effects on agricultural production. For this reason, it would be useful to analyze the scenarios with models focused on agricultural production.

Table 3. Change in sediment, Nitrate-N, TN and TP with scenarios

Scenario No	Scenario Description	Change			
		Sediment	Nitrate-N	TN	TP
1	10% reduction in fertilizer use	0.2%	-3.4%	-3.0%	-0.2%
2	20% reduction in fertilizer use	0.1%	-6.5%	-5.5%	-0.1%
3	Conservation tillage	-0.1%	0.3%	0.0%	-5.2%
4	No tillage	0.6%	-0.2%	0.1%	0.8%
5	Vegetated filter strip (2 m)	-7.4%	-0.1%	-5.7%	-44.3%
6	Vegetated filter strip (5 m)	-10.3%	-5.8%	-12.3%	-58.1%

Scenario 3 and Scenario 4 were run to evaluate the effects of the tillage operations in the Develi Basin (Table 3). For this purpose, conservation tillage and no tillage scenarios were simulated with the model. The conservation tillage practice (Scenario 3) did not have a significant effect on pollutant transport. Only TP transport decreased by 5.2%. With no tillage, TP transport increased by 0.8%. The increase may be due to the fact that easily degradable N and P products do not mix with the soil, so they accumulate on the surface and pass to the surface flow more easily (Tuppad et al., 2010). Previously, Tuppad et al. (2010) found that conservation tillage caused a decrease in the range of 1.4-4.6% in terms of sediment, TN, and TP. Lampa et al. (2016) showed that although the change in the plowing technique had an effect on the downstream basins, it did not have a great effect on the basin outlet. In some previous studies, it was preferred to change some

hydrological parameters (for example, the manning coefficient) together with the change in soil tillage method (Lamba et al., 2016) and it was stated that a higher effect was obtained. Hydrological parameters were not changed in this study.
In Scenario 5 and Scenario 6, the effects of filter strips were evaluated (Table 3). Two different filter strip options (2 m and 5 m) were simulated with the model. Filter strips provided the most effective technique for controlling pollutant transport. The 2 m filter strip (Scenario 5) reduced sediment transport by 7.6%, Nitrate-N transport by 0.1%, TN transport by 5.7% and TP by 44.3%. The 5 m filter strip (Scenario 6) reduced the sediment transport by 10.3%, the nitrate transport by 5.8%, the TN transport by 12.3% and the TP transport by 58.1%. Filter strips reduce nutrient transport by processes such as biodegradation, plant uptake, nitrification and denitrification. However, the difficulties in creating filter strips and protecting them over the years and the fact that some of the agricultural lands will be lost for this purpose show that this scenario can be difficult to implement.

Conclusion

A water quality and pollutant transport model for developed for the Develi Basin. The water quality model was calibrated and validated for sediment, Nitrate-N, TN, and TP. The effects of best management practices were simulated with the model. The results showed that SWAT was successful in simulating sediment and nutrient transport. Scenarios that cover reductions in fertilizer use, the change in tillage operations and application of vegetated filter strips were evaluated based on simulations with the model. The analyzes showed that the application of filter strips provides the most reduction in pollutant transport. This practice was followed by reductions in fertilizer use. It can be useful to make a cost-benefit analyses about which application will be beneficial and consider feasibility of the applications through social assessments.

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PESTICIDES POLLUTION FROM AGRICULTURE ACTIVITIES IN THE ALTINAPA RESERVOIR WATERSHED, TURKEY

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Abstract

The Altınapa Reservoir is a drinking water reservoir, which supplies water to the city of Konya (Turkey) with population of about 2.5 million. In this study, water quality and pesticide concentrations were monitored at 4 points on the river and at reservoir effluent during one year between 2019 and 2020. Water quality parameters including pH, electrical conductivity (EC), total hardness, alkalinity, total organic carbon (TOC), total nitrogen (TN), nitrate, and total suspended solids (TSS) were monitored monthly. Pesticides concentrations were measured at the seasonal samples collected at the same sampling points. The annual mean value of pH and EC were 8.04 and 417 µS/cm, respectively. While annual mean TOC concentrations varied between 1.35 and 1.69 mg/L in samples collected from the stream, it was 2.69 mg/L in the reservoir outflow. The annual mean TN and NO_3^-–N values were 2.39 and 1.25 mg/L, respectively. Pesticide monitoring results for four periods showed that 71 different micropollutants including pesticides were detected. Among these micropollutants, 19 pesticides were observed in at least two sampling points and/or reservoir effluent. Acetamiprid, permethrin, piperonyl butoxide and terbutryn were observed at least two seasons. The concentrations of DDD-op, DDE-p.p' and diflufenican exceeded the environmental quality standards. Managing agricultural activities in the basin is important in order to protect human health and aquatic life from pesticide pollution. Within the scope of the project, the effects of reducing the use of fertilizers, changing the plowing technique, and applying filter strips to the stream sides were evaluated in agricultural practices.

Keywords: Agriculture, Altınapa Reservoir Watershed, pesticide pollution, water quality.

Introduction

Pesticides are widely used in agriculture, industry, forests, and household to repel harmful pests (FAO/WHO 2016). Pesticides are classified according to the target pest, chemical structures, toxic levels, and active ingredients. The most common classification of pesticides is based on target pests where pesticides are classified as herbicides, insecticides, rodenticides, fungicides, various other compounds (de Souza et al. 2020). On the global basis, 4.2 million tons of pesticides are used, and nearly half of pesticide use is herbicides (FAO 2022). In 39 main rivers, 11 herbicides, 4 insecticides, and 1 fungicide were found in higher concentrations than the chronic reference limit for aquatic life, according to nationwide research in the USA. Pesticide levels in rivers and lakes ranged from 7 to 121222 ng/L, in groundwater from 20 to 1060 ng/L, and in drinking water from 141 to 14629 ng/L (Mojiri et al. 2020, Stone et al. 2014). In surface waters, the most commonly detected herbicides are atrazine (Peng et al. 2018), metolachlor (Glinski et al. 2018), diuron (Carazo-Rojas et al. 2018), terbutryn (Peng et al. 2018). On the other hand, chlorpyrifos (Affum et al. 2018), diazinon (Fadaei et al., 2012), imidacloprid (Peng et al., 2018), and malathion (Sumon et al., 2018) are insecticides that are detected the most commonly at the highest concentrations worldwide. Pesticides are mainly transported into the surface and ground water bodies by spray drift, infiltration through soil, and surface runoff, alongside runoff and erosion (Larsbo et al. 2013). Pesticides have negative effects on aquatic organisms, wildlife, animals and human health due to their carcinogenic, mutagenic and teratogenic properties (Sharma et al., 2020). In the Altınapa Reservoir Watershed, the effects of agricultural activities on water quality and pesticide pollution were examined through 1-year water quality monitoring campaign. Altınapa Reservoir is an important surface water source in terms of providing drinking water to a city of approximately 2.5 million people. The reservoir was established in 1967 on Meram Stream and has been feeding the Konya Drinking Water Treatment Plant, which has been purifying 37.8 million m^3 of water annually since 1994. The reservoir is located in the semi-arid region of Turkey, where water resources are scarce. The mean air temperature in the basin is 11.6°C and mean annual precipitation is 338 mm based on 1975-2019 data.

Materials and Methods

Water quality and pesticides were monitored at 4 sampling point (K1-K4) where governmental flow and sampling stations, and reservoir outflow (RE) (Fig. 1). Among water quality parameters, pH, electrical conductivity (EC), total hardness, alkalinity, total organic carbon (TOC), total nitrogen (TN), nitrate, and total suspended solids (TSS) were determined according to Standard Methods (APHA/AWWA/WEF 2012).

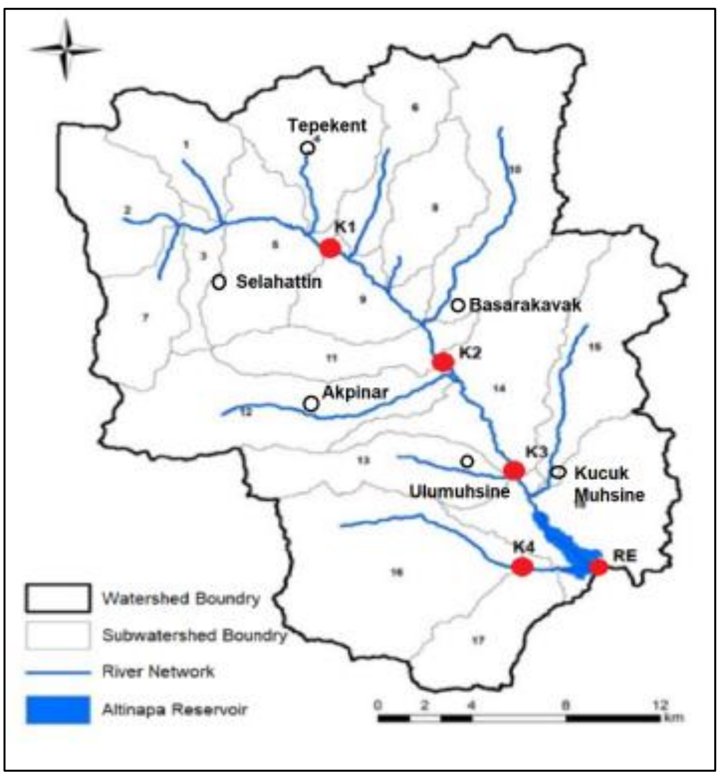

Fig. 1. Watershed boundary and water quality monitoring stations in the Altınapa Reservoir Watershed.

Pesticides were monitored seasonally during the year (Autumn, Winter, Spring, Summer). 2 L water samples were collected from the sampling points and sent to the TUBITAK Marmara Research Center Laboratory for pesticide analyses. The samples were shipped as soon as possible keeping in ice box and stored in refrigerators at +4°C in the laboratory until the analyses were completed. Pesticide analyses were performed according to Standard Methods by LC-MSMS and GC-MSMS instruments. Water samples were scanned for 71 organochlorous and organophosphorus pesticides.

Results and Discussion

Altınapa Reservoir Watershed consists of a mainstream (Meram) and several creeks feeding this river. There are 6 small villages with a total population of 6018 people. The water quality of Altınapa Reservoir and the streams in the basin are affected by both livestock and agricultural activities as well as wastewater discharges. In the Altınapa Reservoir Watershed, the plants cultivated commonly include tomatoes, squash, apple, pear, quince, walnut, cherry, sour cherry. The pesticide commonly applied for these crops include acetamiprid, copper sulfate pentahydrate, hexythiazox, deltamethrin, cypermethrin, thiacloprid, and thiophanate methyl. A total of 4906 bovine and 66054 ovine animals are raised in the basin within the scope of animal husbandry activities.

Monthly water samples collected from five points for 12 months were evaluated to reveal temporal and spatial changes in water quality and pesticides occurrence. Table 1 lists annual means for each sampling points for nine parameters and Table 2 presents the pesticides variations in sampling points. We also showed the mean, minimum, maximum, and standard deviations of means of five sampling points. As shown in Table 1, annual pH and EC values at sampling points ranged from 7.82 to 8.26 and from 335 to 487 µS/cm, respectively. The annual mean values of alkalinity and total hardness were 247 and 242 mg/L $CaCO_3$. Mean TOC values varied between 1.35 and 1.69 mg/L at the sample points located on the stream (K1-K4), however, mean TOC value of reservoir effluent (RE) was 2.69 mg/L, much higher than the values measured in rivers. The highest TN and NO_3^-N values were observed at point K2 with mean concentrations of 4.81 and 2.02 mg/L, respectively.

Table 1. Physico-chemical characteristics of water samples

Sampling Point	pH	EC (µS/cm)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	TSS (mg/L)	TOC (mg/L)	TN (mg/L)	NO_3^-N (mg/L)
K1	7.90	457	267	261	5.36	1.35	1.98	1.33
K2	7.99	476	268	277	4.00	1.69	4.81	2.02
K3	8.01	449	267	258	9.27	1.51	3.32	1.82
K4	8.26	344	217	210	2.25	1.46	0.63	0.43
RE	8.05	360	218	204	6.05	2.69	1.23	0.69
Min	7.82	335	209	200	5	1.24	0.55	0.31
Max	8.26	487	277	279	20	2.68	4.94	2.33
Mean	8.04	411	246	240	9	1.65	1.67	0.86
SD	0.18	66	30	34	7	0.58	1.83	0.89

Table 2. Pesticides detected in Altınapa Watershed and their concentrations at sampling points

Pesticides	Type of Pesticides	EQS (µg/L)	Autumn 2019 (µg/L)					Winter 2020 (µg/L)					Summer 2020 (µg/L)				
			K1	K2	K3	K4	RE	K1	K2	K3	K4	RE	K1	K2	K3	K4	RE
Acetamiprid	Insecticide	42	0.0020	0.0058									0.0007		0.0011		0.0005
Aldrin	Insecticide	0.01											0.0007	0.0007	0.0054		0.0011
Azoxystrobin	Fungicide	0.20	0.0005	0.0007													
BHC-alpha	Insecticide	-											0.0023	0.0014	0.0015	0.0003	0.0003
Bromopropylate	Acaricide	0.12										0.074	0.0075	0.0006	0.0208	0.0000	0.0036
DDD-op	Insecticide	0.025						0.017	**0.065**		**0.058**	**0.061**					
DDE-p.p'	Insecticide	0.02											0.0098	0.0025	**0.0372**	0.0022	
Diflufenican	Herbicide	0.010							**0.044**			**0.070**					
Ethoprophos	Nematicide, insecticide	0.21	0.0311	0.0278		0.1410	0.0198										
Imidachloprid	Insecticide	0.14	0.0543	**0.3577**													
Metalaxyl	Fungicide	17	0.0169	0.0123													
Metrafenone	Fungicide	12							0.937			0.038					
Permethrin	Insecticide	0.12						0.011	0.036	0.012	0.039	0.084	0.0010		0.0179		0.0021
Piperonyl butoxide	Pesticide synergist	3.3						0.011	0.041		0.046	0.059	0.0254		0.1337		
Prometryne	Herbicide	0.3	0.0022	0.0027													
Tebuconazole	Fungicide	23	0.0289	0.0463													
Terbuthylazine	Herbicide	0.2	0.0061	0.0054													
Terbutryn	Herbicide	0.065	0.0091	0.0183	0.0073	0.0045	0.0071						0.0036	0.0024	0.0282	0.0026	0.0020
Tolclofos Methyl	Fungicide	1.2											0.0005		0.0024		

NOT 1: Selected pesticides were not detected in Spring.
NOT 2: The pesticides concentrations exceed Environmental Quality Standards were typed bold.

As a result of the pesticides analyses for 4 seasons in the 2019-2020 period, 71 different micropollutants including pesticides were detected. Pesticides detected in the Reservoir Effluent and/or at least at two sampling points were selected and presented in Table 2. The data belong to Spring period is not shown in table, because any of compounds except Acetamiprid could not be detected at least at two sampling points. 8 of these pesticides are insecticide, 5 of them are fungicide, 4 of them are herbicides, 1 of them are acaricide and 1 of them is pesticide synergist.

Conclusion

Among the monitored pesticides, acetamiprid, aldrin, BHC-alpha, bromopropylate, ethoprophos, permethrin, piperonyl butoxide, and terbutryn were detected at almost all sampling points as well as reservoir effluents. Moreover, acetamiprid, permethrin, piperonyl butoxide, and terbutryn were observed at least two seasons. Besides, the concentrations of DDD-op, DDE-p.p' and diflufenican were exceeded the Environmental Quality Standards of Turkey. Pesticides have harmful impacts on human health and aquatic organisms because of their carcinogenic, mutagenic, and teratogenic nature. Moreover, they are significantly toxic due to their ability to bioaccumulate in organism tissue and migrate to higher organisms. Therefore, it is inevitable to manage agricultural activities and pesticide applications to prevent pesticide entry and pesticide pollution in water resources. As a result of the evaluation of the results, three main objectives for water management in the Altınapa Reservoir Watershed and the viable management strategies of the guards to achieve these objectives were determined: i) Preservation of the hydrological status, ii) Protection of the water quality, iii) Improvement of drinking water quality supplied from Altınapa Dam Lake. The basic precautions that can be applied for pesticide pollution are control of pesticides at source, handling pesticides safely, managing of waste pesticides and empty containers, establishment of catchments for retarding and slowing runoff, construction of vegetated waterways and buffer strips, scheduling irrigation and pesticide application.

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EFFECT OF SOIL PROTECTION TILLAGE ON SOME MICROBIAL PARAMETERS OF MODERATELY ERODED CALCAREOUS CHERNOZEM

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Abstract

Water erosion is the most widespread soil degradation process in Bulgaria. As a result of its action, the surface soil layer is disturbed, nutrients and organic matter are lost and the soil structure deteriorates. To counteract these negative processes, it is necessary to apply soil protection tillage. The assessment of the efficiency of soil protection tillage can be done not only on the basis of soil physical and chemical parameters, but also on the parameters of soil microbiological activity. Enzyme activity and microbial biomass carbon contents are sensitive indicators for changes in soil conditions and are widely used as parameters characterizing soil quality. The aim of the present study is to establish the effect of soil protection tillage (tillage across the slope) in wheat growing on the amount of microbial biomass carbon and the activity of the enzymes alkaline phosphatase, protease and urease. The experiment was carried out in field conditions on moderately eroded Calcareous Chernozem. The microbial parameters were determined twice (in spring and autumn) at two soil depths (0-10 cm and 10-20 cm). It was established that the effect of the studied soil protection tillage was different for the individual parameters, soil depths and sampling seasons. A positive effect was obtained for microbial biomass carbon contents for both layers in spring, protease activity for the surface soil layer in both seasons of soil sampling and urease activity for the same layer in autumn.

Keywords: *Calcareous Chernozem, Erosion, Enzyme activity, Microbial biomass carbon contents.*

Introduction

Water erosion is the most widespread soil degradation process in Bulgaria. A significant part of the arable land (about 60%) in the country occupies sloping terrains and due to the soil and climatic conditions is at risk of erosion (Kuncheva, 2019; Ivanova, 2021). As a result of its action, the surface soil layer is destroyed, nutrients are lost, soil structure deteriorates and the activity and biodiversity of soil biota change (Orgiazzi et al., 2017). Soil microbiological activity as one of the factors of soil fertility is also influenced by the intensity of erosion processes, which lead to a reduction of organic matter in the soil. Hamer et al. (2009) and Park et al. (2014) reported an adverse influence of the erosion on the activity of soil microorganisms. To counteract these negative processes, it is necessary to apply soil protection measures and technologies. According to Dimitrov (2016) agrotechnical measures are of greatest importance for the protection of agricultural lands from erosion. Rousseva et al. (2009) divided the main agrotechnical measures for protection of soils in arable lands with risk of water erosion into the following groups: soil protection tillage, soil protection crop rotations and fertilization, suitable for sloping terrains. Soil protection tillage includes tillage transversely to the slope, no-till planting, flat-cutting tillage, vertical mulching and others. The effectiveness of soil protection technologies can be assessed not only on the basis of soil physical and chemical parameters, but also on the parameters of soil microbiological activity. Microbial biomass carbon contents and soil enzyme activity have been proposed by many researchers as potential indicators of soil quality because they change rapidly with alteration of soil management and conditions (Bending et al., 2004; Roldan et al., 2005; Nedyalkova et al., 2017; Stone et al., 2016; Schloter et al., 2018).

The aim of the present study is to establish the effect of soil protection tillage (tillage across the slope) in wheat growing on the amount of microbial biomass carbon and enzyme activity of moderately eroded Calcareous Chernozem.

Material and methods

The study was conducted in field conditions on moderately eroded Calcareous Chernozem (Epicalcic Chernozem, IUSS Working Group WRB, 2015) on the slope 5^o (8.7%) in the village of Trastenik, Rousse region, Bulgaria. The agrochemical properties of the soil at the beginning of the experiment were as follows: humus contents – 2.09%; mineral N - 11.06 mg/kg soil; P(P_2O_5) – 7.66 mg/100 g; K (K_2O) – 40.95 mg/100 g; pH (H_2O) - 7.70. Two types of tillage in wheat cultivation - conventional technology, including main tillage by turning the layer, applied along the slope (B1) and the same technology, applied transversely to the slope (B2) were compared as regard to their effect on some microbial parameters. The experiment was carried out on block method in four replicate (Shanin, 1977). The experimental plots were 34 m long, 22 m wide and 748 m^2 in area. The wheat was sown in October 2020.

On the experimental area basic fertilization with $N_{15}P_{10}K_8$ kg/da was performed, importing the entire amount of phosphorus (in the form of superphosphate) and potassium (in the form of potassium chloride) fertilizers before sowing. The nitrogen fertilizer (ammonium nitrate) was applied separately - 1/3 of the whole quantity before sowing and the remaining 2/3 - in spring. In 2021 in spring (tillering phase) and autumn (3 months after harvesting the plants) samples from two soil depths (0-10 cm and 10-20 cm) were taken for microbiological analyses. Fresh soil samples were stored in a refrigerator at 4^oC. Before analyzing they were processed through 2-mm sieve and root fragments were removed. The microbial biomass carbon (C) contents was determined by the method of Anderson-Domsch (Anderson *et al*., 1978). The method is based on determination of soil CO_2 evolution after adding easily-decomposable carbon source to the soil. The activities of protease, urease and alkaline phosphatase were determined by adding the appropriate substrate to soil samples, incubation under optimal conditions and determining the concentration of the reaction product on a spectrophotometer. The methods used for determination of the activity of the enzymes mentioned above were as follows: for protease - the method of Hoffman-Teicher (Hasiev, 2005), for urease – the method of Hoffman-Teicher (1961) and for alkaline phosphatase activity – the method of Tabatabai-Bremner (Haziev, 2005). All analyses were done in three replicates.

The statistical processing of the data included determination of the least significant differences (LSD) ($P \leq 0.05$) between the treatments. For the two depths studied LSD are determined separately. A statistical software package (StatGraphics Plus, version 5.1 for Windows, USA) was used.

Results and Discussion

Microbial biomass is an important component of soil organic matter, as it is a labile source of nutrients needed for plant growth. Microbial biomass carbon contents serve as an indicator of the dynamics in the amount of essential nutrients and an ecological marker for changes in soil conditions (Anderson *et al*., 2010; Schloter *et al*., 2018). The data in Fig. 1 show that in the studied moderately eroded Calcareous Chernozem the amount of microbial biomass C varies in the range of 20-37 mg / 100 g dry soil. In the spring, statistically proven higher values of this indicator were obtained for both soil layers in the soil protection treatment (B2) as compared to the control (B1). However, in the autumn, no differences were found between the treatments. For the surface soil layer, the amount of microbial biomass C in both variants was significantly higher than that in the first stage of the study. This is probably due to the intensive mineralization of plant residues of the stubble after harvesting of wheat, in the process of which microbial biomass increases.

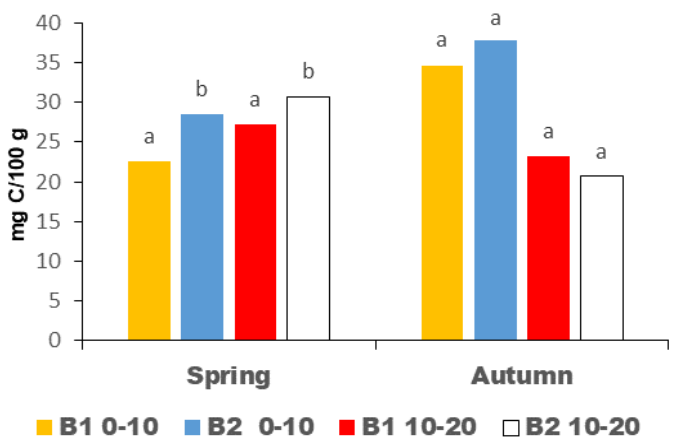

Fig. 1. Microbial biomass C contents of Calcareous Chernozem in conventional tillage (B1) and with tillage across the slope (B2)

Urease is an enzyme catalyzing the hydrolysis of urea to NH_3 and CO_2. The origin of urease in soil is connected with plant residues, manure and mineral fertilizers. It is also formed in the soil as an intermediate product during the breakdown of proteins and nucleic acids. During the spring season for the variant with soil protection treatment (B2), no urease activity was registered for the 0-10 cm layer, and lower values were obtained compared to the control (B1) for the 10-20 cm layer (Fig. 2). In the autumn, statistically higher values of urease activity were obtained for the surface layer of treatment B2. For the 10-20 cm layer, such differences between variants were not established. The registered values in both variants were three times higher than those obtained in the first stage of the study.

Phosphatases catalyze the breakdown of some organic phosphorus compounds (glycerophosphates, sacharophosphates), which represent from 30 to 70% of the total phosphorus resources in the soil. Since the inorganic compounds of phosphorus in the soil are characterized by low solubility, the mobilization of phosphorus from microorganisms is of considerable importance for plant nutrition. Alkaline phosphatase activity in the present study assumes values from 2.58 to 8.30 μmol nitrophenol/g/ h (Fig. 3). In the first stage of the study no proven differences were obtained between the variants and two soil layers studied. In autumn the soil layer with a depth of 10-20 cm was with lower phosphatase activity in the variant with soil protection treatment (B2) compared to the control (B1). Probably this can be related to fluctuations in the amount of mobile phosphorus in the soil as a result of the applied mineral fertilization. It is known that phosphatase activity decreases with increasing content of mineral forms of phosphorus in the soil (Ikoyi *et al*., 2018; Basselt *et al.* 2022).

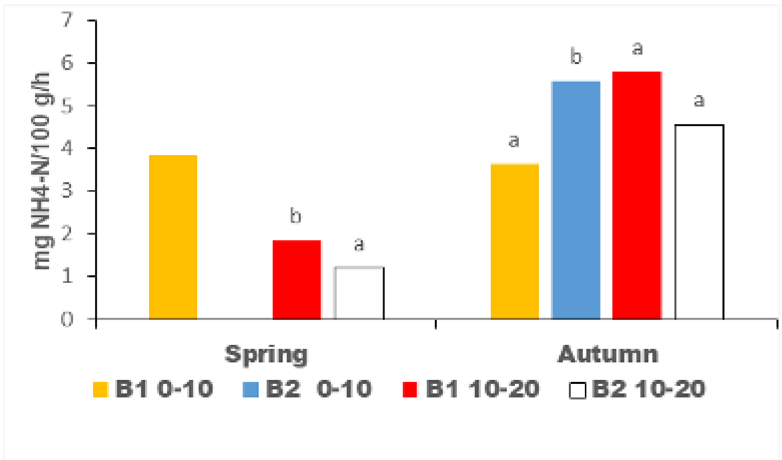

Fig. 2. Urease activity of Calcareous Chernozem in conventional tillage (B1) and tillage across the slope (B2)

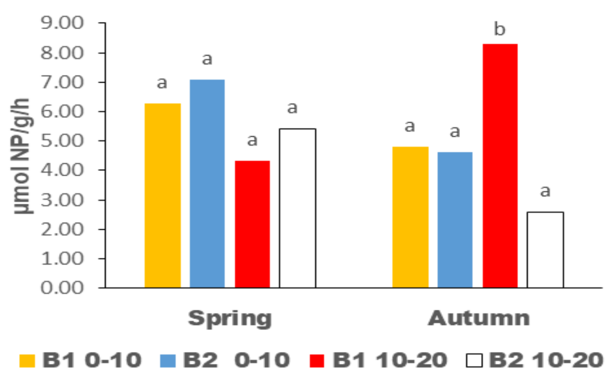

Fig. 3. Alkaline phosphatase activity of Calcareous Chernozem in conventional tillage (B1) and tillage across the slope (B2)

Proteases are produced mainly by bacteria. They participate in the mineralization of organic nitrogen in the soil by catalyzing the hydrolysis of proteins and nucleic acids, during which ammonium nitrogen is released. In the variant with soil protection treatment (for the surface soil layer) for both seasons of the study higher values of protease activity were obtained in comparison with the corresponding soil layer of the control (Fig. 4). For the layer with a depth of 10-20 cm no differences were registered between the variants. Of note are the higher values of protease activity for both variants and both soil depths during the autumn which proves the active mineralization of nitrogen-containing organic compounds in plant residues.

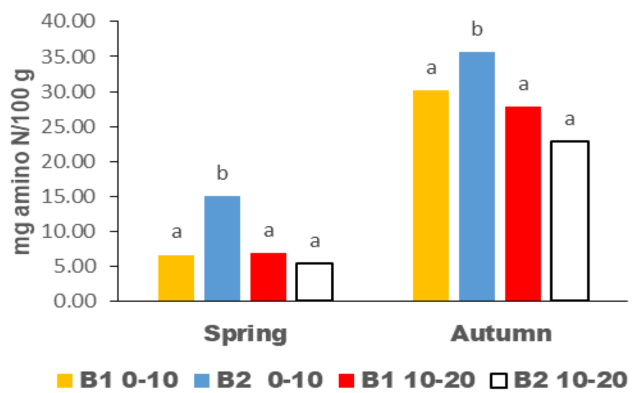

Fig. 4. Protease activity of Calcareous Chernozem in conventional tillage (B1) and tillage across the slope

The presented results show that the effect of the applied soil protection treatment on the studied microbiological indicators of moderately eroded Calcareous Chernozem is different for different parameters, depths of studied soil layers and sampling seasons. A positive effect of tillage across the soil slope was found with respect to microbial biomass carbon contents during the spring for both soil layers. The applied soil protection treatment affects the activity of enzymes related to nitrogen transformation. A favorable effect on protease and urease activity was obtained for the surface soil layer during the autumn.

Conclusion

The effect of the applied tillage across the slope in wheat growing on microbial biomass C contents and protease, urease and phosphatase activity of moderately leached Calcareous Chernozem depends on the sampling season, soil depth and individual microbial parameter.
A positive effect on microbial biomass carbon contents for both layers in spring, protease activity for the surface soil layer for both sampling seasons and urease activity for the same layer in autumn was established.

Acknowledgement

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SOIL AND CLIMATIC CAPACITY OF THE SILISTRA REGION (BULGARIA) FOR AGRICULTURE

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Abstract

This article describes the soil-climatic resources in the Silistra region, North-Eastern Bulgaria. The research presents the main climatic factors on which the development and yield of crops depend and the balance of soil resources by main soil types and with a brief physicochemical characteristic of the soils. Soils in Bulgaria are mapped and classified by soil specialists in M 1:10,000. The existing methodology groups soil differences into agro-soil groups and subgroups. These data classify soil resources via Bulgarian classification and World reference base and they cover area and soil type. The manuscript presents statistical data of 723 soil-cartographic units, united into 5 agro-soil groups and 11 subgroups. The information used covers 92 soil-cartographic surveys (these are soil maps with one or more settlements) for the entire area. The data contains information about basic physicochemical indicators characterizing soil fertility: texture class, the content of soil organic matter of the surface horizon and sub-horizon, and soil acidity. The conclusion finds out that climatic conditions divide the Silistra region into two zones - with "hot" and "moderately hot" temperatures during the growing season and a deficit in the balance of atmospheric moisture (the main limiting factor in agriculture). The territory of the Silistra district is occupied by soils suitable for agriculture approximately 76% of the soil resources but mostly climatic conditions limit their effective agricultural use.

Keywords: *agrosoil group, agrosoil subgroup, climate, soils.*

Introduction

Silistra Region is one of the 28 Bulgarian regions, located in North-Eastern Bulgaria, on the banks of the Danube River. It borders the regions of Ruse, Razgrad, Shumen and Dobrich, and also with Romania by land and via the Danube River. The territory of the region includes parts of the Danube Hilly Plain, Dobruja and the lowlands of Ludogorie. The Danube River is a natural waterway connecting Silistra with Central and Eastern Europe. Silistra region is distinguished by a high potential for the development of agriculture. The primary sector is the leader in the local economy and creates 23% of the gross added value in the district, 31% of the output and 16% of the employment. It is the main source of income and employment for a large part of the population and will continue to play an important role in the development of the area in the future. The favorable combination of fertile soils, climatic conditions and relief are a prerequisite for the designation of the Silistra region as an intensive agricultural area, and the long-standing traditions in the field of crop production and animal husbandry determine agriculture as a priority sector. The climate of the district is characterized by hot summers, early onset of spring and severe cold in winter. The snow cover in the area lasts relatively long - about 60 days a year. Due to the proximity of the Danube River, fogs and strong winds are often observed. For the most

part, the relief is flat, with an altitude of up to 200 m., but the land oriented towards the Danube River gives it a hilly character. A typical loess hump is formed parallel to the coast. The agricultural land in the district is a total of 200 137 ha, of which 179 799 ha are for agricultural purposes, including: arable land – 151 594 ha, permanent plantations 5 980 ha, meadows and pastures – 18 239 ha and other categories of land – 3 986 ha. As of 2021, the arable agricultural land is 84% of the areas with agricultural purpose, the sown/harvested areas are 129 517 ha or 85% of the arable land. Plant growing is the main agricultural branch in the Silistra region. Priority is given to grain, technical and fruit crops - mainly apricots, plums, cherries and vineyards. The structure of crop production in the Silistra region is oriented towards grain production- 60% of the harvested areas in the district (77 199 ha) are sown with wheat, barley and corn. Oilseed crops in the district are represented by rapeseed and sunflower. They occupy 35% of the harvested areas – 45 386 ha. Permanent plantings of wine and dessert vines occupy 1.6% of the harvested area or 2 110 ha, the area occupied by apricot orchards is 2 109 ha.
Most apricot groves are located in the lands of the municipalities of Dulovo, Glavinitsa and Silistra. The remaining 1.8% of the harvested area in the district is occupied by other fruit crops (apples, plums, peaches, cherries) and vegetable crops (pepper, tomatoes, beans, potatoes). Watermelons and melons are grown in the area, which occupy only 0.3%.
Fertile soils are a prerequisite for obtaining high yields of cereals, vegetables, perennial crops (cherries and apricots) and vineyards. Water supply in the district is carried out by water sources from the terrace of the Danube river and deep wells of the "Raney" type.
The aim of the present development is: (i) to present an assessment of the main climatic factors on which the development and yield of crops depend (ii) to present the balance of soil resources in the district and their main agro-soil characteristics.

Materials and methods

To characterize the climatic conditions, data from climate reference books, averaged over long periods – 40 or 50 years, depending on the available sequence of observations were used. Climatic conditions are presented with the indicators of heat and humidity. Heat security is estimated by the annual sum of the air temperature above 10 C, measured in each meteorological station located on the territory of the Silistra region. Moisture security is represented by the deficit in the atmospheric moisture balance for the June-August period. The moisture balance was calculated using the evapotranspiration values according to the equations shown below (Hershkovich, 1984):

$$\mathbf{E} = 0.0018\,(25 + \mathbf{t})^2\,(100 - \mathbf{a}) \quad (1),$$

where:

E - Monthly evaporation (mm);

t - Average monthly air temperature (°C);

a - relative air humidity (%).

$$\mathbf{B} = \mathbf{W_S} + \mathbf{P_I} - \mathbf{E_I} + \mathbf{P_{II}} - \mathbf{E_{II}} + P_{III} - \mathbf{E_{III}} + \ldots \qquad (2),$$

where:

B - humidity balance (mm/m^2);

$\mathbf{W_S}$ - winter soil moisture reserve (mm/m^2);

$\mathbf{P_I, P_{II}, P_{III}}$ ... - amounts of precipitation for each month (mm/m^2);

$\mathbf{E_I, E_{II}, E_{II}}$ … - evaporation for each month (mm/m2).

The soil resources in the area were studied and classified during the large-scale soil mapping in M1:10 000, carried out by specialists-soil scientists of IPAZR "Nikola Pushkarov" (Soil characteristics and soil maps of the lands of Silistra region (1970-1990)). The present article uses the information from 92 soil-cartographic studies, which cover the lands of all settlements on the territory of the district. The soil differences are grouped into agro-soil groups and subgroups, using the extended systematic list for agro-grouping of soils based on the soil-cartographic studies and the classifications of (Antipov-Karataev *et al*.,1960; Koinov *et al.*, 1964).

This list continues to be used in practice in the country to this day (Agrogrouping of soils in the Republic of Bulgaria. Extended systematic list. Code of groups and soil differences, 1976). The main criteria for this grouping are the genetic and production characteristics of the soils. The agrosoil group brings together soil differences at the type-subtype level.

The names of the soils are defined according to the Bulgarian classification (Koinov *et al*., 1964) and the World Reference Base for Soil Resources (Teoharov, 2004) (Table 3).

In fig. 1 shows the location of the Silistra district on the territory of Bulgaria.

Figure 1. Location of Silistra region on the map of Bulgaria

Results and discussion

Silistra region falls into two climatic regions: the northern climatic region of the Danube Hilly Plain covers the lowest part of the district bordering the Danube River. Its climate is sharply continental: cold winter with minimal rainfall and hot summer with maximum rainfall. The annual amount of precipitation is 500 - 611 mm, and the average annual temperature is 11.6°C (Tables 1, 2). The meteorological station in the city of Silistra and the rain gauge stations listed in Table 2 are located in this climatic region (Climatic reference book for the Republic of Bulgaria, 1979, 1983, 1990).

Table 1. Average monthly and annual air temperature (^{0}C)

Station	Months												Average value
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Silistra	-1,7	0,8	5,3	11,8	17,3	20,8	23,0	22,4	18,2	12,6	7,1	1,5	11,6

Table 1 shows that the average multi-year air temperature in the area is 11.6^0 C. Seasonal temperature fluctuations are most pronounced in spring and autumn, when, depending on the shape of the relief, conditions for colds and frosts are created.

Table 2. Average monthly and the annual amount of precipitation (mm/m^2)

Station	Months												Average value
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Silistra	37	29	32	39	51	66	55	39	31	36	47	39	500
Tutrakan	40	43	38	50	70	72	58	53	46	39	53	50	611
Dulovo	45	40	37	53	73	74	52	43	41	39	60	49	607
Nojarevo	38	36	34	46	71	72	50	50	42	42	52	45	579
Alfatar	36	30	32	47	66	73	52	48	41	41	50	39	556

Precipitation during the year is characterized by a pronounced summer maximum (May and June) and a winter-spring minimum (February, and March). The total annual amount is 556-611 mm/m2 [4], and their distribution by seasons is as follows: winter - 105-135 mm/m2; spring – 122-165 mm/m2; summer – 159-173 mm/m2; autumn – 113-140 mm/m2. Table 2 shows that rainfall during the growing season cannot provide the necessary moisture for the development of some crops, such as vegetable and technical crops, corn, soybeans, etc.

The zoning of the country according to the degree of heat supply [the sum of air temperatures above 10 0C for the period June-August] divides the territory of the Silistra region into two regions - "hot" with a temperature sum of 4100 0C, located in a narrow strip along the Danube river, between Tutrakan and the town of Silistra, and "moderately hot" with a temperature sum of 4100-3700 0C, which covers the territory to the south, in the municipalities - Zafirovo, Sitovo, Glavinitsa, Alfatar, Dulovo, Kaynardzha.

In terms of moisture, the area falls into an arid zone with a pronounced deficit in the balance of atmospheric moisture from -200 to -300 mm in the June-August period (Hershkovich, 1984), which in dry years is a limiting factor for the development of certain crops.

The soil diversity in the Silistra region is represented by 723 soil-cartographic units, united into 5 agro-soil groups (APG) and 11 subgroups and some soil-cartographic units (Table 3).

The largest area (71.66%) is occupied by the soils of the second agro-soil group Leached Chernozems, non-eroded, slightly eroded and eroded (Haplic Chernozems and Eutric-Aric Regosols) - 150729.6 ha. The data for 392 soil profiles were processed showing that the depth of the soil profile was average 100 cm in non-eroded soils and 58 cm in eroded soils. According to the depth of the humus horizon, they are classified as low to medium depth (31-48 cm). These soils have favourable physical and chemical characteristics and are suitable for intensive agriculture. They are characterized as "very good lands" for wheat, corn, sunflower, sugar beet, large-leaf tobacco, alfalfa, etc.); permanent plantations - vineyards and fruit crops (cherries and apricots).

In second place by area (13.53%) are the soils of the first agro-soil group - Carbonate and Typical Chernozems, non-eroded, slightly eroded and eroded (Calcic Chernozems and Calcic-Aric Regosols), which occupy an area of 28453.3 ha.

Table 3. Agro-soil grouping of lands in the Silistra region

Agro-soil group and subgroup	Name of the soil according to the Bulgarian classification and WRBSR	Soil mapping units		
		Number of soil profile	ha	% of the total area of Silistra
01.1	Carbonate and typical chernozems, uneroded and slightly eroded /Calcic Chernozems/	17	2173,5	
01.2	Carbonate and typical chernozems, eroded /Calcic-Aric Regosols/	103	24452,6	13,53
01.3	Carbonate and typical chernozems - shallow, on hard rocks / Calcic-Aric Regosols/	34	1827,2	
02.1	Leached chernozems, uneroded and slightly eroded /Haplic Chernozems/	292	113640,5	71,66
02.2	Leached chernozems, eroded /Eutric-Aric Regosols/	100	37089,1	
03.1	Highly leached chernozems, non-eroded and slightly eroded /Luvic Chernozems /	45	15504,4	8,02
03.2	Highly leached chernozems, eroded /Eutric-Aric Regosols/	11	1356,8	
10.1	Alluvial and deluvial /District and Eutric Fluvisols/	99	10021,4	
10.2	Meadow-chernozems /Gleyic Chernozems/	9	3148,0	6,69
10.3	Meadow - swampy, marshy /Eutric Gleysols/	9	911,0	
11.3	Rendzina (humus-carbonate), shallow, on hard rocks /Rendzic Leptosols/	4	220,0	0,10
Total		723	210344,5	100

This group unites soils with similar pedogenesis, but with different productive potential. Data from 154 soil profiles were summarized. The thickness of the soil profile for non-eroded soils is average 78 cm, and for eroded soils - 29-30 cm. They were formed on loess. The thickness of the humus horizon is 25 to 47 cm. In terms of mechanical components, they are medium sandy clay with a physical clay content of 39-42% (particles <0.01mm %).

The content of humus on the surface horizon of arable lands is 2.2-2.4% (weak humus). The soil reaction is slightly alkaline (pH in H_2O – 7.6). Shallow soils and those formed on hard rocks have a shortened soil profile with a total thickness of about 18 cm (Table 4). They are unsuitable for agriculture, although they have a favorable humus content (3.3%). They are usually used as lawns-meadows and pastures. The remaining soil types are most suitable for growing vines, cherries, wheat, sunflower, corn, large-leaf tobacco, etc.

Highly leached chernozems (Luvic Chernozems-3rd agrosoil group) occupy an area of 16861.2 ha (8.2%). These soils have a well-developed soil profile with a thickness of 105 cm in the non-eroded and 62 cm in the eroded varieties. The thickness of the humus horizon is in the range of 28-39 cm (weakly thick). The content of humus on the surface horizon is 1.8-2.2% (weak humus). In terms of mechanical components, they are heavily sandy-clay throughout the depth (physic clay content 44-46%). The soil reaction is neutral (pH in H_2O – 6.3-6.6). These indicators characterize them as "very good lands" for growing wheat, corn, sunflower, alfalfa, plums, cherries, etc.

Table 4. Average values of physicochemical indicators by agro-soil groups and subgroups for the region of Silistra region

Agro-soil group and subgroup	Content of Physical clay (%) (particles <0.01mm %)		Depth (cm)		pH	Humus	Groundwater level
	surface soil horizon	sub horizon of soil	humus	soil	(H_2O)	(%)	(cm)
01.1	42	39	47	78	7,6	2,4	deep
01.2	39	36	25	29	7,6	2,2	deep
01.3	40	40	18	18	7,6	3,3	deep
02.1	45	46	48	100	6,8	2,4	deep
02.2	44	43	31	58	7,0	2,3	deep
03.1	44	49	39	105	6,3	2,2	deep
03.2	46	46	28	62	6,6	1,8	deep
10.1	43	45	117	133	7,3	2,7	deep
10.2	46	47	71	105	7,5	2,6	350
10.3	52	52	76	84	7,7	3,0	88
11.3	47	47	17	20	7,5	5,6	deep

The soils of the 10th agro-soil group occupy 6.69% or 14080.4 ha of the total area of the district. The data from 117 soil cartographic units are presented and summarized. The soils of this group have different profile depths and different productive potentials for growing crops. The deepest soil profile has Alluvial-meadow soils (133 cm). Followed by the Meadow chernozems Soils - 105 cm and the smallest depth of the soil profile- Meadow-swamp soils (medium swampy and swamp soils) - 84 cm. The depth of the humus horizon is average 71 to 117 cm. The content of humus on the surface horizon is in the range of 2.6-3.0% (this ranks them in the group of medium humus soils). In terms of mechanical components, they are medium to heavy sandy clay on the surface horizon (physical clay content 43-52%). Alluvial-meadow soils (Dystric and Eutric Fluvisols) and Meadow Chernozems (Gleyic Chernozems) are some of the most fertile soils in the district. They belong to the group of "very good lands" for growing vegetable crops - pepper, tomatoes, followed by wheat, vines, corn, sugar beet, sunflower, large leaf tobacco, alfalfa, etc.

The Rendzic soils (humus-carbonate soils - Rendzic Leptosols) occupy very small areas - 220.0 ha (0.1%) of the total area. The soils of the 11th agro-soil group are eroded and shallow with a soil profile (20 cm) developed on hard rocks. The thickness of the humus horizon coincides with the thickness of the profile (20 cm). Their agro-soil characteristics are presented in the table. 4 define them as suitable for meadows and pastures.

Conclusion

According to the degree of heat supply, the territory of the Silistra region is divided into two regions - "hot" with a temperature sum of 4100 0C, located in a narrow strip along the Danube river, between the town of Tutrakan and the town of Silistra, and "moderately hot" with a temperature sum of 4100- 3700 0C, which covers the territory in the south, in the municipalities of Zafirovo, Sitovo, Glavinitsa, Alfatar, Dulovo, Kaynardzha. In terms of humidity, the area falls into a zone with a pronounced deficit in the balance of atmospheric humidity from -200 to -300 mm for the period June-August. This is a limiting factor for obtaining reliable yields from agricultural production. It is necessary to revive the irrigation systems that were destroyed in the last 30 years. In such areas, irrigation gives extremely good results, and the area is rich in water resources (using the waters of the Danube). The agricultural lands in the Silistra region are fertile and suitable for growing a large number of crops. Almost 95% of the agricultural lands in the district have very good physical and chemical indicators. With proper zoning of crops, fertilization and application of irrigation (when necessary), very good yields can be obtained. The balance of soil resources shows that the territory of the district is occupied by fertile soils that must be protected from degradation (erosion and other unfavourable factors resulting from their intensive use).

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MULTIFACETED APPLICATION OF SUPERWORM (ZOPHOBAS MORIO F.) IN THE ZERO-WASTE CIRCULAR ECONOMY APPROACH

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Abstract

Insects are the most abundant group of animals in the world. About 1 million species have already been recorded in this group, and this number increases by several hundred new items every year. They perform many functions that can positively affect the surrounding environment. One of the most exciting families is Tenebrionidae, numbering about 20,000 species (Nabozhenko, 2019), of which 86 species live in Poland (Iwan et al., 2010). Many species are found in the natural environment, specifically in rotting wood and litter. One of the species, found in decaying wood is the superworm (*Zophobas morio* F.). *Zophobas morio* reaches 3-4 cm in length. The adult insect has a massive, elongated, black, slightly dull body. Larvae are light cream, with brown around the head and on the border of the segments. They have three pairs of short legs and almost invisible antennae. The paper presents preliminary studies involving superworm larvae, demonstrating the possibility of its comprehensive use in the surrounding environment. Due to their high protein content, superworm can be used as food for humans and animals. Post-cultivation residues (frass) can be composted and then used as fertilizer. The digested residues can also be used to produce ethanol. Obtained digestate can be used to fertilize trees such as poplar and pine. The superworm, like the mealworm, can also digest difficult-to-digest waste such as plastics. Although Superworm is a wood pest, this insect has a wide range of applications in science and economy.

Keywords: *insects, Zophobas morio, fertilization, protein source, ethanol.*

Introduction

People's consumption oriented lifestyle constantly contributes to the massive use of resources and the production of goods. This causes resource depletion and environmental problems. Items such as clothing and electronics used to be luxury goods, and now they are at everyone's fingertips (Crocker, 2013, Moore et al., 2011). The amount of plastics introduced into the ecosystem is over 8,300 million tons, still an increasing trend (Geyer et al., 2017). Due to dynamic changes at the economic, environmental and social levels, the food system is also subject to constant intensification. A growing population forces an increase in food production. Therefore, given the current social, environmental and economic changes, there is a need for continuous improvement in food efficiency, quality and safety (Dubey et al., 2008, Smith and Gregory, 2013). Together with our actions, we are getting closer and closer to the climate crisis. Therefore, reducing global greenhouse gas emissions, rational waste management, preventing incineration and reducing plastic production are increasingly important. The zero-waste idea is to rebuild ecosystems and design ways to grow with them, not against them. In addition, the program promotes ways to fight negative environmental changes that are satisfactory for both people and the planet (https://zerowasteeurope.eu).

Zero waste is a holistic approach to solving waste problems in the 21st century. This program is still in the development phase. Scientists constantly propose various plans and strategies to achieve the goals set under this program (Zaman, 2015). One such idea might be to use insects to reduce waste and obtain a food source in a way that is less degenerating to the planet. Insects are one of the most diverse and widespread groups of animals. They are characterized by a multitude of forms, colours and functions. We can find them in many environments around the world. Insects and arachnids account for 60% of global biodiversity (Kim, 2017). It is such a large group of organisms that many species are still undocumented, and those included in the list of discovered species are still not well characterized and described. Almost a million species of insects have already been described. This is probably much less than half of the representatives of this cluster living in the world (Boczek and Pruszyński, 2015). Insects play various roles in many aspects of human life. They are pests and parasites; that are generally associated with something negative. They also have positive functions. Most insects found among cultivated plants do not adversely affect their development. Some species may limit the number of pests found in arable fields (Pruszyński, 2013). Insects can also be bioindicators showing the condition of a given environment. Representatives of this group of organisms are also pollinators of many plants. They are also used in forensic medicine (Boczek and Pruszyński, 2015). They are also often a source of inspiration as a model of cleanliness, diligence, or defence against danger. In many parts of the world, slowly also in Poland, they are becoming a food source for animals and people. They are often characterized by high protein content and easy breeding, which brings less environmental losses than other animals (Kosewska, 2019).

An example of an insect that plays many roles in the environment is the superworm (*Zophobas morio* F.). This insect is mainly associated with a wood pest. However, it also plays a positive role in the environment and may contribute to its improvement. The study aimed to analyze the multifaceted use of insects, the example of superworm (*Zophobas morio* F.), mainly as utilization of waste with a long decomposition period, the use of its faeces for fertilization, its use as a substrate for the production of biodiesel, or as a source of protein (Figure 1). The analysis was based on the literature data and own experiment.

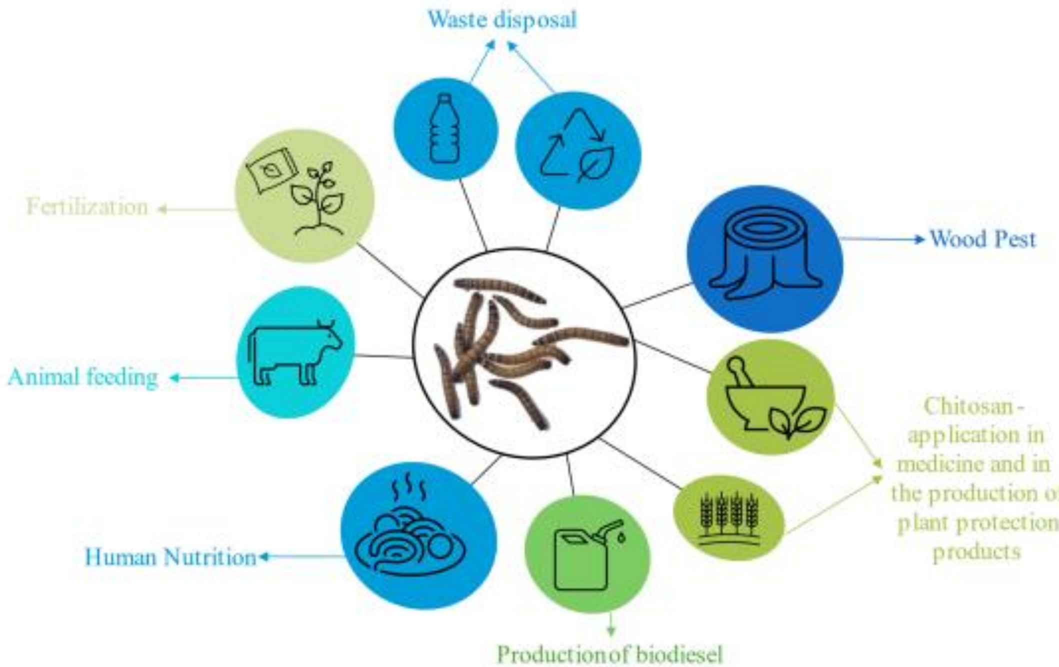

Figure 1. Areas in which superworm (*Zophobas morio*) larvae are used - own study

Material and Methods

The first stage of the work was a meta-analysis of the available information on the use of the super worm in various economic aspects. Various public browsers (Google, PubMed, Scopus) were used to create a database of publications on superworms. The study defined scope and used reliable sources to identify relevant scientific literature. In the study, the available literature was classified and critically assessed in terms of its accuracy (e.g. environmental protection, agriculture, food safety, greenbiotechnology, circular economy). Multivariate segregation was performed in MS Excel using pivot tables. After the analysis, the most important possible applications of the super worm in the zero-waste economy were selected and discussed. The MS Excel package and graphic programs: PowerPoint and Paint 3D were used to develop and visualize the results.

Results and Discussion

The results of a thematic search using a google search engine for topics related to superworm and its use in various areas of the economy were significant, which proves that this topic is more and more popular. The total number of views for *Zophobas morio* was 351,000, with a significant proportion of topics related to nutrition, plastics, waste, and biotechnology (Figure 2).

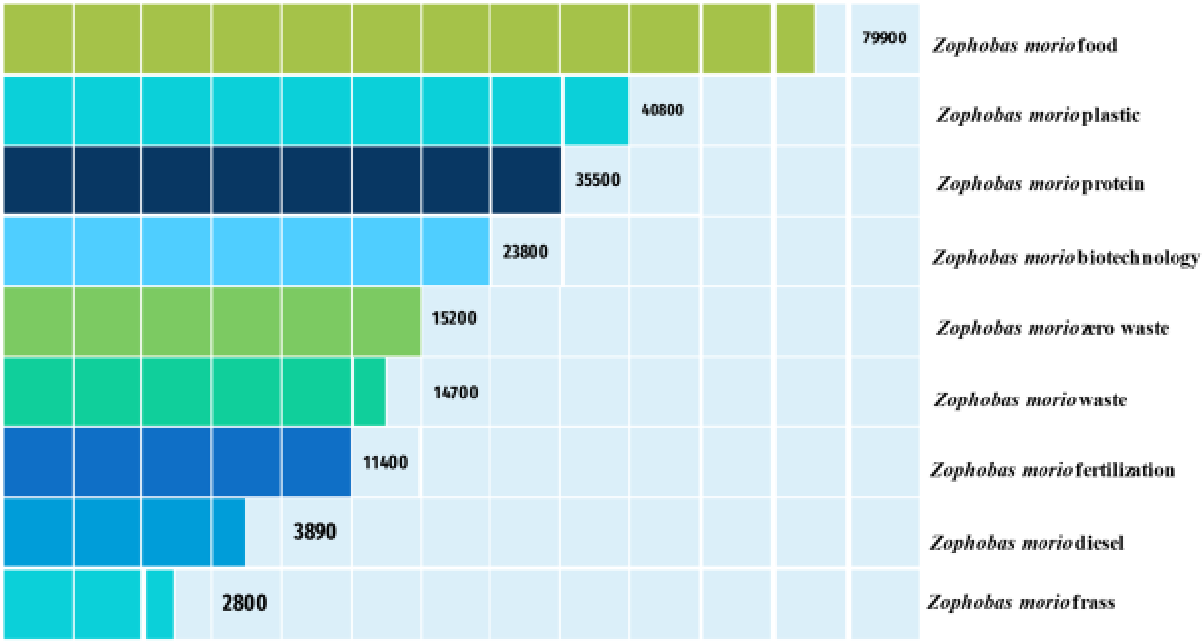

Figure 2. The number of views for the topic related to superworm (*Zophobas morio*) based on the google browser

Zophobas morio production of biodiesel

Due to the increase in energy demand and the limited supply of conventional diesel fuel from fossil fuels, biodiesel production is becoming a global problem (Rayan et al., 2006, Manzano-Agugliaro et al., 2012, Rastogi et al., 2018). Biodiesel is an environmentally friendly energy source and is a blend of fatty acids, methyl esters or ethanol esters that are usually obtained from renewable raw materials such as animal fat, vegetable oil and waste fat (Leung et al., 2010). As insects are one of the wealthiest biological resources in the world, many of which have shorter life cycles than plants and animals, they began to be used as a potential biodiesel substrate (Wang et al. 2019, Nguyen et al. 2019). Unfortunately, there is no scientific research on insects in biodiesel production. However, this is changing. An example is the company EcoSystem Corp., which has been researching a new biofuel production scheme: growing fly larvae with food waste and converting the fat they produce into fuel. They currently produce oil under the name MAGOIL ™ by EcoSystem, which goes to the Biofuel Industries Group in Michigan (Manzano-Agugliaro et al., 2012). One of the insects used in the production of biodiesel is the superworm. These insects can be bred all year round and quickly reach their maximum body size under good conditions. Scientists report that superworm larvae have an oil content of about 1/3 of their dry weight, which is higher compared to *Bombyx mori* (silkworms) and *Lumbricus terrestris* (earthworms) (Finke, 2002). Research has shown that from 1 kg of fresh larvae, about 145.5 g of larvae oil can be obtained. These studies suggest that superworm larvae oil can be used as a raw material for biodiesel production. After specific processing of this raw material, we obtain biodiesel that meets the requirements of ASTM D6751 (Leung et al., 2012).

Zophobas morio – Chitosan

Chitin is a linear biopolymer with β- (1 → 4) -N-acetyl-d-glucosamine monomer. It is biocompatible, biodegradable, non-toxic and highly insoluble in most polar and non-polar solvents (Kumirska et al., 2010). Chitin fibre is abundant in the epidermis of insects, including the epidermis of superworms. Chitin bio sources contain a significant amount of protein. Therefore, a deproteinization process is performed to ensure the high purity of chitin (Soon et al., 2018). Chitosan is used to improve health. This is due to its biocompatibility, antibacterial effect and properties as an environmentally friendly polyelectrolyte. Chitosan has been used successfully in various fields, such as environmental applications. It is used to produce paper or textiles to obtain antimicrobial properties. It is also used in medicine, e.g. in tissue engineering, wound healing, obesity treatment and the prevention of vascular diseases. In the food industry, it is used to produce a film for packaging food (Shin et al., 2019). Chitosan is also used in agriculture. It can chelate various organic and inorganic compounds, making it excellent for improving the stability, solubility and biocidal activity of chelated fungicides or other pesticides. Research into chitosan in agriculture also concerns the creation of plant growth regulators, soil conditioners, anti-staling agents in vegetables and fruits, and seed coating agents, especially in crop disease management (Kashyap et al., 2015).

Zophobas morio – Waste disposal

Superworm larvae feed mainly on rotted deciduous wood but survive on a diet consisting of plastics, such as polystyrene, which can break down into biodegradable components (Yang et al. 2020). Therefore, they can also be used as a natural utilizer of waste with a long biodegradation period. Scientists (Yang et al., 2015, Yang et al., 2020) have found microplastics and plastic monomers in the frass or body of the larvae, but this can be overcome by prolonged incubation with insects, given that PS degradation by the larvae increases with time. This gives hope to creating a better plastic waste management system. It should also be considered that polystyrene waste is very often in food packaging. They contain food remnants that need to be cleaned before further recycling. Natural degradation of plastic waste using insect larvae eliminates the need to clean dirty packaging, and food debris can provide an additional source of nutrients for the larvae. This disposal method is further supported by the fact that superworms can be a food source, and the frass produced is a natural fertiliser. This enables waste-free conversion of PS waste, a goal defined by the Zero Waste International Alliance and the Eco-Cycle Solutions Hub (Gan et al., 2021).

Zophobas morio – Fertilization

Superworm larvae can be bred to excrete nitrogen, and the frass they produce can replace traditional NPK fertilizers (nitrogen, phosphorus, and potassium) in a circular economy (Houben et al., 2020). Insect frass can be a substitute for commercial fertilizers. An example is the fertilization of maize or ryegrass (Beesigamukama et al., 2020, Menino et al., 2021). Based on our results from the experiment with superworms, in which the larvae were fed with poplar, it was observed that frass had almost 14 times more total protein than wood, 2.4 times more water-soluble sugars, half the content of indigestible fibres, and more than two times higher content of the mineral fraction than wood (unpublished data). The results indicate the ability to digest wood by the larvae and the possibility of obtaining a substrate that is bioconverted to protein and easily fermentable sugars and residues that can be used as mineral fertilizer.

Zophobas morio – Animal feeding and Human nutrition

The food system is essential to the world economy and society (Béné et al., 2019). Dynamic changes at the economic, environmental and social levels, and especially the growing number of people, force an increase in food production. Therefore, there is a strong need for new protein sources, both in human and animal nutrition. Additionally, the animal feed industry is looking for new protein sources to reduce dependence on soybean and fish meals. The search for other protein sources is also related to the need to produce food that would not destroy the environment as much as conventional livestock farming (reducing greenhouse gas emissions, water consumption, and deforestation) (Rumbos and Athanassiou, 2021). Over the past few years, treating certain insect species as a source of food for humans and animals has become increasingly popular. Insects are highly nutritious and efficient substrates for feed production (Siemianowska et al., 2013). Their breeding is not very complicated. They can be fed with plant residues and agricultural waste, thus in line with the circular economy strategy. One insect species with great food and forage potential overlooked by scientists and insect producers is *Zophobas morio* (Rumbos C. I., Athanassiou). Superworm contains 46.80% proteins, 43.64% lipids, 8.17% ash and 1.39% carbohydrates. Their nutritional value can be safely compared to other food sources known to be nutritious, providing good indicators to support and promote the importance of the food potential of these insects (Araújo et al., 2019).

Conclusions

Insects are a group of organisms that can be used in many areas of our lives. They can be both bioindicators, waste utilizers and food sources. One of the representatives that are used in many areas is the superworm. Based on the analysis of articles concerning this species, it was possible to conclude that it can be a raw material for the production of biodiesel and chitosan. This insect can recycle plastic waste, and the frass produced can be used to promote plant growth, while superworm larvae can be a source of food for humans, poultry and fish. Perhaps superworms can be the answer to solving the problem of environmental pollution by plastics and problems related to food production (aquaculture and agriculture). An additional advantage is a fact that an insect used at different stages of its development and in various fields can contribute to the creation of a circular economy.

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DEVELOPMENT OF AGRICULTURAL WATER QUALITY INDICES FOR UKRAINE

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Abstract

The cultivation of environmentally friendly agricultural products is limited by the quality of soils, water resources, etc. The results of monitoring the state of natural waters, organized according to the existing scheme, which is based on the comparison of water composition with MPCs, do not answer the main question: whether water sources are suitable for a particular type of water use; how, based on long-term data, to give a valid forecast of quality change for decades, to determine critical parameters. We propose a conception of national water quality indexes for agriculture applications – irrigation, including drip, fertigation, greenhouse soils, for low-volume substrates. Idea is to unite the agronomic, ecological, and technical requirements in a 100-scale numerical assessment using Harrington’s desirability function. In contrast to SAR, or WQI NSF, it is possible to combine any quantity of quality parameters in one result. In addition, it is possible to differentiate the requirements by taking into account the types of soils and plants. The most controversial moment is how to determine the scale of water quality parameter differentiation (very good-good-satisfactorily-bad-very bad). The defect is that different normative documents and authors give different recommendations. It is clear because different countries have different environmental conditions, soils, waters, etc. We are based on Ukrainian requirements for the different types of surface waters, soils, and climate zoning and a few methods such as SAR, and Irrigation coefficient according to Stebler. Finally, we developed the Python WODA application for the calculation of WQI based on Harrington's desirability function.

Keywords: *National indexes, water quality assessment, agriculture, Ukraine.*

Introduction

Ukraine is one of the most important food producers in the world. As was shown by the last tragic events, Ukrainian corn, sunflower oil, and wheat are critically needed for the prevention of global hunger. But the many other factors have a negative influence on agriculture production. One of them is climate change which leads to the increasing lack of water sources (Safranov, 2016, Khilchevskyi, 2020a). In many farms, irrigation is the only limiting factor of yield. Global climate change has led to significant shifts in the local climatic conditions of Ukraine (Fig. 1), with a tendency to widening of low soil moisture agriclimatic zones. According to obtained data, the general trend toward increased aridity has been detected. It has been established (Lykhovyd, 2021) that semi-arid, dry sub-humid, and humid zones are observed not only in the southeastern regions of Ukraine as 30-50 years ago but in central and western regions too. Based on the analysis of the aridity index and soil moisture regimes, it was established that near 46 %

of Ukrainian arable land cannot provide sustainable crop production without irrigation, 42.65 % need irrigation to cultivate crops with high water consumption, and only 11 % of agricultural land can provide enough yield without irrigation.

Figure 1. The shifting of the agroclimatic zones of Ukraine during 30 years: A – 1990; B – 2020 (source: landlord.ua)

Water in agriculture is used not only for irrigation, drip irrigation, and fertigation but in many other fields: for livestock and poultry drinking, for human consumption, fisheries, and aquaculture. It is clear, that the requirements for water quality are different for these applications. For example, the content of dissolved oxygen isn't important for drinking consumption, but it is the main qualitative criterion for fisheries.

Materials and methods

Water quality indexes (WQI) may be very useful for water management in agriculture (Gitau, 2016, Gruère, 2021). They are the communication models for making decisions in the planning of agricultural production, for the comparison of water quality from different sources, etc.
Existing WQI for agriculture are based on different criteria and are based on the local water supply guidelines, water compositions, types of water sources, etc. (Fernandez, 2004, Tyagi, 2013, Shajedul Islam, 2022). Developing of WQI, there are a few principal solutions that must be done by the authors: (i) how to choose the most important parameters of water quality: (ii) how to transform them into dimensionless values, (iii) how to determine the scores and/or weighting coefficients of each parameter; and (iv) how to aggregate them as an overall WQI value (Banda, 2020).
We propose the next approaches to the above mentioned problems:

- A list of water quality parameters can include all existing water properties or concentrations of dissolved and/or suspended components, which are regulated/controlled for the different supplies of water used for agriculture;
- We propose the possibility to choose for WQI calculation the most important parameters or the ones which are controlled for the concrete water body depending on the user's requirements;
- The transformation of the measured values of water parameters and their aggregation in one numerical assessment on a 100-score scale we have done using Harrington's desirability function (Bikbulatov, 2011, Voitenko, 2017).

The general concept of WQI compilation is presented on the Fig. 2. In addition, we propose the separate blocks for the calculation of the previous assessment of irrigation water quality according to Parker & Israelson’s approach, SAR, Stebler’s irrigation coefficient (Tanji, 1990), Budanov’s approach for irrigation of south Ukrainian soils (Budanov, 1962), Antipov-Karataev approach for the assessment of soil salinity risk (Antipov-Karataev, 1959); risk of magnesium salinity according to Szabolcs, Varallyay and Darab approach (Szabolics, 1982) method of water quality assessment based on the concentration of toxic salts expressed in Chloride equivalents (FAO Handbook, 2018). Separate program blocks permit to calculate WQI for drip irrigation, greenhouse soils, and low-volume substrates, which are very sensitive to the composition of water.

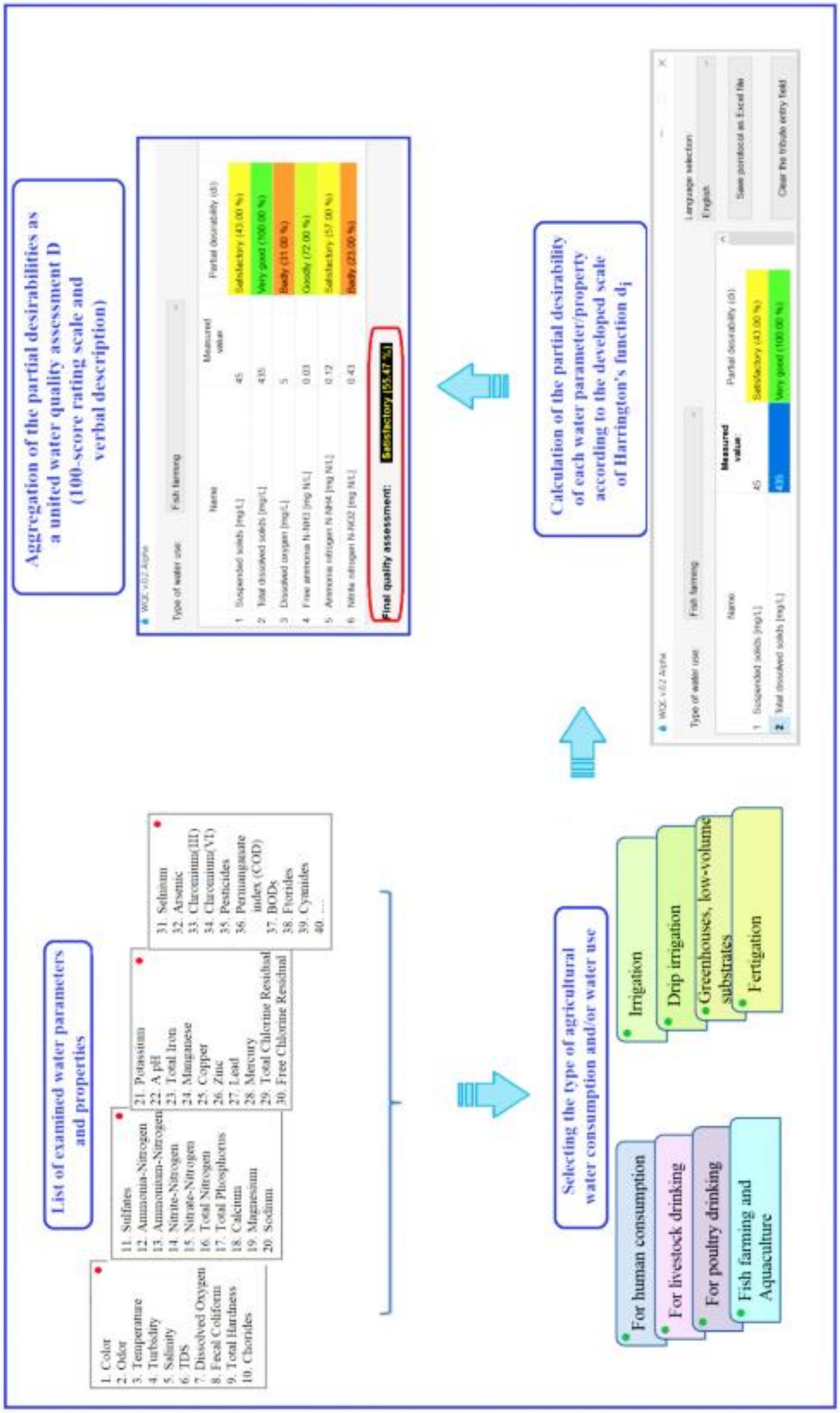

Figure 2. The structural-logical scheme of WQI compilation for different areas of water using in agriculture

Analogically, it is possible to choose the varieties of animals or poultry, if the user wants to assess the water quality for livestock/poultry drinking.

Results and Discussion

The main element of the WQI structure, which can cause justified criticism, is the way of compiling the rating scale for each parameter or the weighting coefficients for individual parameters (Uddin, 2021).

It should be noted that water quality indexes are widely used throughout the world, as they are a convenient tool for a comprehensive assessment and monitoring of the aquatic environment. But for good reason in different countries they have their own names: for example, BC WQI (British Columbia water quality index), CCME WQI (Canadian council of ministers of the environment water quality index), DWQI (Dinius Water quality index), etc (Gupta, 2021). It should be noted that water quality indexes are widely used throughout the world, as they are a convenient tool for a comprehensive assessment and monitoring of the aquatic environment. But for good reason in different countries they have their own names: for example, BC WQI (British Columbia water quality index), CCME WQI (Canadian council of ministers of the environment water quality index), DWQI (Dinius Water quality index), etc (Gupta, 2021). Obviously, such specifics have reasons for the significant differences in the composition and properties of local natural water sources, soils, applied irrigation methods, the availability of water treatment systems for watering livestock and poultry, and so on. Hydrochemical features of Ukrainian natural waters are their high hardness, alkalinity, and excess of iron, manganese, nitrates, and ammonium (Khilchevskyi, 2020b). Therefore, in our opinion, it makes sense to develop water quality indices for agricultural use, taking into account the national characteristics of both agricultural production and the composition and properties of water resources.For the compilation of the quality scale ranges we have used Ukrainian guidelines and standards which are developed for different water supply purposes (DSTU 4808:2007; DSTU 2730;2015; Normative VND 33-5.5-02-97; Standards of Ukrainian organisations SOU 41.00-37-422:2006 SOU 05.01-37-385:2006).

When developing scales for the assessment of individual parameters, it is important to consider that their limitations can be of a different nature - one-sided (when the MPC value is set) or two-sided, when the parameter is limited by a certain range of optimal values.

Tables 1-3 are shown the principles of scale compilations of a few parameters of water quality assessment for irrigation and fisheries. We would like to stress the special attention to the principles of heavy metals concentration scales. It is well known, that soils, animal and fish bodies can collect and accumulate toxic metals (Jezierska, 2006, Gworek, 2020). Unfortunately, the analytical methods of heavy metal concentrations are characterized by high relative errors as usual, which can reach 20-30 % (Shimizu, 2019). Therefore, for the "very good" range of trace metals and other toxic microelements, we limited their concentration to the value of half the MPC value, and "good" - to the MPC value, taking into account the possible errors of their detection.

Table 1. Assessment of water quality for irrigation: Two-sided limitation for parameters having an optimal range for the calculation of Harrington’s desirability function.

Parameters, units	Partial desirability d_i of a parameter									
	0-0.20 very bad	0.20-0.37 bad	0.37-0.63 satisfactory	0.63-0.80 good	0.8 0-1.00 very good	1-0.80 very good	0.80-0.63 good	0.63-0.37 satisfactory	0.37-0.20 bad	0.20-0 very bad
TDS, mg/L	0-80	80-100	100-150	150-320	320-400	400-480	480-1000	1000-3500	3500-5000	5000-10000
Temp, °C	0-8	8-10	10-12	12-15	16-18	18-22	22-25	25-29	29-32	32-45
a pH, pH units	4.5-5	5-5.4	5.5-6	6-6.5	6.5-6.8	6.8-7.2	7.2-7.5	7.5-8.5	8.5-9	9-10.5
SAR	0.1-0.8	0.9-1.2	1.2-1.5	1.5-2	2-2.5	2.5-3	3-6	6-12	12-20	20-40

Table 2. Assessment of water quality for irrigation: One-sided limitation for parameters (i.e., having maximum permitted concentration (MPC) for the calculation of Harrington’s desirability function.

Parameters, units	Partial desirability d_i of a parameter				
	1-0.80 very good	0.80-0.63 good	0.63-0.37 satisfactory	0.37-0.20 bad	0.20-0 very bad
Stebler's irrigation coefficient A	25-18	18-6	6-4	4-1.2	1.2-0.2
Permanganate index (COD), mg/L	0-15	15-25	25-40	40-60	60-200
Turbidity, nephelometric turbidity units, NTU	0-5	5-10	10-50	50-200	200-5000
Nitrogen of nitrates, mg $N\text{-}NO_3^-$/L	0-4	5-15	16-20	21-30	30-1000
Total iron Fe, mg/L	0-0.05	0.05-0.2	0.2-1	1-5	5-20
Boron B, mg/L	0-0.4	0.4-1	1-2	2-5	5-30

Table 3. Calculation of WQI for fisheries: one-side limitation for parameters (i.e., having maximum permitted concentration (MPC) for the calculation of Harrington’s desirability function.

Parameters, units	Partial desirability d_i of a parameter				
	1-0.80 very good	0.80-0.63 good	0.63-0.37 satisfactory	0.37-0.20 bad	0.20-0 very bad
TSS, mg/L	0-10	10-25	25-50	50-100	100-2000
TDS, mg/L	0-500	500-1000	1000-5000	5000-15000	15000-30000
Dissolved oxygen, mg/L	14-9	9-7	7-6	6-3	3-0

Free ammonia N-NH_3, mg N/L	0-0.005	0.005-0.05	0.05-0.2	0.2-0.5	0.5-5
Ammonia nitrogen N-NH_4^+, mg N/L	0-0.005	0.005-0.025	0.025-0.39	0.39-0.5	0.5-5
Total nitrogen, mg N/L	0-0.03	0.03-0.15	0.15-0.9	0.9-5	5-200
Total phosphorus, mg P/L	0-0.2	0.2-0.5	0.5-1.5	1.5-4	4-15
Permanganate index (COD), mg/L	0-10	10-15	15-30	30-50	50-200
Total Alkalinity, mmol/L	0-2.5	2.5-6.5	6.5-10	10-15	15-25
BOD_5, mg O_2/L	0-2	2-4	4-6	6-10	10-50
Total iron Fe, mg/L	0-0.05	0.05-0.2	0.2-1	1-5	5-20
Cadmium Cd, mg/L	0-0.001	0.001-0.005	0.005-0.01	0.01-0.1	0.1-1
Lead Pb. mg/L	0-0.005	0.005-0.01	0.01-0.05	0.05-0.5	0.5-2
Mercury Hg, mg/L	0-0.0002	0.0002-0.0005	0.0005-0.0010	0.0010-0.0025	0.0025-0.1000

Conclusion

Thus, the conditions of climate change require the search for new scientific solutions in the field of water management, since water is the second most important factor in agricultural production after soil. Ukraine is one of the most significant producers of food in the world. Rationing water quality for different types of consumption requires to development of a convenient, scientifically correct tool for a comprehensive assessment of its suitability for a particular type of water consumption. We have proposed such a tool in the form of a water quality index built using the Harrington Desirability Scale. To create desirability scales, we used not the traditional approach in the form of the Delphi method (peer review), but Ukrainian standards, which present the requirements for water quality for various agricultural industries.

Based on the above-described ideas, we developed the Python application “WODA” (Water Of Different Application) for the calculation of the united water quality assessment for different areas of water consumption.

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APPLICATION OF HIGH SALT-TOLERANT MICROORGANISMS IN ORDER TO IMPROVE THE QUALITY OF WASTEWATER FROM AQUACULTURE IN TIEN GIANG, VIETNAM

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Abstract

In Vam Lang district of Tien Giang province, in South Vietnam there is a high density of aquaculture with 15,749 ha area, in which the saltwater aquaculture area is 9,223 ha. The environmental problem is that the large amount of saline wastewater has been discharged to the river from this activity. The salinity of this wastewater can be calculated according to NaCl concentration of 3-30g/L. In saline wastewater with such high concentration there are few organisms, which can survive because of the protoplasm contraction. The aim of this study was to isolate and select high salt-tolerant microorganism in order to improve the quality of such saline wastewater. Therefore a method had to be developed for isolating and selection of the best salt-tolerant microorganism strains at a minimum salinity of 3%, with the treatment efficiency of NH4+ > 50% and COD (chemical oxygen demand) > 70%. The best salt-tolerant microorganisms are isolated from the saline wastewater itself and also from high salinity wastewater sources such as from seafood processing factories, e.g. fish sauce factories. The idea was that these strains can have a good adaptation on salty water environment. As the result of the investigations it can be said that the saline aquaculture wastewater could be classified as well the appropriate strains of the microorganisms. Based on the findings group of strains of salt-tolerant microorganisms or a mixture of selected strains could be detected and described. A method for practical use for the best wastewater treatment could be described.

Keywords: *saline wastewater, aquaclture wastewater treatment, salt-tolerant microorganisms.*

Introduction

In Tien Giang, the fishing output reaches over 101,000 tons/year (Department of Natural Resources and Environment of Tien Giang Province, 2018). Along with this development, the seafood processing industry or the aquaculture activities discharge into water environment a large amount of saline wastewater, causing pollution of water sources (Ap Bac Journal, 2018). Currently, biotechnology is being widely interested in wastewater treatment, which highlights the potential application of salt-tolerant bacteria in the treatment of saline wastewater (Sohair I. Abou-Elela et all, 2010). However in Vietnam, it is a new field for studying. Many strains of salt-tolerant microorganism have been isolated and selected in some researches mainly for application of aquaculture, just only a minority of them for application in environmental protectio (Tran Minh Chi and Ma Song Nguyen, 2016)

In the other hand, a large number types of biological products using for wastewater treatment are traded in Vietnam (Thanh Nien Journal, 2018). However, purified bioproducts of high saline-tolerant microorganism are still not available in the market or just some bioproducts of the low salt-tolerant microorganism (tolerate at salt concentrations of 1-2%). While, the demand for high salinity wastewater treatment has not been met with the salinity up 3%, such as wastewater of tanning, textile and dyeing factories (Department of Science and Technology of Binh Duong province, 2017).

In this situation, the research was performed to meet the needs of eco-friendly products for wastewater treatment as well as for high salinity wastewater treatment systems in Mekong Delta region, particularly in Tien Giang. Salt-tolerant microorganisms was isolated mainly from saline aquaculture wastewater.

Material and Methods

1. Selection of wastewater samples for isolation

Saline wastewater was sampled in Vam Lang districk Tien Giang province, in different sources: The centralied wastewater area of Vam Lang fishing port and 3 ponds of fish culture. These samples were used for isolation salt-tolerant microorganism as well as for performing experiments at Biology laboratory of Institute of Science, Engineering and Environmental Management belong to Industrial University of Ho Chi Minh City.

Isolation, selection of salt-tolerant microorganism

Isolation: The salt-tolerant microorganisms was isolated by basal mineral salt medium (BMS) with the component of 1 liter medium, include: 3g $NaNO_3$; 1g KH_2PO_4; 0.5g $MgSO_4$; 0.5g KCl; 5g NaCl; 1g yeast extract and 20g agar dissolved with deionised water until 1000 ml medium, then pH was adjusted to 7.0 (Xiaohui Zhang, Jie Gao, Fangbo Zhao, Yuanyuan Zhao, and Zhanshuang Li, 2014). The medium was then sterilised in an autoclave at 121°C in 30 minutes. Besides, the saline wastewater was diluted by sterile physiological saline, then inoculated onto the surface of the BMS agar petri-plate with the amount of 0.1ml, after that incubating them at 30^{o}C during 24- 48 hours. Finally, the isolated single colonies was purified with plate streaking method through several times inoculation.

Selection: For the experiment of salt-tolerant capability, sodium chloride was added to BMS medium to make different salinity concentration: 1%, 3%, 5% and 7%. Monitoring the growth ability of bacterium colonies to determine the salinity tolerance of them. Afterward, the high salinity-tolerance microorganisms was selected.

2. Improving the quality of aquaculture wastewater by high saline-tolerant microorganisms

Before using for experiments the selected saline-tolerant strains was cultivated to get the biomass at 10^9cells/ml. On the other hand, in order to evaluate the ability of selected microorganisms for saline wastewater treatment, the experiment was performed with 3 individual experiments, in which the same amount of high saline-tolerant microorganisms were used in different combination, in different time.

<u>Experiment 1</u>: Using separately strain of saline- tolerant microorganisms to treat COD of aquaculture wastewater: from 10 selected high saline-tolerant strains, 10 tanks were prepared with the same volume of aquaculture wastewater (5000ml/tank) and the same amount of saline-tolerant microorganisms (250ml biomass/5000ml wastewater). COD was treated in aerobic condition in 96 hours. After 24h, 48h, 72h and 96h, the COD concentration was measured to estimate the treatment efficiency in different time. Carry out the experiment in parallel with the control sample without saline- tolerant microorganisms.

Experiment 2: Using the mixture of salt-tolerant microorganisms in the ratio 1:1 with 3 different groups: G1- using 4 highest efficiency strains; G2- using 4 best salt- tolerance strains; G3- using mixture of 8 strains from G1 and G2. In which, 3 tanks were prepared with the same volume of aquaculture wastewater (5000ml/tank) and the same amount of saline- tolerant microorganisms (250ml biomass/5000ml wastewater). The COD concentration was measured before and after 96 hours.
Experiment 3: Using the mixture of salt-tolerant microorganisms with three commercially wastewater treatment probiotics with the ratio 1:1. In which the amount of probiotic was used as recommended by the supplier.
The actual efficiency of treatment microorganisms for COD in wastewater is calculated according to the formula:

$$H = \frac{COD_{in} - COD_{out}}{COD_{in}} . 100\%$$

In which: H is the treatment efficiency of salt-tolerant microorganisms at a certain time; COD_{in} is input COD concentration of wastewater; COD_{out}: output COD concentration of wastewater.

Results and discussion

1. Evaluating the saline tolerance of microorganisms

Concentration of salt was selected at 1%, 3%, 5% and 7% in order to estimate the saline tolerance of 45 microorganism strains, were isolated from the saline aquaculture wastewater. The evaluation results and the saline tolerance of microorganism strains on different salt concentration are shown in Table 1. The evaluation results of saline tolerance of microorganism strain is shown in Fig.1.

Table 1. The saline tolerance of microorganism strains on different concentration

Microorganism strains	Salt concentration (%)				Microorganism strains	Salt concentration (%)			
	1	3	5	7		1	3	5	7
1A1	+++	++	-	-	3A1	+++	++	-	-
1A2	+++	+	-	-	3A2	+++	+	-	-
1A3	+++	+	+	-	3A3	+++	++	+	-
1B1	+++	+++	+++	++	3A4	+++	+	-	-
1B2	+++	++	-	-	3A5	+++	+++	++	++
1B3	+++	+	-	-	3B1	+++	-	-	-
1B4	+++	++	-	-	3B2	+++	-	-	-
1B5	+++	++	+	-	3B3	+++	+++	++	+
1C1	+++	+	-	-	4A1	+++	+++	++	+
1C2	+++	+++	+++	++	4A2	+++	-	-	-
1C3	+++	+	-	-	4A3	+++	-	-	-
1C4	+++	-	-	-	4A4	+++	-	-	-
2A1	+++	+	-	-	4A5	+++	+	-	-
2A2	+++	+++	+++	++	4A6	+++	+	-	-
2A3	+++	+	+	-	4A7	+++	+++	++	+
2A4	+++	-	-	-	4B1	+++	+	-	-
2A5	+++	-	-	-	4B2	+++	+	-	-
2B1	+++	+++	++	+	4B3	+++	+	-	-

2B2	+++	++	-	-	4B4	+++	-	-	-
2B3	+++	+++	++	+	4B5	+++	+	-	-
2B4	+++	-	-	-	4B6	+++	+++	++	+
2C1	+++	-	-	-					
2C2	+++	-	-	-					
2C3	+++	++	-	-					

Notes: (+++) well growth; (++) normal growth; (+) weak growth; (-) none growth

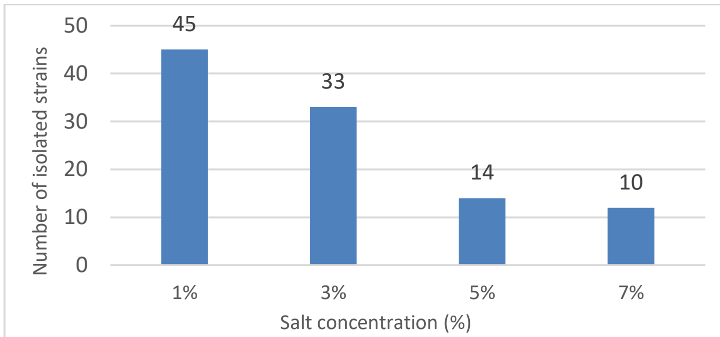

Fig. 1.The evaluation results of saline tolerance of microorganism strains

According to the results, 45 isolated strains grew well at salinity 1%, while 33 strains could grow at salinity 3% after 24 hours cultivation. At higher salt concentrations almost of strains grew slowly. After cultivate 48 hours, there are 14 strains could grew at salt concentration 5% and 10 strains could grow at salt concentration 7% (Fig.1). By this high concentration of salt, the process of ions transport through the cell membrane as well as the osmotic pressure of the membrane were directly affected. Based on the growth ability of these strains at salinity 7%, 10 best growth strains was continue selected for identification: 1B1, 1C2, 2A2, 2B1, 2B3, 3A5, 3B3, 4A1, 4A7, 4B6. Before using to treat aquaculture wastewater, these 4 high saline-tolerant microorganism strains were proliferated by shaking culture during 48 hours to obtain the suitable cell biomass. The results are showing in table 3.

Table 3. The result of proliferation after 48 hours

Microorganism strains	Cell density (cells/ml)		
	First time	Second time	Average
1B1	$3,43 * 10^9$	$3,73*10^9$	$3,58*10^9$
1C2	$1,82 * 10^9$	$2,42*10^9$	$2,12 * 10^9$
2A2	$0,25 * 10^9$	$0,19*10^9$	$0,23*10^9$
2B1	$2,25 * 10^9$	$2,41*10^9$	$2,33*10^9$
2B3	$1,97 * 10^9$	$2,12*10^9$	$2,05*10^9$
3A5	$0,30 * 10^9$	$0,29*10^8$	$0,29*10^9$
3B3	$2,00 * 10^9$	$1,70*10^9$	$1,85*10^9$
4A1	$3,55 * 10^9$	$3,15*10^9$	$3,35*10^9$
4A7	$3,15 * 10^9$	$3,15*10^9$	$3,15*10^9$
4B6	$2,56 * 10^9$	$2,15*10^9$	$2,36*10^9$

Stable density keeps bacteria balance with organic matter content in wastewater, thereby promoting faster decomposition of organic matter. The cell density of all strains reached at 10^9 cells/ml after 48 hours of proliferation culture. This is a suitable density to applicate in treatment systems.

2. Improving the quality of aquaculture wastewater by high saline-tolerant microorganisms

The main feature of aquaculture wastewater is it contains a high concentration of organic (COD, BOD_5) and nutrient (N, P) due to an excess of feed and antibiotics. Therefore, they can cause organic pollution or cause eutrophication as well algal blooms of surface water.

In the frame of this article we refer to the experiments treating one of main parameters of aquaculture wastewater, COD. The value of COD concentration were measured before and after treatment to evaluate the treatment efficiency of salt tolerant strains, and the salinity reach at 1,17%.

During treatment process, the addition of microorganisms requires a certain ratio. If there are too many microorganisms compared to the total organic matter, the F/M ratio is low, the microorganism's growth is slow, it may switch to a dormant state, spore production, the treatment time is long, and the efficiency is not high. If there are too few microorganisms, high F/M ratio, excess organic matter will cause reverse inhibition, quickly pushing the microbial population into the dead phase, increasing the COD of the output wastewater. Therefore, before adding microorganisms to the treatment system, it is necessary to conduct surveys to select the most suitable rate.

After investigating the rate between the volume of microorganisms and the volume of wastewater at 1:5; 1:10; 1:15 and 1:20 for each strain of salt-tolerant bacteria, it is determined that at the rate of 1:10 the density of microorganisms is the most suitable for the treatment system.

2.1. The saline wastewater treatment efficiency of each strain of salt-tolerant microorganisms

After investigating every strain of salt-tolerant microorganism to treat COD at the value 320mg/L, the result showed that almost salt-tolerant strains have the best COD treatment efficiency after 72 hours. In which, 9/10 strains can treat COD well with the efficiency 73.08%-89.74% (Fig.2.).

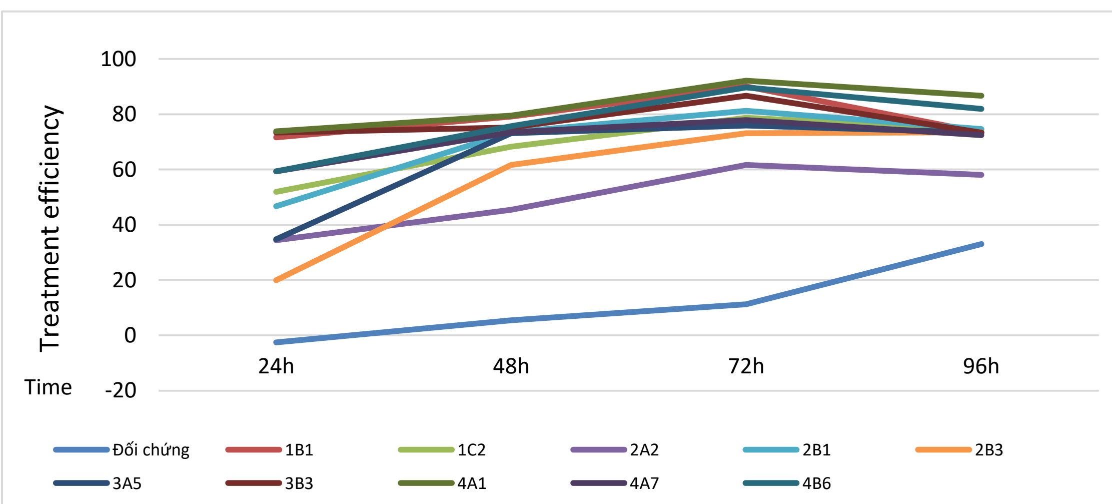

Fig.2. The COD treatment efficiency of 10 salt-tolerant microorganism strains

2.2. The saline wastewater treatment efficiency of mixture strains of salt-tolerant microorganisms: In this experiment, it showed that the best treatment performance came from group G1 with value 84,92- 86,58% after 72-96 hours, while groups G2 and G3 reach the value < 70% (Fig. 3). Based on this result, we selected bacterial strains of group G1 (1B1, 2B1, 3B3, 4A1, 4B6) to use for the next experiment.

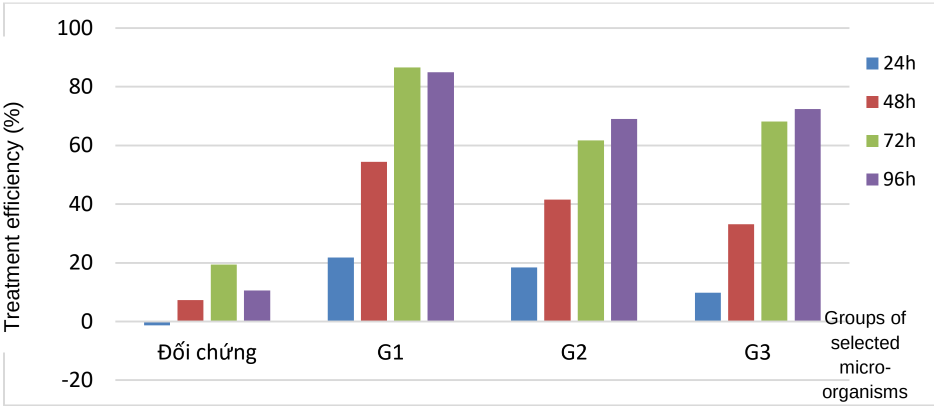

Fig.3. The COD treatment efficiency of mixture salt-tolerant microorganism strains

2.3. The saline wastewater treatment efficiency of mixture strains of salt-tolerant microorganisms with three commercially wastewater treatment probiotics

In this experiment, the mixture salt-tolerant microorganism G1 was used to mix with 3 different commercially probiotics as following model:

- Tank 1: Aquaclean ACF SC
- Tank 2: G1+ Aquaclean ACF SC
- Tank 3: EcoClean™
- Tank 4: G1+ EcoClean™
- Tank 5: Microbe-Lift IND
- Tank 6: G1+ Microbe-Lift IND

The result showed that, almost tanks got the high treatment efficiency, in which the best one can see in tank 4 with the value 72.58%- 90.97% (fig. 4).

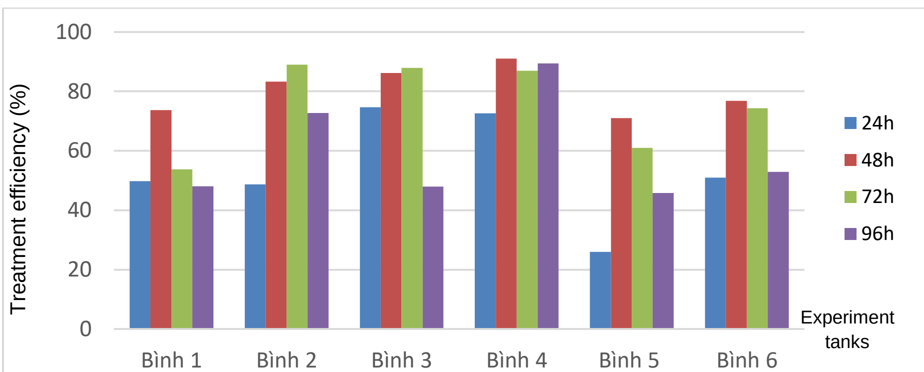

Fig.4. The COD treatment efficiency of mixture salt-tolerant microorganism strains with different probiotics

Conclusion

With the aim of salt tolerance, 10 strains of microorganisms with the highest salt tolerance have been selected (at 7% salinity), include: *Staphylococuss epidermidis; Micrococcus luteus; Bacillus lichenniformis; Bacillus lichenniformis; Bacillus subtilis; Arthrobacter creatinolyticus; Lactobacillus pentosus; Bacillus cereus; Bacillus subtilis; Bacillus subtilis*

Based on the treatment efficiency (COD treatment efficiency >70%), 5 best strains of salt-tolerance microorganism was selected to continue producing as commercially wastewater treatment probiotics. It can be suggested that these production can be used for saline wastewater treatment.

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IRRIGATION NETWORK DIAGNOSIS: CASE OF KARFIGUELA PADDY FIELD IN BURKINA FASO

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Abstract

In Burkina Faso (BF), irrigation systems suffer from numerous technical, organizational, land and structural problems, that strongly undermine their performance as the case of irrigated perimeter of Karfiguéla. This research has contributed to the reflection on the performance and operation of gravity irrigation networks on irrigated perimeters. An approach based on a: (i) mapping using remote sensing of hydraulic networks (ii) a detailed visual inspection of hydraulic infrastructure (iii) estimation of canals' hydraulic efficiency (iv) and surveys of the various actors involved in the management of the perimeter was used. These results show that Karfiguéla perimeter has an area of 360 ha and is supplied by a network made up of a supply canal, a primary canal, four (4) secondary canals, 37 tertiary ones, and quaternary ones for plots water supply, all in ordinary concrete except the quaternaries which are in rammed earth. The efficiency of the canals in rainy season, greater than 100%, shows that the irrigation network in rainy season functions as a drainage network it is disorderly and non-functional. The damage to the irrigation network, siltation, grassing, erosion, overturning, cracks are severe and persistent. The Strickler roughness coefficient is 68 for the feeder, 47 for the primary, between 32 and 52 for the secondaries and 31 for the tertiaries which also reflect a degradation of the channels. A complete rehabilitation of the hydraulic network, is essential to ensure the sustainability of the rice-growing activity, which has a concentration of nearly 1,200 farmers.

Keywords: *irrigation, hydraulic, water, remote sensing.*

Introduction

Agriculture is a major component of BF's economy, a landlocked country in West Africa (Zidouemba, 2014). However, it's completely subject to the weather hazards which greatly influence his production (Ghattas, 2014; Kambou, 2019). Nowaday, irrigated agriculture has emerged as a solution for adapting to the effects of climate change in compensating for rain-fed production deficits and promoting off-season production (IIMI, 1997; Nebie, 1993; Tapsoba et al., 2018). In BF water resources become scarce: 1750 m^3/year/inhabitants in 1998, then to 852 m^3/year/inhabitants in 2001 and finally 703.4 m^3/year/inhabitants in 2017 (DGH and IWACO, 2001). In this context, irrigated systems should to reduce their water consumption while producing more; in other words, it is important to improve irrigation performance (Kambou, 2019; Nebie, 1993). Unfortunately the irrigated perimeters in BF suffer of poor agronomic and economic performances highlight by several studies and caused by the inadequacy of speculations, non-compliance with itineraries and good irrigation practices and techniques, the irrigation networks malfunctioning and many other reasons (Daré et al., 2019; Kambou, 2019; Pale et al., 2016; Tapsoba et al., 2018). However, there remains a factor that is rarely discussed, the aspects related to efficiency and water management in the irrigation network and on the plot (Amarasinghe et al., 2021; Dembele et al., 2011; Kambou, 2019). The present study of irrigated perimeter of Karfiguela paddy field assess the impact of the current network on the supply and management of the irrigated perimeter, by adopting cartographic, physical, hydraulic and participatory methods.

Study Area

The study area (Figure *6*) is in the south-west of BF, between the longitudes 4° 49 ’and 4° 48 ′ West and the latitudes 10° 38 ’and 10° 41 ′ North in the alluvial plain of Karfiguéla. The” Cascade” region is one of the best watered places in the country with an annual rainfall between 1000 mm and 1200 mm (DRAHRH Cascades, 2009). It’s crisscrossed by lakes, perennial ponds, waterfalls and many rivers including the Comoé and the Léraba (Loye et al., 2009). The perimeter is also built a few meters from a transboundary river in BF and Ivory Coast called Comoé and has a gravity network, supplied by a water intake on this river. Out of 750 ha of planned development, only 350 ha been developed in the years 1973 to 1977 for rainfed rice cultivation only.

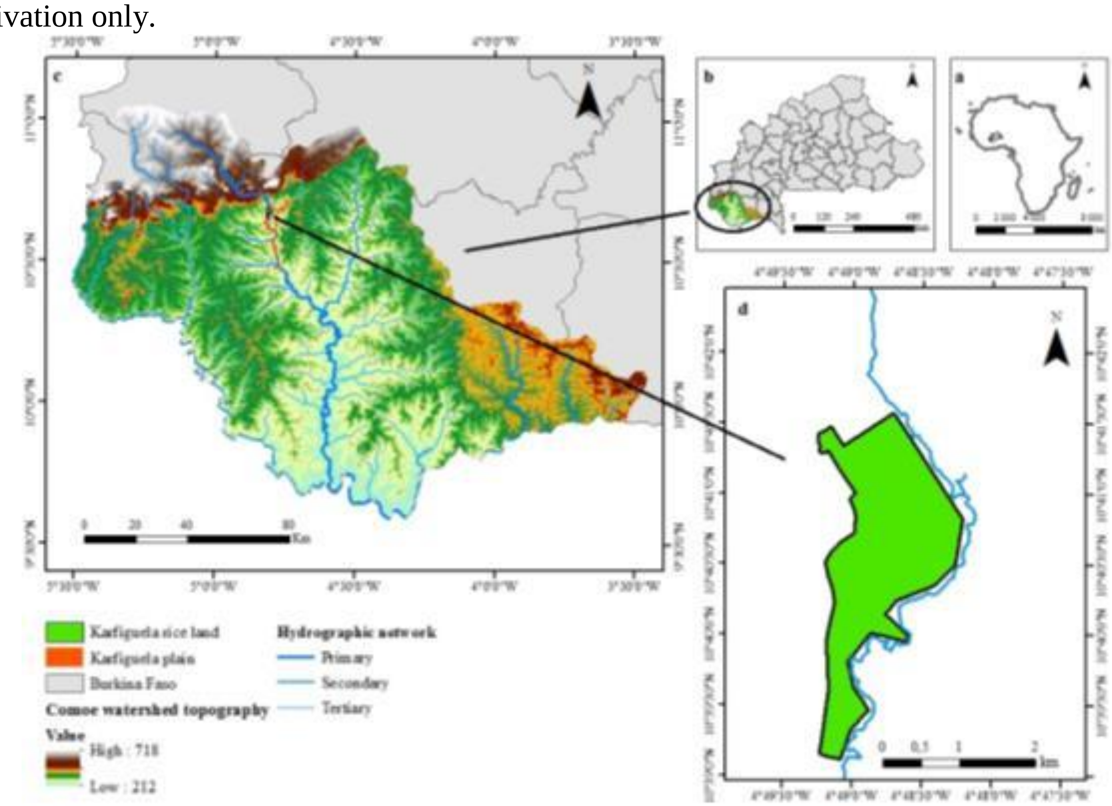

Figure 6: location of the study area (**a**) Burkina Faso (**b**) Comoé watershed in Burkina Faso (**c**) Karfiguela paddy field in Comoé watershed (**d**) Global view of Karfiguela paddy field

Material and methods

The diagnosis of the network was both quantitative and qualitative based on a participatory multi-method approach: (i) mapping of the hydraulic network (ii) detailed visual inspection of the hydraulic infrastructure (iii) estimation of the canals’ hydraulic efficiency. Before carrying out any diagnosis, it’s important to have a recent and reliable map of the network which will serve as a basis for diagnostic, and the planning of interventions on the network. The network mapping was carried out based on high-resolution Google Earth images ranging from 30 m to 60 cm. The images used for this study are provided by CNES SPOT satellites dating from November 2019. The steps of mapping of Karfiguela perimeter are presented on the Figure 2.

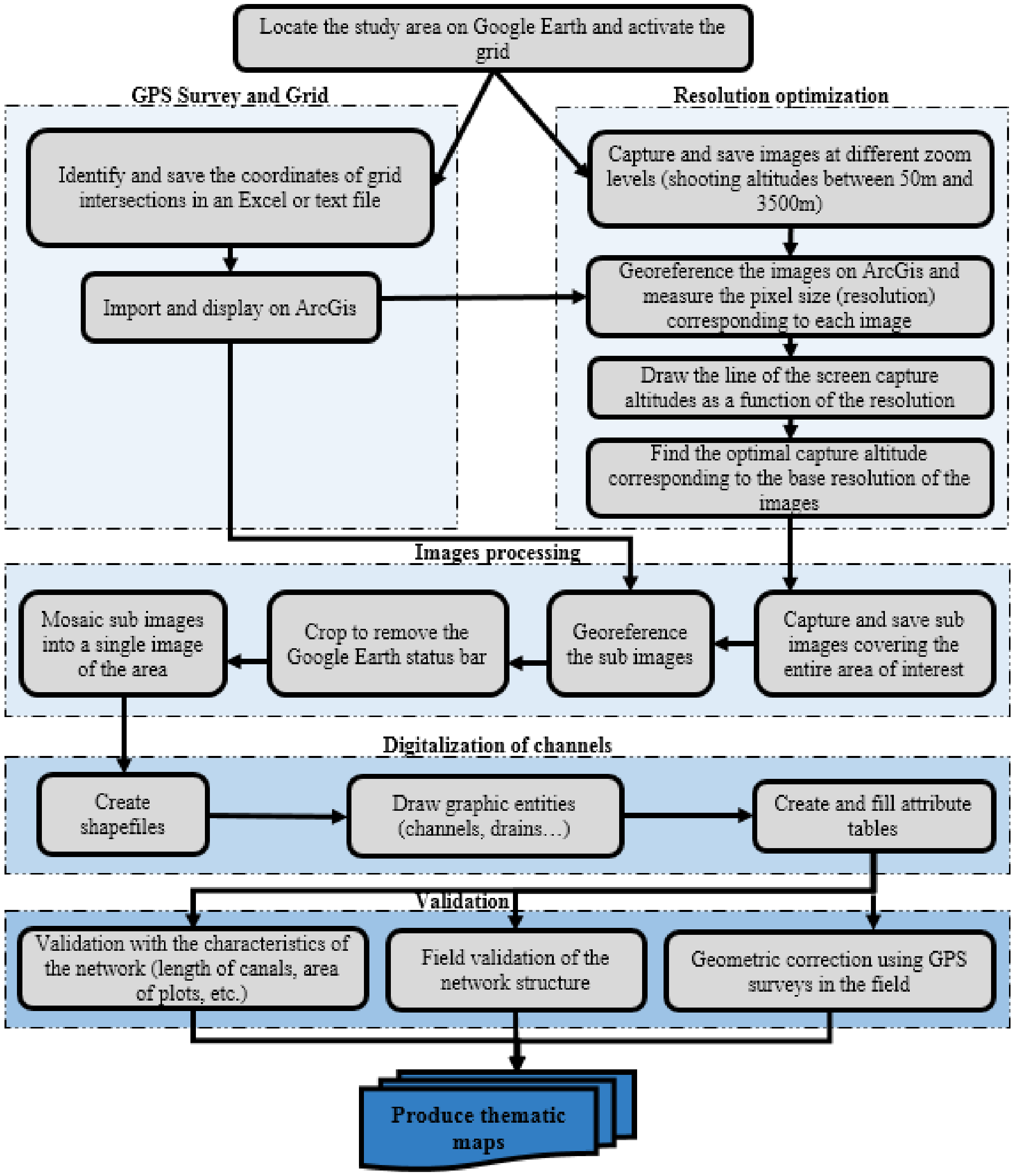

Figure 7: Flowchart of the mapping methodology

To examen the hydraulic infrastructure of perimeter the visual diagnosis has been used, it is recommended method for irrigated areas in BF (Berase, 2018; Dembele et al., 2011; Faso et al., 1996). It’s a field phase which allowed to analyze in detail the physical limits of the perimeter, the irrigation channels (feeder, primary, secondary, tertiary and quaternary), the drainage network, the flow regulation infrastructure, the plots and engineering infrastructure (bridges, slabs, roads…) (IIMI, 1997; Illiassou et al., 2017; Kambou, 2019). The visual diagnosis also constituted a validation’s step of the mapping of irrigation network by remote sensing based on satellite images.

To study of hydraulic efficiency of the irrigation channels an estimation of water flows and their roughness have been carried out. In the literature, several methods have been developed to estimate the flow rates of free surface water depending on whether we take into account the channel size, type of flow, flow velocity, required accuracy, safety consideration (MELCC, 2019; Perzyna, 2016). For this study, the current meter gauging and the float gauging methods with two types of floats (weighted bottles and stick) thrown on the two banks and the middle of the channel was used. Indeed, these methods, which are simpler in their implementation and have already been used for flow measurements on several irrigated perimeters in Burkina Faso (IIMI, 1997; Wellens et al., 2008). In this study, the section is defined as any portion of the network where there is no water intake, no service on the way and no water supply. According to the continuity equation, the sum of the incoming flows must be equal to the sum of the outgoing flows plus or minus the losses (Bazarov et al., 2019). Thus, for a section:

$$Inflow = outflow + \sum losses \qquad eff\ [\%] = \frac{outflow}{Inflow} \times 100 = \frac{Qdownstream}{Qupstream}$$

To estimate the channels roughness the Manning and Stricker equations were used. For greater reliability, on the same reach, the calculation was made on the basis of two flow measurements. The goal is to compare the current roughness of the channels with that of new channels and to assess the current quality of the channel.

$$U = K_S R_H^{2/3} \sqrt{I} \Rightarrow \ K_S = \frac{Q}{R_H^{2/3}\, S\, \sqrt{I}}$$

$$R_H = \frac{S\ wetted}{P\ wetted}$$

I: slopes of the canal
S: wetted section [m^2].
P: wet perimeter [m]
R_H: hydraulic radius [m]
Q: flow rate [m^3/s]
K_s : Manning’s roughness coefficient [m$^{1/3}$/s]

Results and discussion

Irrigation and Drainage Network Mapping

Validated mapping (Figure 8) determined that the irrigation canals have varying lengths (Table 3) depending on the geometry of the area they serve. The irrigated perimeter of Karfiguéla has a gross area of 400.4 ha and a net area of 360 ha. It’s served by a gravity network supplied by a feeder located on the Comoé River far of 2.230 km from the perimeter. The hydraulic district, called “block” on the perimeter, generally has an area of 15 ha which may vary depending on the configuration of the network (Table 3). The block has 30 plots of 0.5 ha. Each farmer generally divides his plot into ten boxes of 25 m by 20 m each. The hydraulic districts are grouped into four large blocks representing the areas dominated by the secondary canals as presented on the Figure 8.

Table 3: Nature and length of the different irrigation canals in the perimeter

Channels	Type of coating	Symbols	Number	Length [m]
Feed	Ordinary concrete	CA	1	2,451
Primary	Ordinary concrete	CP	1	1,654
Secondary 1	Ordinary concrete	S1	1	792
Secondary 2	Ordinary concrete	S2	1	797
Secondary 3	Ordinary concrete	S3	1	1,568
Secondary 4	Ordinary concrete	S4	1	3 883
S1 tertiary	Ordinary concrete	T1-i	5	320 - 612
S2 tertiary	Ordinary concrete	T2-i	4	612
S3 tertiary	Ordinary concrete	T3-i	10	110 - 410
S4 tertiary	Ordinary concrete	T4-i	18	190 - 585
Quaternaries	Clay	Q	-	87 - 332

As presented on the Figure 9, the drainage of the lower part of the perimeter isn’t really assured. The secondary drains aren’t connected to any collector, thus making drainage non-existent. Also, during the mapping, interruptions on several secondary drains were noticed. The results of the visual inspection, presented below, explain these remarks and validate the map.

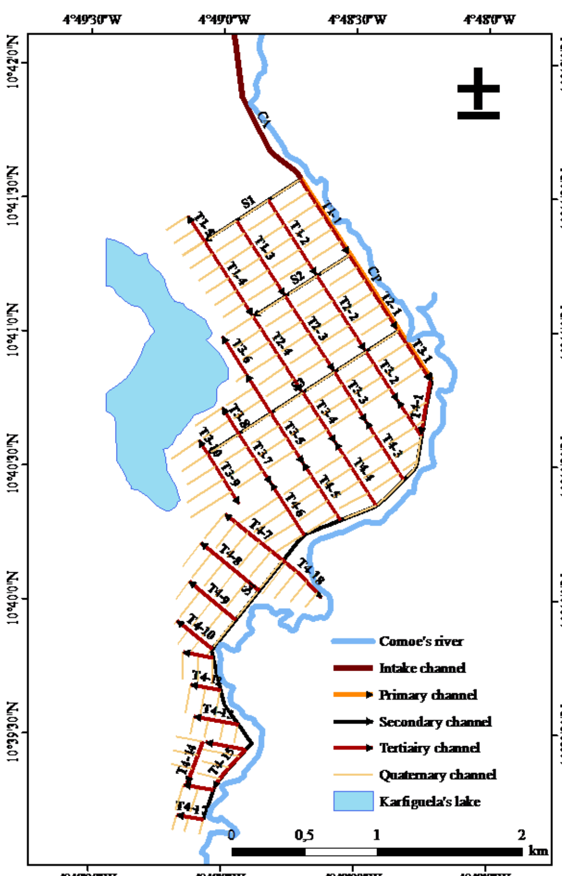

Figure 8: Architecture of the irrigation network of the Karfiguéla perimeter

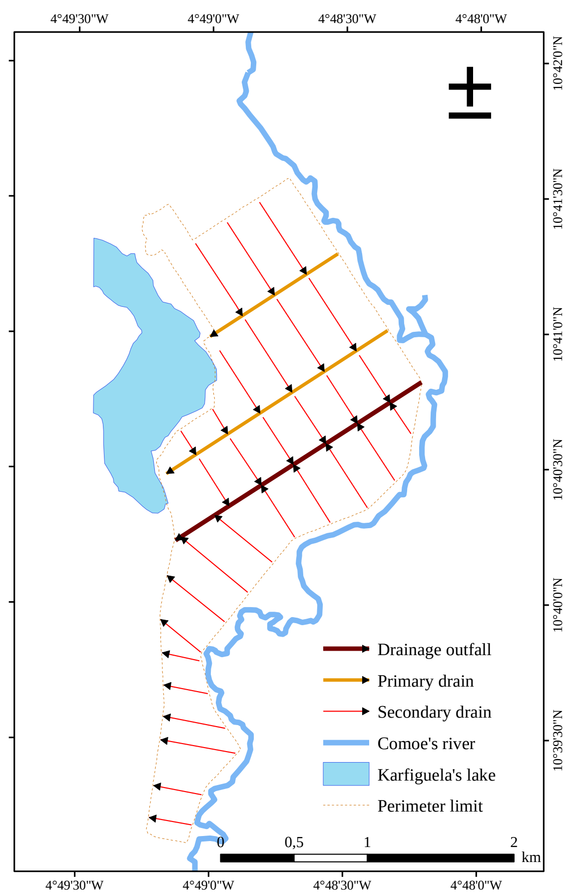

Figure 9: Mapping of the drainage network

Physical state of irrigation and drainage network

In general, the irrigation network suffer from a few slight pathologies ranging from grass cover to cracks in the panels, including silting up. The eight (8) main impairments observed on the different channels can be summarized as follows (Figure 10): (i) transverse, vertical, oblique or any other cracking of the majority of the panels; (ii) complete degradation of the panels joints on all the channels and grassing of these joints; (iii) settlements and overturning of certain canals; (iv) advanced erosion of the canals lining, especially in the tertiary canals; (v) erosion of the backfill of canals and weirs certainly due to overflows caused by improper handling of valves; (vi) presence of shrubs and large trees in close proximity of the canals leading to serious cracks and even overturning of the concrete modules over a length of several meters for the secondary and tertiary canals; (vii) significant siltation and grassing of the various canals and (viii) intentional filling of the last sections of certain tertiary canals with a view to redirecting all the water towards the plots upstream. As for the drainage network it is re-traced each campaign by the producers, but in an anarchic and disordered. Several malfunctions hampering its proper functioning. These are: (i) the interruption of the drainage network in certain places, by some producers who by this sabotage hope to gain a few additional square meters of irrigable area; (ii) voluntary widening of drains in order to engage in fishing activities; (iii) rejecting the rice straw directly inside the drains after threshing; (vi) the diversion of drainage water for the irrigation of plots outside the consensus irrigation schedules


Cracks present on all channels


Problem of backfill erosion


Breakage due to the tree roots


Overturning of panels


Grass and siltation of canals


Intentional filling of canal

Figure 10: Some of the most recurring degradations on the irrigation network of the perimeter


Drain deepening


Fishing practice


Flooding of a plot without a drain

Figure 11: Some malfunctions of the perimeter drainage network

Water flow transport efficiency

Current meter and float gauging were carried out in the irrigation network during the July and August months (peak rainfall) of 2020 year (Table 2). The general observation is that during the wet season, the role of irrigation network is reversed, it receives more water into the different channels than it supplies and becomes, a drainage network. Indeed, between July and August the amount of water entering in the feeder is practically constant and the flow values in the feeder are consistent with these carried out during 2018 dry season (Berase, 2018) during which the efficiency of the network was estimated at 52%. On the other hand, the flow values in the primary, secondary and tertiary networks vary enormously due to the decline the crop’s water requirements in rainy season and, the rainfall variability. For the secondary channel S4, the flow goes from 0.053 m^3/s in July to 0.24 m^3/s, this canal constitutes a drainage channel for unused flow and not consumed by the plants during the rainy month of August. For 2020 wet season, the efficiency during the month of July in the feeder and primary channel seem quite good for an old gravity network (Table 4). It is greater than 100% in August suggesting water unforeseen inflows into the canals from (i) surface water runoff coming from unplanned plots, and the degradation of canals (ii) the drainage of plots which are flooded or the presence of large cracks in the canals (iii) the drainage of surface water.

Table 4: value of flow rates and efficiency of canals in wet season

<table>
<tr><th rowspan="3">Channels</th><th colspan="2">July 2020</th><th colspan="2">August 2020</th></tr>
<tr><th colspan="2">Float gauging</th><th colspan="2">Current meter gauging</th></tr>
<tr><th>Q upstream [m³/s]</th><th>Efficiency [%]</th><th>Q upstream [m³/s]</th><th>Efficiency [%]</th></tr>
<tr><td>Feeder</td><td>0.231</td><td>88%</td><td>0.26</td><td>123%</td></tr>
<tr><td>Primary</td><td>0.121</td><td>89%</td><td>0.25</td><td>167%</td></tr>
<tr><td>S1</td><td>0.083</td><td rowspan="4">124%</td><td>0.07</td><td rowspan="4">136%</td></tr>
<tr><td>S2</td><td>0.033</td><td>0.05</td></tr>
<tr><td>S3</td><td>0.01</td><td>0</td></tr>
<tr><td>S4</td><td>0.053</td><td>0.24</td></tr>
</table>

Channel roughness

The aim of estimating the roughness coefficients is to evaluate the level the channel degradations. The values Ks1 and Ks2 (Table 5) represent the Strickler roughness coefficients, calculated using two flow measurements in July and August 2020. An average Ks is calculated when the variation between Ks1 and Ks2 is acceptable and if not, the measurements are repeated. Indeed the table 4 shows that all the values of Strickler coefficients of channels are lower than that of the estimated new concrete at about 70 $m^{1/3}/s$ (Chow et al., 1988) except the feeder which has a Ks of 68 $m^{1/3}/s$. This situation is explained, on the one hand, by the increase in the solid load and the organic and chemical water pollution as one as moves forward in the network and, on the other hand, by anthropic activities creating mechanical and chemical erosion of channels which are closest to the plots. Indeed, the operators use clay mixed with herbs to plug leaks at the valves and to replace the non-existent valves at the tertiary level.

Table 5: values of the roughness coefficients of canals of the Karfiguela irrigated perimeter

Channels	Ks1 [$m^{1/3}/s$]	Ks2 [$m^{1/3}/s$]	Average Ks [$m^{1/3}/s$]
Feeder	64	72	68
Primary	44	49	47
S1	36	35	35
S2	31	32	32
S3	43	43	43
S4	51	52	52
Tertiary	32	31	31

Conclusion

Karfiguéla perimeter is a typical example of paddy fields reality in Burkina Faso (Kambou, 2019). With an irrigated land potential of 750 ha, only 370 ha have been currently schemed due to scarity of the Comoé River water which feeds it. The consequence is that only a small part of perimeter is developed during each dry season, and even that in the event of pockets of drought, the productivity remains mediocre. In addition to the scarity of water, major dysfunctions of irrigation network, hampers the local increase in the production of rice and, and the effective and substantial improvement of farmers' incomes. The different methodological approaches used in this study prove to be complementary in the process to explain the situation of water management on the perimeter with a view to purpose solutions and appropriate action plans for improving water productivity of the perimeter. The mapping results constitute a basis for the management and intervention planning of the irrigated perimeter. The advanced degradation of the irrigation network highlighted not only by flow measurements but also by roughness values lower than the roughness of concrete and approaching that of clay are so many indicators that show the inaptitude network for the effective and efficient distribution of water. Thus, the insufficiency of water on the perimeter isn't justified only by the distribution of the resource from Comoé River but also by a waste of water (Pale et al., 2019) obvious which finds its explanation in the defective state of the irrigation network, but also in the water management. Indeed, this study reveals problems of efficient and equitable management of water allocation between irrigators of the same block, sub-block or agricultural plot: the quantities of water allocated can't be known because of the state of network failure, leading to uncontrolled distribution through fractures and cracks in the channels. While some plots further downstream dry out from lack of water, others die from excess water due to an uncontrollable supply of water and an inefficient drainage system. Thus, beyond the rehabilitation of the perimeter, an establishment of sophisticated and adequate water information and management systems is necessary. Studies in this direction have already been carried out within the framework of research projects on the efficient management of water in the perimeter in the Kou, Bagré basins, etc. Thus, Traoré (2012), Wellens (2014) and Palé (2020) show that easy-to-use, routine and financially accessible management tools could be applied and used by agricultural administrations in irrigated perimeters (Pale, 2020; Traore, 2012; Wellens, 2014; Wellens et al., 2013). Agricultural water management using free remote sensing, the decision support tools as CropWat, Google Earth Engine, and communication platforms such as WhatsApp have been proposed. .

Acknowledgement

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RECOVERY AND UTILIZATION ANALYSIS OF PAPER AND PAPERBOARD BY YEARS IN TURKEY

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Abstract

The production of recycled paper shows an increasing trend in our country as well as in the world. Raw material production of recycled paper is provided by collecting waste paper cardboard. The aim of the study is to provide recovery and utilization analyzes of recycled paper which reflects the progress of the waste paper management process in the short-term process. The use of paper-cardboard and its products, which is increasing day by day, shows a rising trend due to the more intense use of online commerce during the pandemic. Despite the increase in the demand for paper-paperboard and its products, the amount of paper raw materials needed is also increasing linearly. Some problems are encountered in the supply wood supply, which is the primary lignocellulosic raw material, and in the production of virgin paper obtained from this raw material. The decrease in forest resources and the inability to ensure sustainability also cause the primary paper production unit cost to be high and have higher environmental pollution parameters resulting from production. As an alternative to the primary raw material source, waste paper and its products are needed as secondary raw materials. Recovering of waste paper its products as recycled paper has an important contribution to ensure environmental protection and sustainability of waste paper-paperboard resources. Recycled papers were analyzed to and discussed between the years 2009-2021 using the formulation of recovery and utilization rates. In the two-year period including Covid-19 2020-2021, there was a total increase of 12.63% in waste paper consumption.

Keywords: *Waste paper, Recovery rate, Utilization rate, recycled paper.*

Introduction

One of the most important problems in solid waste management today is waste minimization. The solution of this problem includes recycling and reuse of wastes basic operations. Resource reduction and recycling are the two main functions of the problem. Generally; Recycle; protects environmental resources and prevents materials from entering the waste group. In this sense, 50% of recycling is supported by EU fund investments, according to 2020 Waste legislation. (Eco-Center, 2014).
Paper Recycling; It is the recycling of paper and cardboard as a new product by using wastes obtained from industrial wastes such as paper making, printing, garment processes that transform paper into different functions, packaging production and collection of expired paper-cardboard from consumers as raw materials (Young 1993, Akyıl 2018, Kırcı 2003).
Recycled paper and cardboard production is carried out after the last second half of the 20th century and showed an increasing momentum in Turkey as in the world.
There are many reasons for the raw material need of wastes of paper and cardboard, which are the source of recycling raw materials.

The increase in paper and cardboard consumption with the increasing population, The fact that virgin paper raw material is obtained from wood obtained from forests and the sustainability of forest resources can not be ensured, resulting in a decrease in raw material natural resources. In order to ensure the sustainability of the raw material, the emergence of a new product with economic added value by applying the recycling process to paper and cardboard waste becomes important. Recycling is produced from the raw material obtained by collecting, classifying and converting individual consumer and industrial paper and cardboard waste into a raw material source. It contributes greatly to the protection of the environment in the process of converting into raw materials.

In addition to ensuring the sustainability of forest and raw material resources in recycled paper production, it contributes to the protection of the environment by reducing air pollution by 74% and water pollution by 34% (Savar, 2001).

The paper industry is one of the industries that generally uses recycling.

According to the annual report of the Pulp and Paper Industry Foundation; While the total amount of recycled (domestic and imported) waste paper was 4,352,106 tons in 2019, it increased to 4,669,326 tons in 2020, showing an increase of 7.3%.

Recycling (total domestic waste paper amount/consumption) in the paper and cardboard industry in Turkey in 2020 is 46.8%. The recycling rate for wrapping paper and cardboard only is 59.5%. Total waste paper use in 2020 is 3,627,052 tons (SKSV 2020, Mertoğlu Elmas and Karabulut 2021)

It has been determined that the online shopping service, which was 2.7% before Covid-19, increased to 13.8% online service after Covid-19 (Güder *et al.*, 2021).

Waste paper sources mainly consist of office papers, corrugated cardboard papers and packaging paper and cardboard. Due to the pandemic epidemic, there has been a great increase in the number of online shopping. The need for corrugated cardboard boxes as a packaging material for online shopping has increased significantly with the online shopping service.

To determine the recycling process in the short term using waste paper recovery and utilization rate formulations, which are important measurement methods, and to discuss the recycling management that is affected by the results obtained. At the same time, it is to determine the impact of Covid-19 on waste management.

Recovery and Utilization Rates

The Recovery (RR) and utilization (UR) rates are two important criteria that determine the recycling process. RR measures the success able to recover used paper and board from the waste stream in the waste-management or supply Equations 1 and 2 showing the recovery and utilization rates formulas are shown (Berglund and Söderholm, 2003).

$$RR = (WP_{CONS} + WP_{EX} - WP_{IM}) / P\,B_{CONS} \quad (1)$$

$$UR = (WP_{CONS}) / PB_{PROD} \quad (2)$$

WP_{CONS}:Waste paper consumptions, WP_{EX};Waste paper export, WP_{IM}, PB_{Cons} ;Paper and board comsumption, PB_{PROD}:Paper board production.

“$(WP_{CONS} + WP_{EX} - WP_{IM})$” are defined waste paper recovering.

The UR rate is standed for a measurement indicator showing the degree of recycling.

Results and Conclusions

According to the annual reports of the Pulp and Paper Industry Foundation, the variables showing the waste management are given in Table 1.

Table:1: Variables showing the waste management by years (Ton)

	WP_{CONS}	WP_{EX}	WP_{IM}	PB_{PROD}
2014	2348727	43329	183834	5806002
2015	2791426	59970	301404	5864979
2016	2900927	46100	450913	6003919
2017	3164772	40130	753440	6326345
2018	3238340	72000	725361	6060330
2019	3387384	91349	1235819	6278846
2020	3627052	44000	1532955	6698216
2021	3815092	44200	1191274	6857015

In the 6 years between 2019 and 2014, there was a 44% increase in waste paper-cardboard consumption, with an annual average increase of over 7%. While there was an increase of approximately 7.43% in the first year of Covid-19 within the scope of 2019-2020, it decreased to approximately 5.2% in the second year within the scope of 2020-2021. In the two-year period including Covid-19 2020-2021, there was a total increase of 12,63% in $WP_{CONS.}$ Although the annual averages of the post-Covid-19 period seem to be the same as the 6-year period before Covid-19, it is clearly seen that the consumption of waste paper was much higher on average. (Table 1). This rising difference is due to the decrease in WPIM and WP EX amounts to “-%” compared to 2019 (Table 1).

The recovery rate and utilization rate, which reflects the progress of the waste paper management process, are calculated according to the measurement formulations and their graphics are given in Figures 1 and 2.

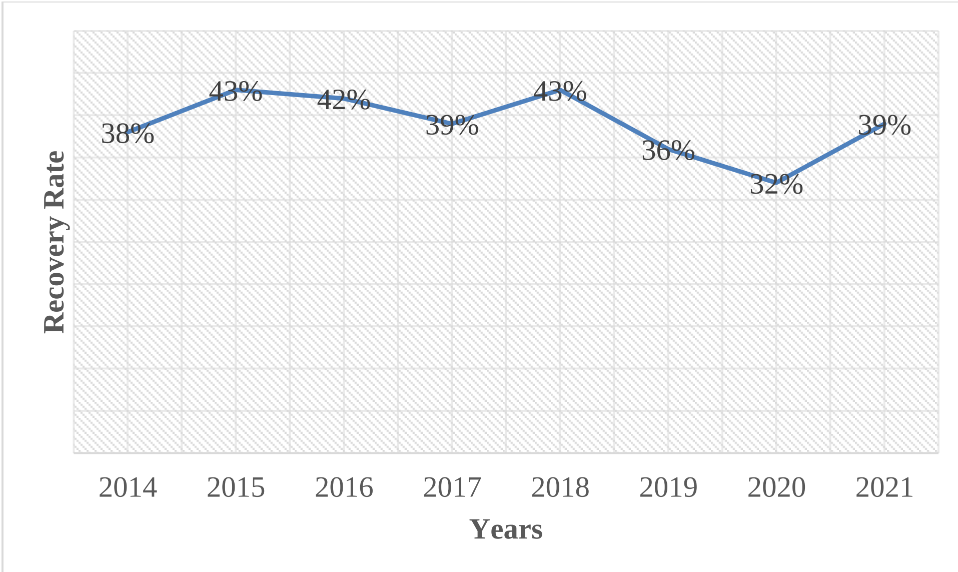

Figure:1: Recovery rate % change between 2014-2021

The Recovery rate results, which measure the progress of recycling between the years 2014-2022, are the minimum value in 2020, and the maximum value in 2015 and 2018. value was observed. It was determined that the recovery rate levels was decreased after Covid-19 (Fig. 1).

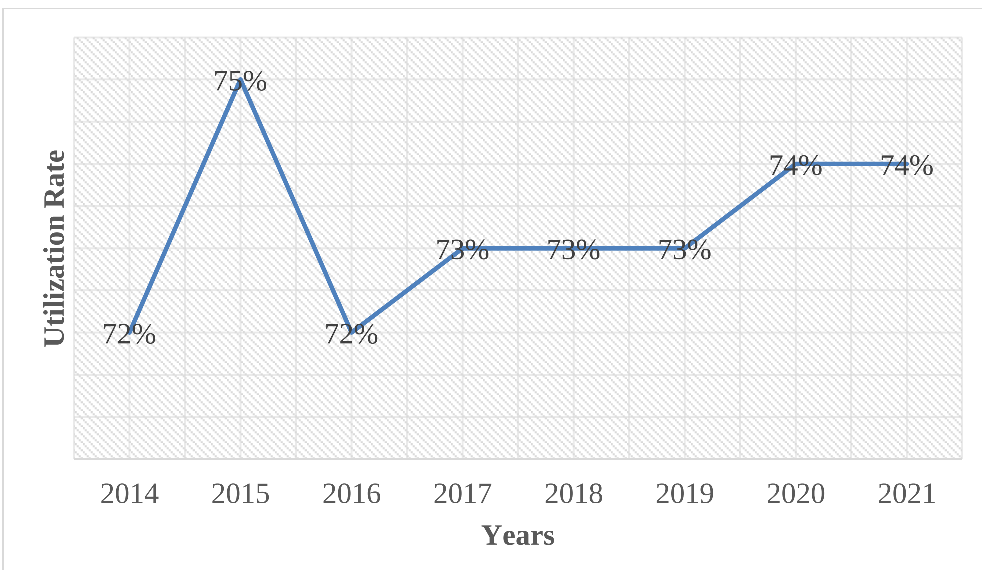

Figure:2: Utilization rate % change between 2014-2021

The results of the usage rate calculations, which measure the recycling progress between the years 2014-2022, were minumum in 2014 and 2016, and maximum in 2015 were reached the value (Fig. 2). Although it was 73% on average between 2014-2019, which includes the pre-Covid-19 period, it was increased by 1% in the post-Covid-19 period (Fig. 2).

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WETNESS OF KARST FIELDS AT EAST HERZEGOVINA REGION (BOSNIA AND HERZEGOVINA)

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Abstract

The article refers to the occurrence of soil moisture in the karst fields of East Herzegovina region, in the south of Republic of Srpska (RS), political entity within Bosnia and Herzegovina (B&H). The Copernicus Water and Wetness (WaW) database has been used, based on the photointerpretation of Sentinel satellite images, in resolutions 10 m and 20 m. Wet zones are divided into four categories, based on the percentage of water and moisture over the year: permanent water, intermittent water, permanent wet areas and temporary wet areas. Compared to other regions of RS, East Herzegovina has the largest area of temporary wetlands in the amount of 3.7% of the territory. The karst fields contribute the most to this, as they cannot receive all the water in the underground channels during the rainy period of the year, so water spills on the surface. However, there are significant differences depending on the altitude of the karst field. Fields at higher altitudes have a high percentage of soil moisture while lower fields are relatively dry. This is largely due to human activities and agricultural production in densely populated areas around lower fields. Also, a correlation was observed regarding matching of humidity and grasslands. The high and medium altitude karst fields are the areas richest in grassland in RS. The aim of the research is to determine types of humidity in the karst fields of East Herzegovina region, as well as to define the zones where soil moisture is most present.

Keywords: *wetness, karst fields, East Herzegovina, WaW database.*

Introduction

Study area covers the East Herzegovina region located in the southeastern part of RS and B&H. It encompasses the part of the Herzegovina region east of the Neretva River, within the boundaries of the RS. The majority of the region is represented by mezozic limstone and dolomite, whereas the youngest paleogen sediments (conglomerates, flysch, clay and sandy limestone) is characteristic for mountainous areas. As a result of prevailing limestone geology, permanent surface river flows are poorly represented, whereas surface runoff is extremely weak and almost does not exist (Popov et al, 2019). According to EEA (2016) East Herzegovina mostly belongs to Mediterranean biogeographical region. Gnjato et al. (2005) called it Adriatic region, which include most of the East Herzegovina, save for its mountain part around Gacko and Nevesinje. The whole area belongs to deep karst with its main features consisting of low karst fields, like Popovo field and Trebinje field, in addition to karst hydrography. This part of the region than is low Herzegovina, is known under the name of Herzegovina's Humine. Name of Humine refers to mild, i.e. altered Adriatic climate, with mild winters where average winter temperature reaches around 5 ^{o}C. Transitional area of Herzegovina, from Humine toward the mountains area is known as Rudine. This region includes the area of high fields such as Dabarsko, Fatničko, Plansko, Bilećko, Ljubomirsko and Ljubinjsko fields, as well as interfiled

karst heights, karst plateaus and karst mountains. The region receive the most precipitation in B&H (up to 2000 mm). In lower Herzegovina there are typical mediterranean precipitation regime with maximum at winter and minimum at summer months. It is the warmest part of B&H with annual temperature average over 14 ^{o}C (Popovo field, Trebinje) (Bajić & Trbić, 2016).
The largest karst field in RS are: Nevesinjsko (189 km^2), Popovo (185 km^2) and Gatačko (60 km^2). Also, karst fields are often divided by altitudes on: low (Popovo, Trebinjsko), mid-level (Dabarsko, Fatnčko, Plansko, Ljubinjsko) and high (Nevesinjsko, Gatačko, Trusino, Cerkničko, Lukavičko, Slatko). Soil moisture plays a pivotal role in vegetation dynamics, considering that soil water availability is a crucial limiting factor for plant photosynthesis (Proietti et al., 2019). The correlation between wetness and grassland will also be analysed, since the karst fields are known as zones with the highest distribution of grassland in B&H. In RS zones of permanent and temporary water cover 0.50% and 0.02%. Temporary wet area cover 1.83% and permanent wet cover 0.03%. In B&H permanent wet areas cover 0.1% and temporary wet areas cover 2.5% of the country territory. The aim of the research is to show distribution of wetness zones in karst fields of East Herzegovina and to analyse conditions that leads to higher surface wetness.

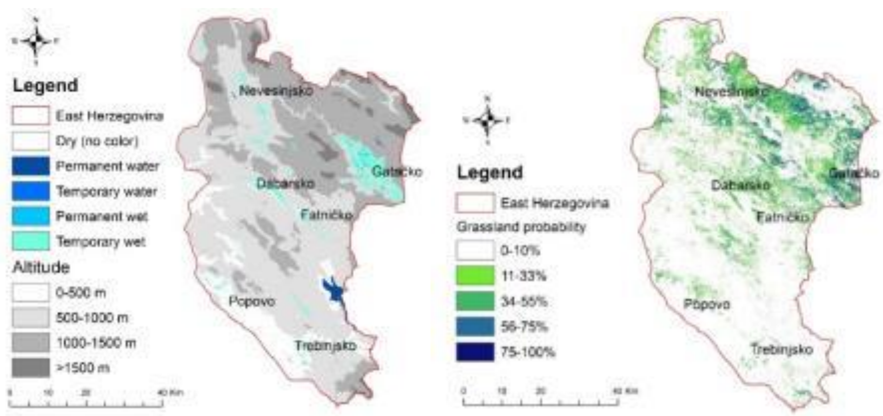
Materials and methods

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. The combined WaW product is a thematic product showing the occurrence of water and wet surfaces over the period 2009-2015. This layer is based on multi-temporal and multi-seasonal optical high-resolution Landsat data and also on radar information (Sentinel-1 data) with a geometric resolution of 10m on a pan-European basis (Laganke, 2016).
The HRL WaW 2018 comprises the product in full spatial resolution of 10m x 10m (instead of the original 20 m x 20 m resolution of the WAW 2015 production). The main product is a classified layer, which contains defined classes of permanent water, temporary water, permanent wet, temporary wet, and dry areas, derived from water and wetness occurrences in the period 2012-2018. It is complemented by the Water and Wetness Probability Index (WWPI), a product mainly dedicated to expert users and indicates the occurrence of water and / or wet areas (with saturated soil moisture content) during a prolonged part of the year over a number of years (CLMS, 2020a).
In this way, it is possible to get an overview and dynamics or variability of the occurrence of water and wetlands within a year or season. Composite images of the seasons are derived from images that cover a period of three months, while annual composite images are derived from images for each season within a given year.
The Grassland 2018 (GRA 2018) database aims at providing a synoptic view on the distribution, extent and dynamics of the pan-European grasslands, comprising natural, semi-natural and managed grasslands and displaying the diversity of grassland types and landscapes. Grassland vegetation probability index (GRAVPI) provide additional information in thematic and technical perspective indicating the soundness of the grassland classification (CLMS, 2020b).
For the analysis of climate conditions, it has been used the data for 7 meteorological stations within East Herzegovina for period 1961-1990. The data refer to annual average of temperature and precipitation during the year. Digital elevation model (EU-DEM v1.1.) with a spatial resolution of 25 m has been used to analyse karst fields wetness by altitude zones. The territory

of the region has been divided into four altitude zones: 0-500 m, 500-1000 m, 1000-1500 m and over 1500 m. All the data has been processed by GIS tools and exported to Microsoft Excel.

Results and Discussion

According to WaW database in 2018 permanent water cover 0.64% (24.33 km^2) and temporary water 0.02% (0.69 km^2) of East Herzegovina region. Temporary and permanent wet area cover 3.7% (140.66 km^2) and 0.07% (2.73 km^2) respectively. The most of wet zones are positioned in the area of mid-level and high karst fields. High amount of precipitation and geologic limestone structure contribute to water table rise up to surface in rainy period of the year. During the vegetation period, the fields are mostly dry and rich with grassland. Therefore, these areas are registered in the database as temporary wet. In WaW nomenclature temporary wet (areas with 25% to 75% wet / total instances and minor instances of water) includes: areas of changing soil moisture, Intermittent wetlands, etc. The rainy period at the region is October-April. Floods occur in the fields because the underground layers cannot receive such a large amount of precipitation. Average annual precipitation within East Herzegovina is 1753 mm. Winter is more humid (556 mm) than summer (233 mm). From October to April monthly precipitation is 217 mm and from April to October 101 mm. Average annual air temperature is 10.8^{o}C. Winter is much colder comparing to summer (2.6^{o}C vs 19.2^{o}C). Autumn is warmer than spring (11.7^{o}C vs 9.8^{o}C). The largest wet area is at Gatačko field (77.9 km^2) with 55% of total temporary wet zones in the region. The subterranean rivers of Gračanica and Mušnica cross the field and rise up surface moisture. Nevesinjsko, Dabarsko and Fatničko fields are covered temporary by wet meadows. All these fields are within 500-1000 m altitudes.



Figures 1-2: Wetness and Grassland probability at East Herzegovina

Figures 1-2 shows that grassland probability raise with surface humidity and higher altitudes. Since there are no large differences in precipitation, the main causes of increasing humidity are geological structure and relief. Analysing the WaW 2018 and GRA 2018, results show that over 140.66 km^2 of temporary wet surface grassland cover 73.5 km^2 or 52.3%. At the Gatačko field correlation rise up to 60.3%. Regarding the differences in wetness between 2015 and 2018, there are almost none. The real changes in the region of East Herzegovina amounted 0.43 km^2 mostly within Gatačko field.

Conclusions

According to WaW 2018 data East Herzegovina region is rich with wetness. Temporary wet areas occupy 3.7% of the region comparing to average in RS with 1.8% or B&H with 2.5%. The largest zones of wetness are medium-high and high level altitude karst fields. Gatačko field cover 55% of total temporary wet zones in the region. The main causes of high surface humidity apart the high amount of precipitation are geological limestone structure and relief. During the rainy period of the year (from October to April) karst fields are exposed to intermittent floods and these zones have been clasificated as temporary wet. Temporary wet zones are covered by grassland over 52%. It is especially case at Gatačko field with 60.3%. High level of soil moisture provides a good condition for grassland development.

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EFFECT OF PHYSICAL SOIL PROPERTIES ON POTATO YIELD IN DIFFERENT AGRO-ECOLOGICAL CONDITIONS

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Abstract

The soil is the basic supstrate, which with its physical, chemical and biological properties greatly affects the productivity of cultivated plants. Two-year period research was carried out in the open field at three locations (East Sarajevo, Rogatica, Bijeljina) in the Entity of Republic of Srpska in Bosnia and Herzegovina, that differ from each other in terms of climate and soil conditions. The factors included in the field research are locality (A) and year (B). The tested soil types differed in physical properties and, in combination with climate factors, had a significant effect on the yield of potatoes. The yield of potatoes in the open field was significantly influenced by locality and year. Potatoes grown in Bijeljina had the highest yield but the lowest was in East Sarajevo. The most favorable physical properties of the soil at the location of Bijeljina affected the yield of potatoes, so the highest yield was achieved at this location.

Key words: *potato, soil, location, physical properties, agro-ecological conditions.*

Introduction

Marković et al. (2006) state that potato represents one of the basic cultivated crops, both because of areas on which it is grown and because of its importance in human nutrition. Given that land is a limited natural resource and in most cases used, increasing food production is possible only by increasing the yield per unit area. The soil is made up of a solid phase, consisting of 45% mineral and 5% organic matter, while water and air are found in soil pores (Altieri et al., 1995). The very composition of soil depends on how the plant will develop, what its structure will be and what its characteristics are (Stevović et al., 2010). The physical characteristics are an important part of the soil quality, as they often cannot be easily improved (Idowu et al., 2008). The solid soil phase is a heterogeneous poly-disperse system composed of mineral, organogenic and organomineral particles that are classified by size into mechanical fractions. With the reduction of the size of mechanical fractions, their activity and effect on the properties and dynamics of the soil increase (Kisić, 2012). The texture (mechanical compostion) of the soil is one of the most important parameters of the soil physical properties which has an effect on almost all physical and individual chemical properties of the soil.
The aim of this study was to determine the importance of soil physical properties and their direct effect on potato yield.

Material and method

The field experiments were set up at three different locations which differ significantly from each other, both in terms of soil types and their fertility as well as altitude and climatic conditions. Research in the field was carried out during two years (2017, 2018), at three locations and on three different soil types: at the territory of the Municipality of East Ilidža (sampling field of the Faculty of Agriculture), altitude of 550 m, soil type fluvisol, in Bijeljina (at the private property of the Petković family, in the village of Kojčinovac) altitude of 90 m, soil type humofluvisol and in Rogatica (at the estate of Solanum product in Borike) altitude of 1100 m, district cambisol soil type. The mid to late Agria variety was used for planting potatoes. The field experiments were set up according to the scheme of randomized block system in four replications with an assemblage of 53333 plants per ha-1. The area of the basic land plot was 15 m^2 (four rows 5 m long with 20 plants in a row, space between the rows 0.75 m and between plants in a row 0.25 m). For the purposes of the experiment, standard agro-technical measures were carried out: basic tillage (ploughing), supplementary tillage (plating after ploughing), pre-sowing preparations (rotator), but without the application of mineral fertilizers. Before setting up the test in the open field, soil samples were taken in a disturbed and undisturbed state from which the most important physical properties of the soil were determined. At the end of the vegetation period, the following was analyzed: the number of potato tubers per hole, avarage mass of tubers (g), yield of potatoe tubers which was calculated per hectare (t ha^{-1}) and the fractional structure of tubers. The samples of twenty holes were taken from each basic plot at the technological maturity of potatoes in order to determine the number of tubers per hole, the avgerage weight of the tuber (g), the yield of tubers and the fractional structure of tubers. The data obtained from the two-year experiments in the field (AxB) were processed using the method of descriptive statistics. Examination of the significance of differences between treatments was preformed by analysis of variance (ANOVA). The significance of differences was tested by Fisher's LSD test. Statistical processing of the obtained data was done using the statistical program STATISTICA 10 (StatSoft, Inc. Corporation, Tulsa, OK, USA).

Results and discussion

The mechanical composition of the soil as an important agronomic factor directly affects the development of the root system and indirectly the level of crop productivity (Bengough et al., 2011). Soil texture affects infiltration, runoff speed and aggregate stability (Lado et al., 2004).

Table 1**.** Mechanical composition (texture) of the tested soil types

Soil type	Sampling depth (cm)	Coarse sand 2-0,2 mm (%)	Fine sand 0,2-0,02 mm (%)	Powder 0,02-0,002 mm (%)	Clay <0,002 mm (%)	Texture class
Fluvisol	0-30	10,99	46,85	23,00	19,16	Sandy loam
Humofluvisol	0-30	10,68	46,62	25,10	17,60	Sandy loam
District cambisol	0-30	23,02	44,18	21,80	11,00	Loamy sand

Analyzing the mechanical compostition of the smaples taken from the alluvial soil (fluvisol) at the depth of 0-30 cm, it was determined that it has a light mechanical composition because the content of the total sand fraction (<0,02 mm) is 57,84 %. The content of powder (0.02 – 0.002 mm) is 23% while the share of clay particles (<0.002 mm) is 19.16%. According to the texture, this type of soil belongs to the sandy loam class (Chart 1). Martinović (2000) came to similar results from the analysis of the mechanical composition of fluvisol-type soil, who stated that the average value of fine powder particles was 23.3%. The analysis of the samples taken from the humofluvisol soil showed that this soil contains 57.30% of the total sand fraction (<0.02 mm), 25.10% of the powder content (0.02 – 0.002 mm) and 17.60 % of the clay fraction (<0.002 mm) and belongs to the textural class of sandy loam (Chart 1). Galić et al., (2000) state that the analysis of the content and distribution of the powder and clay fraction revealed the similarity in the genesis of the loamy form of fluvisol and humofluvisol.
The analysis of soil samples of the district cambisol type showed that this soil belongs to soils with a very light mechanical composition, i.e. into the textural class of loamy sand because it contains 67.20% of the total sand fraction (<0.02 mm), 21.80% of the powder fraction (<0.02 – 0.002 mm) and 11% of the clay fraction (<0.002 mm) (Chart 1).
The percentage of mechanical fractions in soil (sand, powder, clay) can significantly correlate differently with crop yield (Lal, 1997, Tueche et al., 2013).

Table 2. Physical properties of the tested soil types

Soil type	Sampling depth (cm)	Average volumetric soil mass (g/cm^3)	Average density of the solid soil phase (g/cm$^{3)}$	Average soil porosity (%)
Fluvisol	0-30	0,91	2,59	64,87
Humofluvisol	0-30	1,18	2,71	54,66
District cambisol	0-30	1,11	2,73	59,35

The values of the average volume mass differed significantly on the tested soil types and ranged from 0.91 – 1.18 g/cm3 (Chart 2). Our research has shown that all tested soils have an optimal volume mass value and do not belong to compacted soils. A large value of volumetric mass above 1.5 indicates soil compaction because the volumetric mass is an indicator of the amount available pore space within individual soil layers (Adekiya et al., 2009).
The tested soil types had different average values of the specific soil mass which indicates a great heterogeneity among them. The values of the average specific mass on the fluvisol soil type were 2,59 g/cm^3, on the humofluvisol soil type 2,71 g/cm^3, on the district cambisol soil type 2,73 g/cm^3. The specific soil mass can vary significantly for arable soils, most from 2.4 – 2.9 to an average of 2.7 g/cm^3 (Škorić, 1991). The values of the average total porosity, according to the classification of Miljković (1996), show that all tested soils belong to the class of soils with good porosity because their total porosity ranges from 54.66% - 64.87% (Chart 2).

Table 3. Structural aggregates stability of the tested soil types

Soil type	Sampling depth (cm)	3 min	30 min	6 časova
Fluvisol	0-30	2	3	3
Humofluvisol	0-30	3	3	3
District cambisol	0-30	2	3	3

Based on the data shown in Chart 3, it can be concluded that the stability of structural aggregates in soils of the fluvisol, humofluvisol and district cambisol is average, with the fact that the most favorable soil type is humofluvisol. Many factors affect the aggregates' stability, two of which are plant diversity and biological activity (Pohl et al., 2012). Soil with stable structural aggregates are more resistant to aeolian and water erosion, the leaching process of nutrients is slower and they are less sensitive to different types of soil degradation.

Potato yield in different localities

The yield of potato tubers is conditioned not only by the genotype, but also by the conditions of the external environment, primarily by the physical and chemical properties of the soil. Chart 4 shows yield components and potato yield in three locations that differ from each other in terms of agro-ecological conditions and soil types.

Table 4**.** Yield components and potato yield in the years of study at selected localities

		Number of tubers per plant	Average tuber mass (g)	Yield (t ha^{-1})
Locality	East Sarajevo (A_1)	10,9	72,7b	41,2b
	Bijeljina (A_2)	9,1	104,2a	48,8a
	Rogatica (Borike) (A_3)	10,5	87,8ab	42,0b
Year	2017 (B_1)	11,2a	76,3b	41,3b
	2018 (B_1)	9,2b	100,2a	46,7a
2017(B_1)	East Sarajevo (A_1)	11,3b	66,0bc	39,3
	Bijeljina (A_2)	8,3cd	112,7a	47,1
	Rogatica (Borike) (A_3)	14,0a	50,1c	37,4
2018 (B_1)	East Sarajevo (A_1)	10,5bc	79,3bc	43,0
	Bijeljina (A_2)	10bc	95,7ab	50,4
	Rogatica (Borike) (A_3)	7d	125,5a	46,5
Locality		ns	*	*
Year		*	*	*
Locality *x* year		**	**	ns

*Values marked with different lowercases per column for year, locality and their interaction are significantly different at P≤0,05 level according LSD test; **F test significat at level; ns-F test is not significant.*

In two-year trials at three localities, potato yield was significantly affected by the locality and year, while the effect of the interaction was not significant. Potatoes grown in Bijeljina location had the highest yield (48.8 t/ha) and the lowest in East Sarajevo locality (41.2 ha/t). Higher yields were achieved in 2018, compared to 2017 (Chart 4). These results were affected by the agro-ecological conditions at the time of testing as well as the average tuber mass. Yield variation depends on the soil type, topography, physical and chemical soil properties and nutrient availability (Penney et al., 1996) which is consistent with our research. The most favorable physical soil properties at the location of Bijeljina affected the potate tubers yield, so the highest yield was achieved at this location. Favorable physical soil properties affect the yield of plants because the spread of the root system and its adoption depends on the availability of air and water in its zone. (Downie et al., 2009; Devereux et al., 2012).

Conclusion

The mechanical composition is an important feature of the soil, which through the water, air and temperature regime as well as the absortive complex determines the conditions for development, i.e. the supply of plants with water and oxygen. The researched soils belong to the soils with a light mechanical compostion, i.e. in the textural classes of sandy loam (fluvisol, humofluvisol), loamy sand (district cambisol). Optimum values of the avarage of volumetric mass less than 1,4 g/cm^3 were recorded on all tested types. The average specific soil mass ranged from 2,59-2,73 g/cm^3 , while the structural aggregates stability was avarage and the most favorable on humoflovisol soil type (Bijeljina locality). Potato yield was siginificantly affected by the locality and year, while the interaction effect was not significant. Potatoes grown in Bijeljina had the highest yield, while the lowest was in the locality of East Sarajevo. Higher yields were achieved in 2017, compared to 2017, which were mostly influenced by agro-ecological conditions at the time of testing. The most favorable properties of the soil at the location of Bijeljina affected the yield of potato tubers, so the highest yield was achieved at this location.

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MATHEMATICAL MODELING OF PHOTOSYNTHETICALLY ACTIVE RADIATION IN BURGOS, SPAIN, AND THEIR APPLICATION TO OTHER CLIMATES

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Abstract

Photosynthetically Active Radiation is the fraction of the solar spectrum with a wavelength range between 400 and 700 nm, that produces biochemical processes, and also influences vegetation growth. Therefore, PAR is part of the visible light spectrum band (400-780 nm). Due to the scarcity of PAR data from direct measurements at ground meteorological stations, it is common to estimate it using mathematical models that depend on other more commonly measured meteorological and climatic variables: solar global irradiance, temperature, relative humidity. Many of the models developed to estimate PAR, found in the literature, are carried out through linear regressions (considering a single variable) or multilinear regressions (considering the simultaneous influence of several variables). In addition, in recent years, artificial neural networks (ANN’s) have also been used, with different meteorological indices as input variables.
In this work, both procedures, multilinear regressions and ANN’s, have been used for modeling PAR in Burgos, Spain, under all sky conditions. The sky classification has been carried out using the sky clearness classification, k_t. Both procedures have been also used to develop a model with all the experimental data, regardless of the sky type. Once the models were developed in Burgos, which has a continental Mediterranean climate (according to the Köppen classification), they have been fitted in other locations with different climates. The experimental data were obtained from 7 meteorological stations belonging to the SURFRAD network in the USA. The objective of this work is to verify the suitability of the models developed in Burgos to other locations with different climate. The results obtained prove the good fit of the models developed in Burgos to the SURFRAD weather stations.

Key words: *photosynthetically active radiation (PAR), mathematical modeling, machine learning, climates.*

Introduction

Photosynthetic active radiation *(PAR)* is the component of global solar radiation with a wavelength between 400 and 700 nm. It can be expressed as photon flux *(Q_p in $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$)*, or in power units *(PAR in $W{\cdot}m^{-2}$)*. Usually, Q_p is measured and transformed into *PAR* units through the McCree factor 4.57 $\mu mol{\cdot}J^{-1}$ which depends on climatic factors (Akitsu *et al*., 2015).
PAR directly influences vegetation growth (Verma *et al*., 2021), leaf area and evapotranspiration (Saini and Fricke, 2020), renewable energy production from biomass (Deo *et al*., 2019). Likewise *PAR* is also used to calculate the euphotic depth of the oceans (Churilova *et al*., 2020; Jyothibabu *et al*., 2018) and plays a key role in coral bleaching (Jackson *et al*., 2021; Yates *et al*., 2014) and marine viral decomposition (Wei *et al*., 2018) .

Despite its importance, most weather stations do not have sensors for measuring PAR (López *et al.*, 2001). Therefore, its calculation is usually performed with meteorological variables more commonly measured at weather stations. Several authors have developed mathematical models to obtain PAR estimates from other meteorological variables as single variables through linear regressions from horizontal global radiation ($RaGH$) measurements (Majnooni-heris, 2014; Vindel *et al*., 2018), or from relative optical air mass (m) values (Hu *et al.*, 2016). Other authors have modeled PAR considering the simultaneous influence of several variables at the same time. For instance, Zempila et al. developed a model from the horizontal global radiation, the cosine of the zenith angle, the precipitable water vapor column, and the aerosol optical depth (Zempila *et al*., 2016). Ferrera-Cobos *et al.* used $RaGH$, extra-terrestrial irradiance ($G0$), temperature (T) and relative humidity (RH) as input variables for their models (Ferrera-Cobos *et al.*, 2020).
In addition, PAR is related to sky conditions, being the classification of skies a complex problem due to the abstract definition of clear, partial and overcast skies. Therefore, many authors performed the classification of skies according to cloudiness, based on different meteorological indices, such as the clarity index, k_t, (Jacovides *et al*., 2007), the relative insolation *(S)* (Escobedo *et al.*, 2009), and the Perez's clearness index, ε, or brightness of the sky, Δ, (Alados *et al.*, 1996).
In this work, a PAR estimation model was developed for the three sky categories (clear, partial, and overcast), with data collected at the meteorological station of Burgos, Spain. The two modeling procedures used were Multi-Linear Regression (MLR) and Artificial Neural Networks (ANN's). Subsequently, once the models were developed, they were applied to seven stations of the Surface Radiation Budget Network (SURFRAD) in the USA with different climates, according to the Köppen–Geiger climate classification (Peel *et al.*, 2007).

Materials and methods

In this study, data collected at the weather station of the SWIFT research group of the University of Burgos have been used. This weather station is described in previous work (García-Rodríguez *et al.*, 2020). Data were recorded every 10 minutes and filtered according to conventional quality criteria (Gueymard and Ruiz-Arias, 2016). The experimental campaign took place from April 2019 to February 2021. Experimental data recorded at seven weather stations belonging to the SURFRAD network were also used. The following variables or meteorological indices (MI's) were directly measured: horizontal global irradiance ($RaGH$), photosynthetic active radiation (PAR, obtained from Q_p), air temperature (T) and pressure (P). Other necessary variables were derived from meteorological measurements: clearness index (k_t), dew point temperature (T_d), horizontal diffuse fraction (k_d), cosine of the solar azimuth (*cosZ*), sky's clearness (ε) and brightness (Δ). Each meteorological variable has been obtained both at the Burgos station and at the seven stations of the SURFRAD network.
Figure 1 shows the location of the weather station in Burgos, Spain, and the location of the seven SURFRAD weather stations, USA.

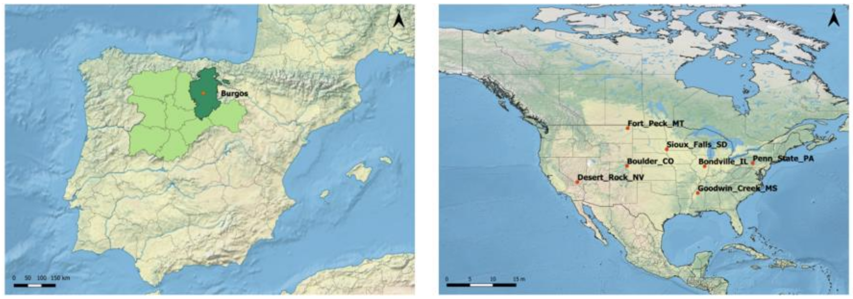

Figure 1. Location of the weather stations (Burgos and USA).

The data obtained were classified according to the sky type, based on the clearness index, k_t, and the values adapted by Suarez-García (Suárez-García *et al*., 2020) considering clear [0.65, 1), partial (0.35, 0.65), and overcast (0 < 0.35] skies. In order to find out which MI’s are more influential in the PAR estimation models, a feature selection was made for each sky type. For this, the Pearson correlation coefficient (r) is calculated to determine the influence or weight that each of variable has on the PAR component.
PAR estimation models have been developed using both multilinear regressions (MLR’s) and Artificial Neural Networks (ANN’s) trained with the Levenberg-Marquardt Back-Propagation (LMBP) algorithm (Du and Stephanus, 2018). The variables considered for these models were those that obtained a Pearson correlation coefficient, r, greater than 0.5. To study the goodness of fit of the models developed using MLRs and ANNs, the coefficient of determination (R^2), the normalised root mean square error ($nRMSE$) and the normalised mean bias error ($nMBE$) were used, Equations (1)-(3).

$$R^2 = \frac{\sum_{i=1}^{n}(PAR_{mod} - \overline{PAR_{mod}}) \cdot \left(PAR_{exp} - \overline{PAR_{exp}}\right)}{\sqrt{\sum_{i=1}^{n}(PAR_{mod} - \overline{PAR_{mod}})^2 \cdot \sum_{i=1}^{n}\left(PAR_{exp} - \overline{PAR_{exp}}\right)^2}}. \quad (1)$$

$$nRMSE\ (\%) = \frac{1}{\overline{PAR}_{exp}} \sqrt{\frac{\sum_{i=1}^{n}\left(PAR_{mod} - PAR_{exp}\right)^2}{n}} \cdot 100. \quad (2)$$

$$nMBE(\%) = \frac{1}{\overline{PAR}_{exp}} \frac{\sum_{i=1}^{n}\left(PAR_{mod} - PAR_{exp}\right)}{n} \cdot 100\,, \quad (3)$$

where n is the number of the experimental data used for model fitting, PAR_{mod} is the modeled value of PAR, PAR_{exp} is the experimental value of PAR, $\overline{\mathrm{PAR}}_{\mathrm{exp}}$ is the mean of the experimental values and $\overline{\mathrm{PAR}}_{mod}$ is the mean of the calculated values.
Based on the Köppen–Geiger climate classification, the climatology has been obtained for each location. In Burgos, the climate type is Cfb, temperate oceanic climate. The climatology type of the seven SURFRAD network stations is shown in Table 1.

Table 1. Geographical data of SURFRAD weather stations and their climatology.

USA Stations	**Latitude (ºN)**	**Latitude (ºW)**	**Climate**
Bondville, Illinois (BON)	40.05192	88.37309	Dfa
Table Mountain, Boulder, Colorado (TBL)	40.12498	105.2368	BSk
Desert Rock, Nevada (DRA)	36.62373	116.01947	BWk
Fort Peck, Montana (FPK)	48.30783	105.1017	BSk
Goodwin Creek, Mississippi (GWN)	34.2547	89.8729	Cfa
Penn State, Univ. Pennsylvania PSU)	40.72012	77.93085	Dfa
Sioux Falls, South Dakota (SXF)	43.73403	96.62328	Dfa

(**Dfa:** humid subtropical; **BSk:** cold semi-arid; **BWk:** cold desert climate; **Cfa**: humid subtropical)

Results and discussion

Table 2 shows the different intervals of Pearson’s coefficients for the different MI’s in each sky type (clear, partial, and overcast), based on data collected in Burgos.

Table 2. Pearson Coefficients, $r(PAR, MI_i)$, based on sky conditions defined by k_t sky classification (clear, partial, and overcast).

$\boldsymbol{\lvert r(PAR, MI_i)\rvert}$					
k_t interval	**[1-0.9]**	**(0.9-0.7]**	**(0.7-0.5]**	**(0.5-0.3]**	**(0.3,0]**
Clear	$RaGH, cosZ$		k_t, ε	T	k_d, Δ, P, T_d
Partial	$RaGH, cosZ$			T	k_t, k_d, $\Delta, \varepsilon, P, T_d$
Overcast	$RaGH$	$cosZ$	k_t, Δ		k_d, ε, P, T, T_d

This table shows that $RAGH$ is the MI with the strongest influence on the PAR estimation for each sky type. These results are in accordance with the results obtained by Ferrera-Cobos *et al*. (Ferrera-Cobos *et al.*, 2020). $cosZ$ also presents a strong influence for clear and partial skies, while this relationship is strong for overcast skies. Once the most influential variables for each sky type, shown in Table 2, were determined, PAR estimation models were developed using multilinear regressions. These models are shown in Table 3.

Table 3. Multilinear regression models of PAR.

Sky type	**Multilinear regression model**	$\boldsymbol{R^2}$	$\boldsymbol{nRSME}$(%)
Clear	$PAR = -18.12 + 0.33RaGH + 83.15cosZ + \ 4.19 \cdot kt + 0.71\varepsilon$	0.990	3.27
Partial	$PAR = -1.81 + 0.40RaGH \ + 13.75cosZ$	0.977	6.80
Overcast	$PAR = -0.03 + 0.42RaGH + 6.88cosZ + 1.58k_t - 6.12\Delta$	0.978	7.33

As can be seen in Table 3, all the models fit very well, with an R^2 greater than 0.97 and an *nRSME* of less than 7.5%, the model for clear skies standing out with an R^2 of 0.99 and an *nRSME* of 3.27%.
The results obtained when developing the PAR estimation models using neural networks were also very good for the 3 sky categories. Again, the best fitting model was for the clear sky type, with an R^2 of 0.992 and an *nRMSE* of 3.01% (Table 4).

Table 4. Statistical results of the ANN models.

Sky type	$\boldsymbol{R^2}$	$\boldsymbol{nRMSE}$ (%)	$\boldsymbol{nMBE}$ (%)
Clear (ANN2)	0.992	3.01	$-4.68 \cdot 10^{-2}$
Partial (ANN3)	0.977	6.80	$4.06 \cdot 10^{-3}$
Overcast (ANN4)	0.978	7.28	$-3.50 \cdot 10^{-2}$

Once the models were developed in Burgos, they were applied to the 7 stations of the SURFRAD network with different climatology, obtaining very good results for all locations and all sky types. Statistical results were obtained for both MLR and ANN models with R^2 higher than 0.98 and *nRMSE* values lower than 10%. The best fitting models were those for clear skies, as shown in Table 5.

Table 5. Model fit values for MLR (left) and ANN (right) models for clear sky conditions.

	MLR			**ANN**		
USA Stations	$\boldsymbol{R^2}$	**nRMSE (%)**	**nMBE (%)**	$\boldsymbol{R^2}$	**nRMSE (%)**	**nMBE (%)**
BON	0.985	4.05	1.61	0.985	4.13	1.28
TBL	0.993	4.50	3.30	0.992	4.67	3.13
DRA	0.994	5.50	4.87	0.994	5.77	4.77
FPK	0.988	5.41	4.16	0.987	5.31	3.74
GWN	0.985	4.25	2.37	0.984	4.46	2.06
PSU	0.987	3.77	1.20	0.986	4.02	0.95
SXF	0.991	5.44	4.49	0.990	5.36	4.03

When analysing the results according to climatic zones, hardly any differences were observed in the values of R^2, all of them being above 0.980. The highest error $nRMSE$ was obtained for overcast skies and corresponds to the desert zone (Bwk), a climatic zone very different from that of Burgos, while the lowest $nRSME$ occurs in continental zones (Dfa) with clear skies.

Conclusions

It has been observed that for the estimation of PAR models according to the sky category, the use of multilinear regressions and neural networks is adequate. In addition, by applying these models to the seven USA weather stations, very good fits are obtained for all sky types, locations and climatologies. The worst fit was obtained for overcast skies, using ANN at Desert Rock station, which has an arid climate.

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CALCULATION OF INTERCEPTED PHOTOSYNTHETICALLY ACTIVE RADIATION (IPAR) FOR INDUSTRIAL CROPS IN CASTILLA Y LEÓN, SPAIN

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Abstract

Agriculture is one of the most important economic activities in the Castilla y León region (Spain), as more than 20% of the land area is used for this purpose. There are many factors involved in its productivity, but a decisive climatic variable which is often not considered is the Intercepted Photosynthetically Active Radiation (IPAR). It is directly involved in photosynthesis process and it is usually estimated from a Beer's Law approach. To apply this law, it is necessary to know other parameters: *PAR* (Photosynthetically Active Radiation), *k* (light extinction coefficient) and *LAI* (Leaf Area Index). Industrial crops, including sugar beet, rapeseed and sunflower, are becoming very relevant in Castilla y León. In particular, sunflower area is increasing every year, reaching 242.432 ha in 2021. The IPAR values of the three types of industrial crops were calculated and spatially represented using Geographical Information Systems (GIS). The highest IPAR values were reached for sunflower and sugar beet. This is because the planophile leaves of sunflowers and sugar beet can intercept more solar radiation than rapeseed. Therefore, the LAI is one of the most influential factors in its calculation, as it varies depending on the development stage of the crop. The ability of canopies to intercept PAR has a positive impact on their growth and, consequently, on their productivity. For this reason, studying variables such as IPAR has a potential application in agronomic management and in improving crop production efficiency.

Keywords: *GIS, industrial crops, intercepted photosynthetically active radiation, sunflower.*

Introduction

The role of agriculture in rural areas is fundamental in many aspects: social, economic and environmental. From a territorial perspective, rural depopulation is originating a serious crisis in the countryside as there is no labour force to work the land. This is a major problem in regions such as Castilla y León, where more than 20% of the territory is destined to this activity. In addition, climate change is having a significant impact on agricultural productivity in Mediterranean areas (Zubelzu *et al.,* 2022). The increase in the frequency and severity of extreme events, such as heat waves or torrential rains, causes severe damage to crops.
Considering the relevance of this sector in our community, over the last few years, the automation of agricultural practices has been implemented to help a gradual transition from being static and manual to dynamic and smart. Precision agriculture (PA) uses satellite navigation systems, geographic information systems (GIS) and sensors installed on crop plots, which help to collect massive information from different sources (soil, crop and weather conditions) and from different parts of the field (Shafi *et al.*, 2019). In this way, farmers can

identify the real needs of crops and apply exactly what they need to optimise productivity and minimise environmental impact.
Therefore, it is fundamental to highlight the relevance of agriculture as a multifunctional activity. Among all the factors that influence crop growth, there is a climatic variable that is not often considered in crop planning and management: Photosynthetically Active Radiation (PAR), in particular, Intercepted Photosynthetically Active Radiation (IPAR). It is directly involved in photosynthesis process and it is usually estimated from a Beer's Law approach defined in Eq. 1:

$$IPAR = PAR \cdot (1 - e^{-k \cdot LAI}) \quad (1)$$

Photosynthetically Active Radiation (PAR) is a component of the global solar radiation with a wavelength between 400 and 700 nm, i.e. within the visible spectrum. It is a term that refers to both photon flux ($\mu mol \cdot m^{-2} \cdot s^{-1}$) and energy ($W \cdot m^{-2}$) capable of transporting electrons from the photosynthesis (Nwokolo and Amadi, 2018).
The light extinction coefficient (k) is a parameter that describes the efficiency of light interception in plant canopies (Kukal and Irmak, 2020).
The Leaf Area Index (LAI) is the leaf area per unit ground projection area (m^2/m^2). This index is a reliable indicator of vegetation state and evolution over time (Asner *et al.,* 2003), as it can be measured at different spatial and time scales.
Despite its importance in the photosynthetic process, most weather stations do not have sensors to measure PAR. There are some initiatives that are starting to install sensors at strategic locations to develop a PAR measurement network (Vindel *et al.*, 2018). For this reason, PAR is usually calculated by determining a constant relationship between PAR and other climatic variables. One of the most common relationships is to determine it by assuming that its value varies between 45 and 50% of the Global Horizontal Irradiance (GHI) value (Tsubo and Walker, 2005).
The main objective of this study is to calculate and represent an estimation of IPAR values for some industrial crops with GIS. In this way, it will be possible to improve the planification of the agricultural year, making optimum use of natural resources such as solar radiation and, therefore, achieve higher yields.

Materials and methods

The Castilla y León community is the largest region of the Iberian Peninsula, with an area of approximately 94.000 km^2. Its orography is distinguished by a wide plateau with an average altitude of 800 metres above sea level, surrounded by three mountain systems (Cordillera Cantábrica to the north, Cordillera Central to the south, and Sistema Ibérico to the east). The climate is temperate, as it is located in the inland northwest of the country.
In order to identify the industrial crops and the area destined to each of them, the "Mapa de Superficies Naturales de Castilla y León 2021" (MSNCyL), elaborated annually by the "Instituto Tecnológico Agrario de Castilla y León" (ITACyL, 2021), was used. Industrial crops were classified into three groups: rapeseed, sunflower and sugar beet. The total area covered by each of them is shown in Table 1.

Table 1. Area destined for industrial crops in Castilla y León in 2021.

Crop	Area (ha)
Rapeseed	33810
Sunflower	242432
Sugar beet	18422

Global Horizontal Irradiance (GHI) data were collected at 95 agroclimatic stations (46 from Castilla y León and 49 from all the neighbouring regions) provided by several networks (SiAR network, ADRASE, Air Quality Control Network in Cantabria, and SOLARGIS). All data between the years 2007-2020 were processed to obtain the annual daily mean value of GHI (MJ/m^2) at each station. Then, they were spatially represented in QGIS 3.16 software with the ordinary kriging geostatistical method as it is the spatial interpolation technique that best performs with GHI data (Rodríguez-Amigo *et al.*, 2017).
PAR was calculated by setting a constant relation of 0.48 (Harbo *et al.*, 2022) with the Community GHI data and the k and LAI values (Table 2) were established after an extensive review of numerous studies performed in different locations and under a wide range of soil and climatic conditions.

Table 2. Light extinction coefficient (k) and Leaf Area Index (LAI) estimated values for each industrial crop.

Crop	k	LAI (m^2/m^2)	References
Rapeseed	0.718	1.609	Fletcher *et al.*, 2013
Sunflower	0.738	4.416	Zarea *et al.*, 2005
Sugar beet	0.694	4.420	Varga *et al.*, 2021

Results and discussion

Firstly, the GHI map of Castilla y León was represented using the ordinary kriging interpolation method to the annual daily mean GHI values of the 95 agroclimatic stations of the Community and the neighbouring ones.
Afterwards, the PAR map of Castilla y León was elaborated by applying the constant coefficient of 0.48 to the GHI map. Its values range are between 5.69 and 8.35 MJ/m^2 and tend to increase as we move southwards in the region (Figure 1). The lowest values are registered in the north of Palencia and Burgos.

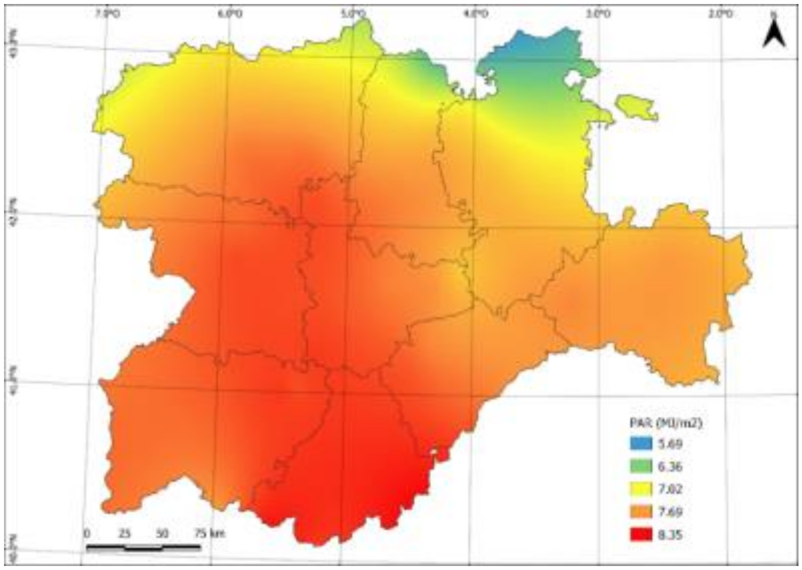

Figure 1. Estimated annual daily average PAR (MJ/ m²) values in Castilla y León.

PAR provides relevant information that can improve crop development, but it is necessary to know the IPAR as it is the one actually used by plants to carry out the photosynthesis. Therefore, with the PAR map of Castilla y León and k and LAI values established after the literature review for each type of industrial crop (Table 2), Beer’s law (Eq. 1) can be applied with the expressions described in table 3.

Table 3. Beer’s law and estimated IPAR ranges values estimated for each industrial crop in Castilla y León.

Crop	**Beer’s law**	**IPAR (MJ/m²)**
Rapeseed	$PAR\ map \cdot (1 - e^{-0.718 \cdot 1.609})$	4.07-5.62
Sunflower	$PAR\ map \cdot (1 - e^{-0.738 \cdot 4.416})$	5.73-7.89
Sugar beet	$PAR\ map \cdot (1 - e^{-0.694 \cdot 4.420})$	5.93-7.74

The values in table 3 show the IPAR ranges for the three industrial crops, rapeseed, sunflower and sugar beet, respectively. The IPAR ranges are quite similar for sunflower (Figure 2) and sugar beet as they can intercept more solar radiation than rapeseed. These values show that canopy light interception is positively related to changes in LAI values.

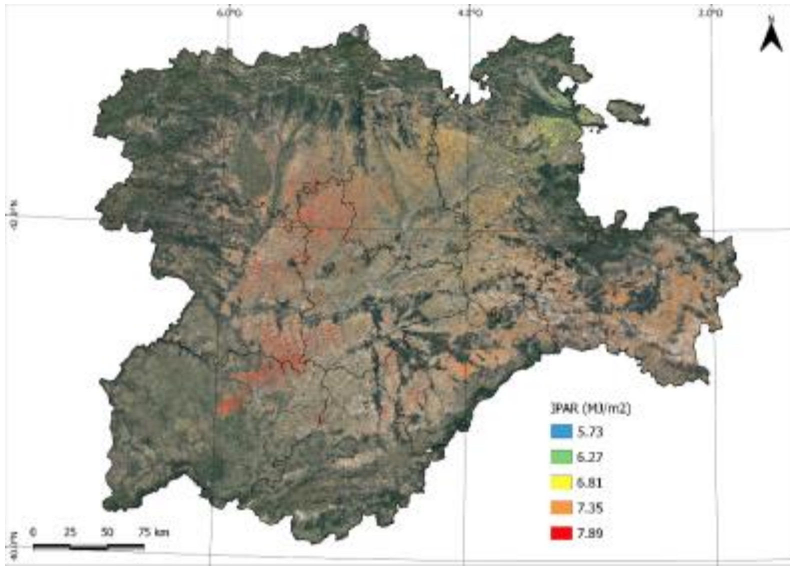

Figure 2. Estimated IPAR (MJ/ m²) values in sunflower.

Moreover, PAR interception usually increases sharply with increasing LAI values up to 2, but increases more gradually thereafter. In the mid-late growing season, when LAI values are around 5, the percentage of intercepted radiation reaches up to 95% (Wajid *et al.*, 2004).
Another way of expressing IPAR is over the entire growing season. Cumulative IPAR (CIPAR) can be estimated at the end of the growing season from measurements based on the number of days since sowing (Liu *et al.*, 2021).

Conclusion

These types of studies, which emphasise the relevance of precision agriculture, are very important, as this is a field that is constantly developing. The use of GIS is very useful for planning agricultural years and thus improving the relative efficiencies of crops, in this case industrial crops. The interception of PAR has a direct impact on biomass accumulation and, consequently in grain production. For this reason, estimating IPAR can be a potential application in the development of new, more sustainable agronomic management techniques.

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CHARACTERIZATION OF GLOBAL IRRADIANCE, ULTRAVIOLET RADIATION AND PHOTOSYNTHETICALLY ACTIVE RADIATION IN BURGOS, SPAIN

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Abstract

Solar radiation (SR) is essential in many areas of human activity. In the energy context, especially in renewable energies, the broadband solar radiation is converted into useful heat or electricity. Ultraviolet radiation (UVR) with a wavelength between 100 and 400 nm is responsible for a huge variety of photochemical reactions (Jacovides et al., 2009) and, especially in its shorter wavelengths, has deleterious effects in many biological systems. In people, it can cause damage to the skin (premature aging, burns, skin cancer ...). Finally, photosynthetically active radiation (PAR), with a wavelength between 400 and 700 nm, is a key factor in agriculture, as it is the energy source capable of triggering photosynthesis, which makes food and biomass production possible (I., Alados, I. and L., 1996). Despite the obvious interest in the knowledge of the spectral distribution of SR, the number of measurement sites in most areas of the world is scarce. Therefore, the lack of data means that UVR and PAR are usually estimated as a constant fraction of broadband solar irradiance. However, these ratios are affected by atmospheric conditions, mainly due to absorption and diffusion effects of SR across different regions of the spectrum. The objective of this work is to study the variation of the UvGH-RaGH and PAR-RaGH ratios as a function of the sky type classified according to the CIE standard at different time intervals (ISO, 2004). For this purpose, a 36-month experimental campaign was carried out in which data on atmospheric variables were collected together with illuminance and radiation data (UvGH, PAR and RaGH). After analysing the data, it can be concluded that there is a high correlation between UvGH-RaGH and PAR-RaGH (R2 value of 0.968 and 0.995 respectively), having obtained that the UvGH-RaGH ratio is 4.94% and the PAR-RAGH one is 39.1%.

Key words: *global irradiance, ultraviolet radiation, photosynthetically active radiation, vegetation growth.*

Introduction

Ultraviolet radiation (UVR) is the region of the solar spectrum between 100-400nm. The earth's surface does not receive all the UV radiation emitted by the sun. Atmospheric oxygen and ozone completely absorb the UV-C component (100-280 nm), while the UV-B (280-315 nm) and UV-A (315-400 nm) components are partially and weakly absorbed by ozone, respectively (Alados-Arboledas et al., 2003). This type of radiation has a significant influence on human health. Excessive ultraviolet radiation can be responsible for premature skin aging, skin cancer, cataracts,... (Ahmed et al., 2022; Human and Bajic, 2000; Modenese et al., 2020). In moderate doses, it can be beneficial to health by lowering blood pressure and promoting vitamin D synthesis, ... (Serrano et al., 2017). Since not all meteorological stations have sensors to measure

this type of radiation, many authors have related the UvGH component to RaGH, estimating that this relationship varies between 3% and 5% and it is influenced by cloudiness and solar elevation (Bilbao et al., 2011; Foyo-Moreno et al., 1999, 1998).
Photosynthetically Active Radiation (PAR), with a wavelength between 400 and 700 nm, is responsible for the biochemical processes carried out by plants in photosynthesis to convert light energy into biomass (McCree, 1973). Accurate PAR estimates are needed to model plant productivity and biomass production (Aguiar et al., 2012), to measure forest productivity (Landsberg and Waring, 1997), etc. Furthermore, accurate theyare fundamental to determine the impact of climate change and deforestation on agriculture (Pei et al., 2013). Due to the scarcity of PAR data, this parameter is usually calculated as a function of Global Horizontal Irradiance (García-Rodríguez et al., 2020). Several studies have estimated the PAR/RaGH ratio between 44%-50% (Moon, 1940; Tsubo and Walker, 2005), and concluded that this ratio is practically not influenced by the geography of the place, seasonal trends, meteorological and climatic factors and day length (Meek et al., 1984). However, when the influence of sky conditions on the PAR/RaGH ratio is analysed, several studies conclude that the highest values are obtained with overcast skies (Blackburn and Proctor, 1983; Stigter and Musabilha, 1982).
This work has focused on determining how UVR and PAR ratios in the horizontal plane (UvGH, PAR) vary in the city of Burgos (Spain) with respect to broadband solar irradiance in the horizontal plane (RaGH) as a function of sky conditions, classified according to the CIE standard over different time scales (10-minute and seasonal intervals).

Materials and methods

The experimental campaign was conducted at the meteorological facility (Figure 1) located at the Higher Polytechnic School of Burgos University (42º21′04″N, 3º41′20′W, 856 m.a.s.l.) from 1st March 2019 to 28th February 2022. A complete description of the meteorological facility can be found in Ref.(Suárez-García et al., 2020).

Figure 1. Location of the experimental equipment on the roof of the Higher Polytechnic School building at the University of Burgos, Spain.

The atmospheric variables measured at the weather station are: ambient temperature and relative humidity, precipitation, wind speed and direction, atmospheric pressure (Table 1). The data are recorded every 30 seconds and 10 minute average values are stored, the data obtained are analysed and filtered according to quality criteria (Gueymard and Ruiz-Arias, 2016).

Table 1. Models of the installed sensors.

Type	Units	Model
Temperature	ºC	Campbell Scientific - CS215
Precipitation	mm	Campbell Scientific - 52202 Electrically Heated Rainand Snow Gage
Wind	m s^{-1}	Campbell Scientific - 03002 Wind Sentry Set
Pressure	mbar	VAISALA - PTB110
Irradiance	W m^{-2}	Hukseflux - SR12-T205
PAR	µmol m^{-2} s^{-1}	EKO - ML-020P
UV 5	W m^{-2}	K&Z - CUV 5

Table 2. Characteristics of the installed Sky-Scanner.

Model	MS-321LR Sky Scanner
Dimensions (W × D × H)	430 mm × 380 mm × 440 mm
Mass	12.5 kg
FOV	11°
Luminance	0 to 50 kcd/m2
Radiance	0 to 300 W/m2
A/D Convertor	16 bits
Calibration Error	2%

A Kipp&Zonen CUV5 sensor is used to measure UVGH and RaGH data aremeasured with a (Hulseflux, model SR11) pyranometer (Table 1). The sky type is determined from the luminance data collected by a Sky-Scanner (EKO MS-321 LR) (Table 2) according to the CIE classification (ISO, 2004). The Sky-Scanner takes measurements of the sky from sunrise to sunset by performing a complete sweep of the sky for 4 minutes, and starts a new sweep of the sky vault every 10 minutes. Luminance measurements that are not between 0.1 kcd·m-2 and 50 kcd·m-2, according to the equipment specifications, are discarded to avoid erroneous measurements. Solar height values lower than 7.5º (corresponding to the first and last measurement of the day) are also discarded. Data are considered valid when, in addition to meeting all the conditions mentioned above, they are present in all the equipment used in the study.

Results and discussion

A Sky-Scanner has been used to determine the frequency of occurrence (FOC, %) of each CIE sky type in Burgos during the measurement campaign (Granados-López et al., 2021; Suárez-García et al., 2020, 2018), with clear skies being predominant in Burgos during the experimental campaign. Type 13 was the most frequent type, with a FOC above 20%, followed by type 11 and 12 (Figure 2).

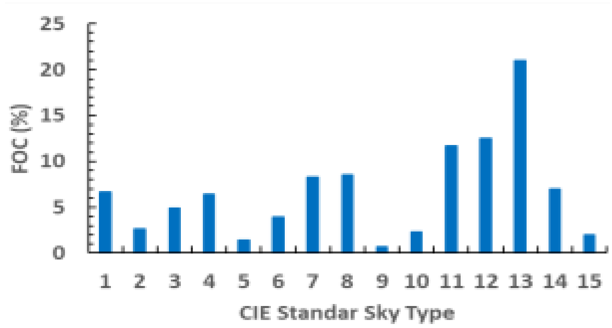

Figure 2. FOC (%) of CIE Standard Sky types in Burgos.

Since weather stations do not usually have sky scanning devices, most authors use a more generic classification to classify skies according to the CIE standard in which 3 sky categories are considered: cloudy, partial and clear skies. According to this classification, the 15 CIE skies are grouped into three categories: 1 to 5 for cloudy skies, 6 to 10 for partial skies and 11 to 15 for clear skies. In this study, the frequency of occurrence of each type of sky has been analysed per season according to the three categories mentioned above, considering spring from March to May, summer from June to August, autumn from September to November and winter from December to February. Figure 3 shows that all seasons are dominated by clear skies with frequencies of 52.4% in spring, 65.5% in summer, 47.8% in autumn and 42.0% in winter.

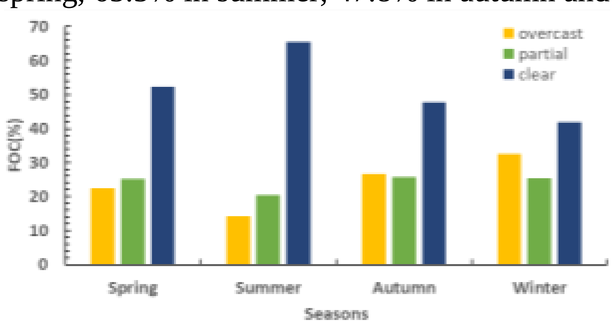

Figure 3. Seasonal FOC (%) of CIE cloudiness classification in Burgos.

This study also analysed the UvGH-RaGH and PAR-RaGH relationship during the experimental campaign.

Figure 4a) shows the UvGH-RaGH relationship, the high positive correlation is observed, with a coefficient of determination (R2) of 0.968.

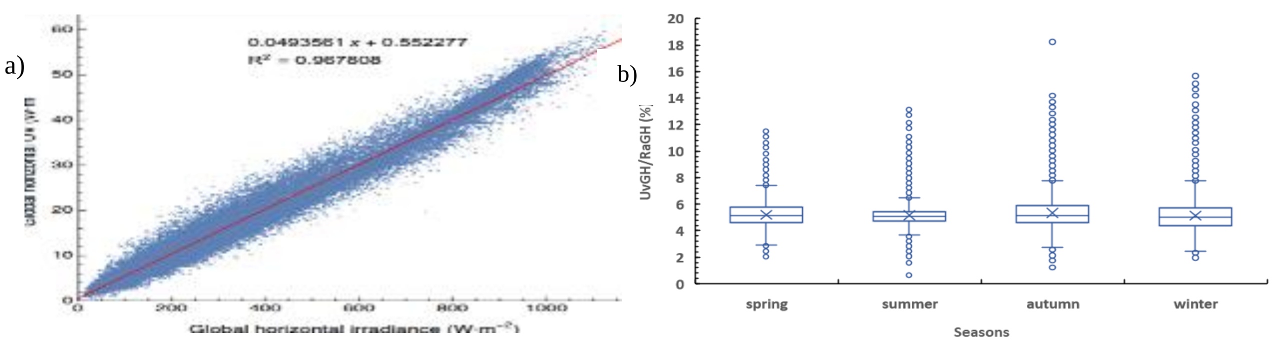

Figure 4 a). UvGH vs RaGH (W/m^2) ten-minute data measured in Burgos.
b) Box-plot ratio UvGH/ RaGH for each season.

Figure 4b) shows the UvGH/RaGH ratio by season. It is observed that in summer the dispersion is very small, increasing slightly in spring and winter, in autumn the largest dispersion occurs (in this season the 3 types of skies have a closer FOC). The highest standard deviation is recorded in autumn (1.12), while in spring and summer the standard deviation decreases to values close to 0.90. The minimum value (0.65) is recorded in summer and the maximum (18.23) in autumn. The UvGH/RaGH ratio during the experimental campaign is 4.9%.

a)

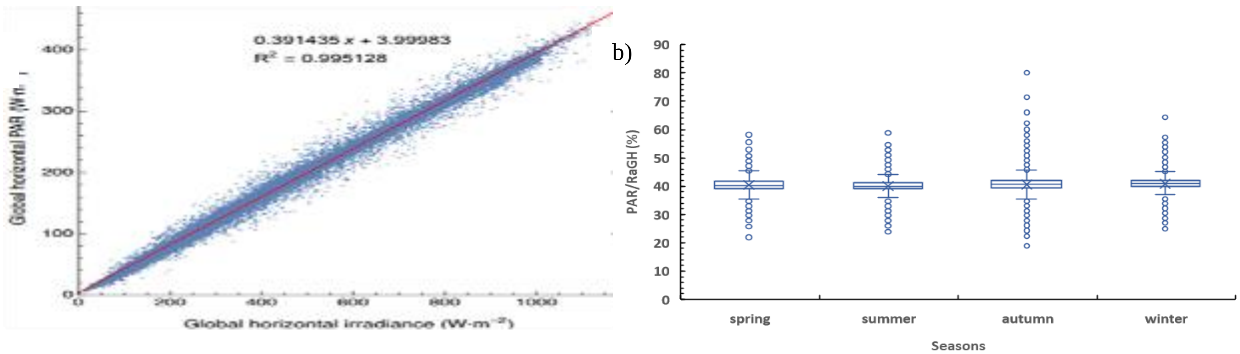

Figure 5 a). PAR vs RaGH (W/m^2) ten-minute data measured in Burgos.
b) Box-plot ratio PAR/ RaGH for each season.

Figure 5a) shows the PAR-RaGH relationship, the high positive correlation is observed, with a coefficient of determination (R2) of 0.995.
Figure 5b) shows the PAR/RaGH ratio, whose dispersion is very small in summer and winter but increases slightly in spring and autumn. The highest standard deviation is recorded in autumn (2.68), while in spring, summer and winter the standard deviation decreases. The maximum (80.06) and minimum (18.86) values are also recorded in autumn.
The PAR/RaGH ratio during the experimental campaign is 39.1%.

Conclusion

UvGH, PAR and RaGH data were recorded at 10-minute intervals in Burgos, Spain, between March 2019 and February 2022, and the variation of UvGH-RaGH and PAR-RaGH ratios were analysed under all sky conditions. It can be concluded that, in the city of Burgos, clear skies predominate, being CIE 13 the one with the highest FOC (21%).It is also observed that clear skies predominate in all seasons of the year, reaching the highest values in spring and summer.
After analysing the ratios, it is observed that there is a high positive correlation in the ten-minute UvGH-RaGH data, obtaining a high R2 value (0.968), and the UvGH/RaGH ratio during the experimental campaign was 4.94%. The correlation of the ten-minute PAR-RAGH data is also high, with an R2 value of 0.995, obtaining that the PAR/RaGH ratio was 39.1%.

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OVERVIEW AND USE VALUE OF VINEYARD WEEDS IN BOSNIA AND HERZEGOVINA

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Abstract

Weeds are an extremely complex, dynamic and very diverse category of plants, whose main feature is under greater or smaller anthropogenic influence. Floristic-phytocenological research of weed flora and vineyard vegetation was conducted during three vegetation seasons (2019, 2020, 2021), from spring to mid-autumn. The aim of the research was to determine the current state of weed flora in vineyards in Bosnia and Herzegovina and to analyze the flora with regard to affiliation to life forms, floral elements, use value. The research included 73 vineyard sites in Bosnia and Herzegovina. During three years of research into the weed flora of Bosnian vineyards, 155 species of vascular flora were recorded, classified into 50 families. Negative properties are most often attributed to weeds, and interestingly, they have a very wide use value. A large number of weed species are used as medicinal, honey-bearing, edible, spicy and fodder plants. They have other interesting and useful use values (decorative, compost, biofertilizer, insecticides, plant stimulants, erosion protection, application in the cosmetics and food industry, etc.). Out of 155 recorded weed species in the vineyards of Bosnia and Herzegovina, the most common use of weeds is for medical (medicinal) purposes (120 weed species or 28%), followed by food (102 weed species - 24%), as honey plants (85 species - 20%), and fodder (38 species - 9%). They are least used as spices (18 species - 4%) while as many as 60 species (14%) have poisonous properties. 117 weed species have a different use value, than applications in various types of industry, such as ornamental species, use for compost, biofertilizer, biocides, plant stimulants, erosion protection and use in cosmetics.

Keywords: *weeds, vineyards, use value.*

Introduction

Weeds are a diverse group of plants whose basic characteristic is being under the influence of man. Definitions of weeds are numerous, and most often we call weeds all plants that are undesirable in a certain area at a certain time, i.e. all plants that grow together with the cultivated plant species.

The word weed is most often associated with undesirable and harmful plants that do cause numerous damages and reduced yields in agricultural production, but the fact is that weeds can also be useful. From a biological point of view, weeds are plants, which form plant communities (phytocenoses) with crops and tolerate agrotechnics. Weeds are partly of autochthonous flora origin, and partly non-native species, which were imported as companions of cultivated plants.

It is important to know the weeds within the vineyard, knowledge refers to the specific biological and ecological characteristics of each important weed species. These characteristics determine its competitive abilities and place it in the interaction culture - weeds. The cultivation of vines in Bosnia and Herzegovina has a tradition of more 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 (Beljo et al., 2018). Today, according to official data, the vine occupies an area of about 3500 ha, some data (Rotim, 2017) state 4000 ha, which is less than 1% of arable land in Bosnia and Herzegovina.

In addition to many negative effects and negative perceptions about weeds, they are important components of agroecosystems and biodiversity and can have positive effects such as antierosive action in grapes, which are most often grown on sloping terrain, then low-growing weeds that protect from erosion, crusting in dry areas, or from excessive evapotranspiration, and are not competitors to nutrients, water, and light. The benefits of weeds can be in the preparation of compost and mulching.

The weed flora has changed over time, therefore some species have decreased in number while others have become more numerous. Research in the UK (Marshall et al., 2003) shows that many weed species support greater diversity of phytophagous insects, so reducing weed numbers reduces the number of related insects and other taxa and thus directly positively affects biodiversity.

Numerous weed species have become very rare and have been included in national red lists (Čerovsky et al., 1999; Moser et al., 2002). The situation is particularly dramatic in Switzerland, where out of 176 taxa of weeds, 137 of them are included in the *red lists* (Richner et al., 2017). The aim of the research was to determine the current state of the weed flora in vineyards in Bosnia and Herzegovina and to analyze the flora with regard to belonging to life forms, flora elements and to show the wide utility value of weeds in vineyards because the most effective measure of weed control is their use, which has economic and ecological advantages.

Material and Methods

The research covered 73 vineyard sites in Bosnia and Herzegovina, from Prijedor, the wider area of Banja Luka, Gradiška, Prnjavor, through Žepče, Tomislavgrad, Mostar, Široki Brijeg, Grude, Ljubuški, Čitluk, Čapljina to Stolac and Trebinje (Figure 1). Latitude, longitude and altitude were determined for each locality using GPS devices. Floristic research of weed flora and vineyard vegetation was performed during three vegetation seasons (2019, 2020, 2021), from spring to mid-autumn. The size of the area per location was 100 m^2, and floristic research was done before application of agrotechnical measures.

Standard keys and iconographies for this area were used for processing and determination of plant taxa: Flora EuropaeaI – V (Tutin, ed., 1964 - 1980, 1993), Flora of Bosnia and Herzegovina (Beck, 1903, 1906–1923). , Flora SR SrbijeI-IX (Josifović, ed., 1970-1977), Flora Hrvatske (Domac, 1994), Ilustrovana korovska flora Jugoslavije (Čanak et al., 1978), Ikonographie der Flora des Südöstlichen Mitteleuropa (Javorka i Csapody , 1979), Flora Italiana (Fiori and Paoletti, 1921) and Flora Croatica database (Nikolić, 2015). The nomenclature of plant taxa is harmonized according to the work Flora Europea I – V (Tutin et al. 1964–1980, 1993), partly according to Pignatti (1982), Trinajstić (1975–1986) and according to the Index Florae Croaticae.

Figure 1. Distribution of the investigated vineyards

Results and Discussion

During the three-year research on the weed flora of the vineyards of Bosnia and Herzegovina, 155 species of vascular flora were recorded, classified into 50 families. The families *Asteraceae* and *Poaceae* stand out with the largest number of species, followed by the families *Cichoridaceae* and *Lamiaceae, Fabaceae, Polygonaceae, Caryophyllaceae*. 90 species of these families or 58.2% of the total number of recorded species, while other species are represented by less than 5 representatives. These families are among the most represented in the flora of Bosnia and Herzegovina (Beck, 1903-1927). Kojić (2005) lists them as the most numerous in orchards, Kovačević et al. (2015) in vineyards and Petrović et al (2021) on arable land in Herzegovina.
Analysis of life forms of weed flora of vineyards of Bosnia and Herzegovina shows the presence of 5 life forms. Equal dominance of therophytes and hemicryptophytes with more than 80% of species is noticeable, which gives this flora a therophytic-hemicryptophytic character (Table 1). Among other life forms, the presence of geophytes is also significant, representing 10.4% of the total number of species in the weed flora of vineyards. The representation of life forms is in line with previous research on the weed flora of Bosnia and Herzegovina (Kovačević et al., 2015, Petrović et al., 2021).

Table 1. Representation of life forms in the weed flora of vineyards of Bosnia and Herzegovina

Life forms	Number	Share (%)
Hemicryptophyte	62	40,3
Therophyte	62	40,3
Geophyte	16	10,4
Phanerophyte	4	2,6
Therophyte / Hemicryptophyte	4	2,6
Nanophanerophyte	2	1,3
Chamaephyt	2	1,3
Phanerophyte/Chamaephyte	1	0,6

Phanerophyte/Nanophanerophyte	1	0,6
Chamaephyte/Hemicryptophyte	1	0,6
Total	155	100

Analysis of floral elements, i.e. phytogeographic analysis of weed flora shows the presence of all 11 floral groups (Horvatić 1963 and Pignatti 1982) with the highest representation of widespread plants (WIDE), 72 plant species. It is followed by the Eurasian floral element with 32 species, cultivated and adventitious species are represented by 13 species. The southern European floral element is represented by 10 species, and the Mediterranean by 9 species. Types of circumcholoarctic distribution, Central European, Illyrian-Balkan and Southeastern European distribution are also represented (Table 2).

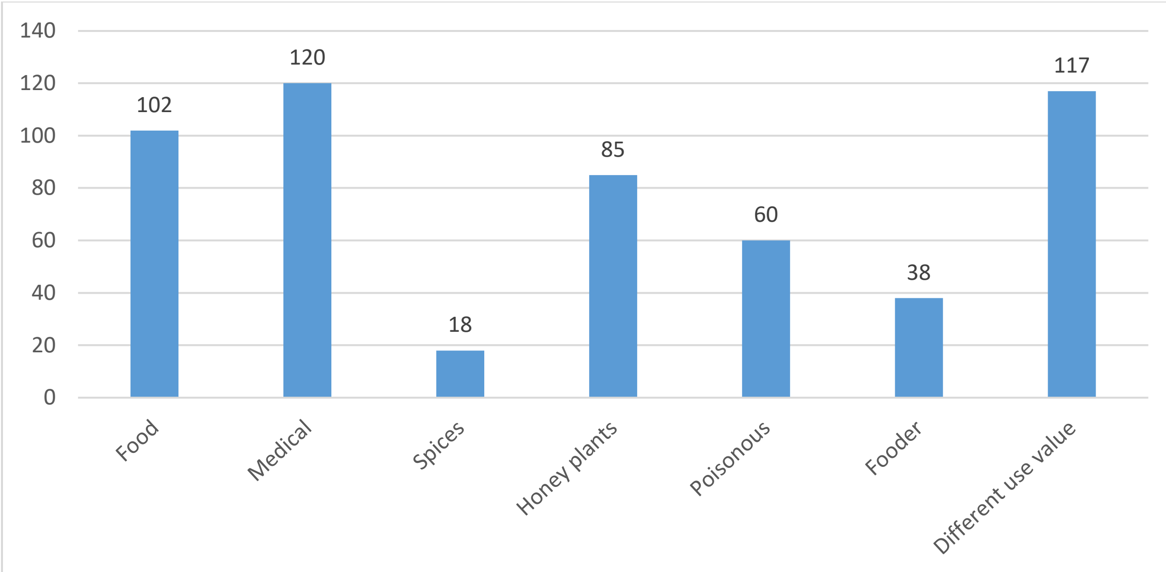
Table 2. Representation of certain types of floral elements in the flora of BiH vineyards

Floral groups	Number	Share (%)
Eastern European - Pontic floral element	2	1,3
Illyrian-Balkan floral element	2	1,3
European floral element	3	1,9
Central European floral element	4	2,6
Plants of circumcholoarctic distribution	7	4,5
Southeast European floral element	1	0,6
Mediterranean (Mediterranean) floral element	9	5,8
Southern European floral element	10	6,5
Cultivated and adventitious plants	13	8,4
Eurasian floral element	32	20,6
Plants of wide distribution	72	46,5

Cultivated and adventitious species are mainly man-made, intentionally or unintentionally, introduced from other continents, as ornamentals, cultivated, and more or less adapted there. They have 13 representatives in the weed flora of BiH vineyards and most of them are species from North America.

Weeds are mostly attributed to negative properties, but they have a very wide use value and a wide range of action. A large number of weed species are used as medicinal, honey, edible, spicy and fodder plants and may also have other interesting and useful values.

Out of 155 recorded weed species in the vineyards of Bosnia and Herzegovina, the most common use of weeds (Graph 1) is for medical (medicinal) purposes (120 weed species or 28%), followed by food (102 weed species or 24%), as honey plants (85 species or 20%), and fodder (38 species or 7%). They are least used as spices (18 species or 4%) while as many as 60 species (14%) have poisonous properties. 117 weed species have a different use value than applications in various types of industry, such as ornamental species, use for compost, biofertilizer, biocides, plant stimulants, erosion protection and use in cosmetics. Some weeds have multiple use and can be used for many different purposes.



Graph 2. Useful value of weed vineyards in Bosnia and Herzegovina

Conclusion

Most people have a repulsive attitude towards weeds based on the knowledge that weeds can cause a number of direct and indirect damages to a cultivated plant, so they need to be destroyed, which significantly increases the cost of plant production. During the three-year research on the weed flora of the vineyards of Bosnia and Herzegovina, 155 species of vascular flora were recorded, classified into 50 families. The families Asteraceae and Poaceae stand out with the largest number of species. Analysis of life forms of weed flora of vineyards of Bosnia and Herzegovina shows equal dominance of *therophytes* and *hemicryptophytes* with more than 80% of species. According to the analysis of floral elements, the most common plants are widespread with 72 plant species, followed by the Eurasian floral element with 32 species, cultivated and adventitious species are represented with 13 species.

The most common use of weeds is for medical (medicinal) purposes (120 weed species or 28%), followed by food (102 weed species or 24%), as honey plants (85 species or 20%), and fodder with 38 species or 9%. They are least used as spices (18 species or 4%) while as many as 60 species (14%) have poisonous properties. 117 weed species have a different use value, than applications in different types of industry, such as ornamental species, use for compost, biofertilizer, biocides, plant stimulants, erosion protection and use in cosmetics. Some weeds have multiple uses, and can be used for many different purposes.

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FERTILISATION POTENTIAL OF DIGESTATE OBTAINED FROM *ZOPHOBAS MORIO* FRASS

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Abstract

Nowadays, considering the exhaustibility condition of energy carriers, renewable and non-renewable energy sources are taken into account. The first group includes fossil fuels: fissile and conventional, which include crude oil, natural gas, peat, thorium, uranium, and coal. The second group comprises rapidly regenerating resources, the amount of which does not end up due to exploitation. This group includes the energy of water, sun, or biomass from which high-calorific biogas is obtained. Biomass consists of agricultural raw materials, agricultural by-products, and animal waste. After undergoing the anaerobic decomposition of organic matter, these products constitute the digestate, hereinafter referred to as the digestate. During the anaerobic decomposition of organic matter, plant components such as nitrogen and phosphorus are mineralised, nitrogen to NH_4^+ and phosphorus to PO_4^{3-}. In these forms, they are much more digestible for plants. One of the alternative methods of obtaining digestate is, among other things, the use of insect frass. One of the insects is *Zophobas morio*, commonly known as superworms, but also due to its body rich in protein and fats, bred and used in feeding fish, reptiles, and birds. The study aimed to obtain a digestate from the excrement of the Tenebrionidae beetle – superworm (*Zophobas morio*) and determine its fertilisation possibilities. The digestate from the frass of *Z. morio* has a moderate nitrogen concentration (<0.2%), but it is in the form of ammoniacal nitrogen, easily absorbed by plants. In addition, over 90% of the solid fraction of the digestate is carbon, which improves the properties and structure of the soil. The soil fertilised with digestate containing lignocellulose and easily decomposable carbohydrate fractions has better air-water properties and more significant microbiological activity. The digestate obtained from *Z. morio frass* can be used to fertilise, e.g. energy plants following the Zero-Waste Circular Economy.

Keywords: *Superworm, Frass, Digestate, Fertilisation.*

Introduction

Agricultural production of the 21st century emphasises farmers are increasing yields while reducing pesticides and mineral fertilisers. This is a crucial strategy of the European Green Deal, which, among other things, assumes a 50% reduction of plant protection products by 2030. With such a significant reduction in permitted pesticides, their alternate application will be complicated, so the phenomenon of resistance to diseases, pests or weeds will increase. On the other hand, excessive use of plant protection products or mineral fertilisers is also a very unfavourable phenomenon. Important here is their proper use and balance. This is important both for the farmer's portfolio and for the environment. The more expensive means of agricultural production and more frequent droughts require the farmer to use them often and adequately introduce changes in the crop rotation. For example, the introduction to the cultivation of legumes capable of nitrogen-fixing from the air allows for reducing the initial dose of this element for the succeeding plant and enriches the soil with organic matter. Due to the relatively high level of acidification of Polish soils, the often low level of organic matter and the deficiencies of micronutrients in the soil, widely used mineral fertilisers do not always work as they should (Vanlauve et al. 2015; Ebanyat et al. 2010; Kihara et al. 2016; Liverpool-Tasie et al. 2017).

However, pesticides in agriculture have their alternative, which is developing more and more dynamically, which are biological solutions and special fertilisers containing organic substances, which can neutralise unwanted bacteria, weeds and pests. An additional advantage of organic fertilisers is reducing the pressure of pathogens on crops without negatively impacting the environment. It is also worth emphasising their positive impact on the final yield, which translates into the economic result of the farm (Compant et al. 2005; Bonilla et al. 2012; Robaˇcer et al. 2016). Soil microorganisms contained in organic fertilisers contribute to stabilising biological processes in the soil solution, humus formation, and enhancing nutrient availability in the soil. Beneficial bacteria in the soil can also protect plants against pathogens. In agricultural ecosystems, the fungicidal activity is mainly demonstrated by: *Actinomycetes*, *Bacillus* spp. and *Pseudomonaceae*. Their development depends mostly on the C:N ratio, the number of nutrients in the soil, its structure, and the amount of microbiota (Ahemad and Kibret, 2014; Kooch et al. 2018). For example, the bacterial species *Pseudomonas fluorescens* is fungistatic to soil pathogens attacking plant roots by producing the 2,4-DAPG antibiotic (Svercel et al. 2009; Ahemad and Kibret, 2014). Growing agricultural plants requires a proper nutrient supply. The most important of these is nitrogen because of its yield-forming effect. To avoid losses caused by leaching ($N\text{-}NO_3$) or oxidation ($N\text{-}NO_4$) of nitrogen, it is worth turning to organic or natural fertilisers in which nutrients are gradually released and transformed into mineral forms using microorganisms. An alternative organic fertiliser of good quality, affordable, and readily available can be fertiliser derived from insects that are bioconverters of organic matter (Van Huis 2013; Van Huis 2016; Makkar et al. 2014; Cheseto et al. 2020; Oonincx et al. 2015; Ngoka et al. 2007; Magara 2021; Beesigamukama 2021).

Material and Methods

The tested material was frass from superworms (*Zophobas morio*) grown under controlled conditions in two treatments. The first variant included superworms whose main feed was poplar wood, and the second was pine wood. Superworms were placed in two insect boxes, 500 in each. The insect boxes consisted of a breeding chamber and a sieve in the bottom part, which was to collect the frass. The diets of both variants were enriched with organic household residues (vegetables, fruits). The frass that was tested was collected and weighed every two weeks. Nutrient content (NPK) measurements were made using the SKW 500 Complete Soil Kit. The reagents and the apparatus included in the kit were used to carry out the tests. The tested frass was weighed in a 2 ml teaspoon and added to the test tube in which, depending on the analysis, Extract N, Extract P or Extract K was placed along with 50 ml of distilled water. After thoroughly mixing the faeces for 1 min, Nitratest was added to the test tube in which nitrogen was determined and mixed. All solutions were filtered using Whatman Filter Paper. The obtained NPK solutions were poured into cuvettes in the amount of 10 ml, 2 ml (made up to 10 ml with distilled water), and 10 ml and placed in the Soiltest 10 apparatus until the standard was obtained. The next step was to add one tablet of each appropriate reagent to each of the analysed cuvettes, crushing it and mixing it thoroughly. The solutions prepared in this way were left for 10 minutes in the case of nitrogen and phosphorus and 2 minutes in the case of potassium for staining. After the time specified in the methodology, the cuvettes were transferred to the Soiltest 10, and the correct results were read.

Results and Discussion

The diversified way of feeding superworms caused a change in the fertiliser composition of the frass obtained from them and its amount (Table 1). Based on the obtained results, the greatest increases in the obtained biomass could be observed between days 28 and 42 in the case of pine and between days 42 and 56 in the case of poplars (Chart 1). The average frass increment in both plant species was around 30 g (pine 32.395 g, poplar 29.231 g). This is confirmed by the trend line presented in Figure 1. The lowest amount of biomass was produced by superworms during the 14th to 28th day. No significant differences were found in the case of the fertilisation values of the obtained biomass. The biomass obtained from poplars showed a higher mineral nitrogen content of 9.9 mg/kg. This was reflected in the ratio of NPK elements to each other.

Table 1. Increase in biomass from superworms and their fertilising value.

Tested feature	Pine	Poplar	Indices
Increase of biomass			
14 days	16.757g	13.493g	Pine wood R^2=0.9622 Poplar wood R^2=0.975
28 days	27.570g	26.820g	
42 days	61.893g	55.874g	
56 days	92.360g	85.282g	
The fertilising value			
N overall	6.2%	6.8%	N:P:K relation Pine wood: 1:4.7:8.5 Poplar wood: 1:3.8:6.6
N mineral	24.2 mg/kg	31.1 mg/kg	
P	114.8 mg/kg	119.5 mg/kg	
K	204.8 mg/kg	204.8 mg/kg	

Treating the biomass obtained from superworms breeding as agricultural fertiliser should be applied in advance. This is necessary due to the low amount of available nitrogen in frass. Adhering to agricultural terminology, its application should be pre-sowing, i.e. before sowing the crop. This application date will allow soil microorganisms to properly process the fertiliser, releasing the nitrogen present in organic tissues. In addition, frass from superworms as an organic fertiliser improves the physicochemical parameters of the soil and its water-air relations. It positively affects the content of organic matter in the soil. A longer release time for macro- and micronutrients is also important, so these substances are available to plants over a broader period. Importantly, soil microorganisms distribute the organic substance into amino acids and humic acids, which are part of soil humus.

Using biomass from superworms manure as fertiliser is worth turning to nitrogen. Considering the most important agricultural plants in terms of their fertilisation needs, frass should be used in the amount of 32 to 806 kg per ton of seeds or fresh weight (Table 2). The calculated doses are relatively high, but their single application before sowing is justified.

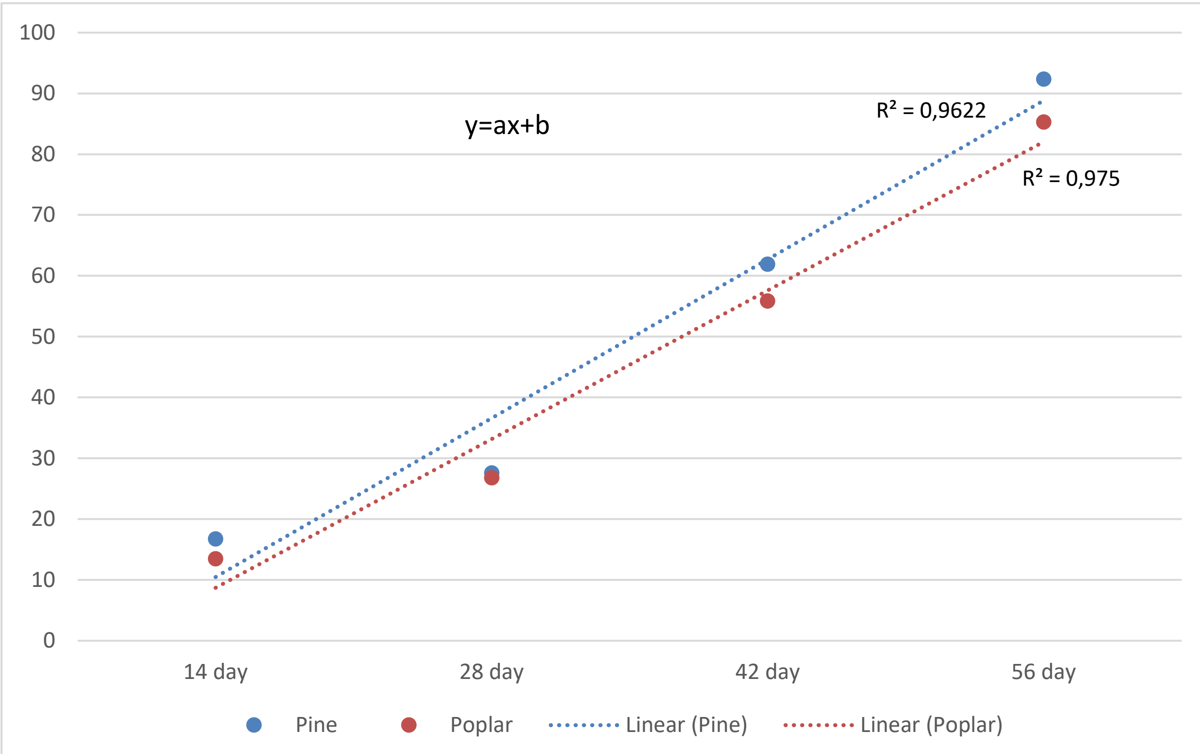

Chart 1. Time increment of biomass from superworms.

Considering the declining number of farms with cattle or pigs, where the by-products are manure or slurry, there may be a shortage of organic fertilisers in circulation. Another important aspect is the often poor crop rotation of farms and the increase in the prices of mineral fertilisers. Especially in recent years, the fertiliser market has been unpredictable.

Table 2. Fertilisation needs and the dose of frass necessary to satisfy them.

	Fertilisation needs N to produce 1t of seeds / fresh weight	A dose of frass to produce 1t of seeds / fresh weight
Rape	50kg	806.45kg
Wheat	27kg	435.48kg
Corn	26kg	419.35kg
Onion	2kg	32.26kg
Cabbage	3kg	48.38kg
Carrot	4kg	64.52kg

However, it is essential to keep the C: N ratio below 20:1 when using this type of fertiliser. This will significantly accelerate the mineralisation of the manure biomass. A higher ratio will cause the microorganisms to collect available nitrogen from the soil, causing its immobilisation, resulting in crop blockage.

Conclusions

The superworms digestate predisposes to be used in agricultural crops, including vegetables, cereal and oilseed. In the coming years, usage of the fertilising value of frass may be an alternative solution to expensive mineral fertilisers that only work interim when needed. The future is to maintain a proper soil structure, increase biological and biochemical activity and not sterilise but enrich it with organic matter. If the soil is not managed correctly, it may translate into the yield or health of crops. When used correctly, frass, an organic fertiliser, allows for a partial resignation from mineral fertilisation in favour of building yield-generating humus.

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AMBIGUITY IN THE RESULTS OF USLE K–FACTOR OBTAINED BY NOMOGRAPH AND ERODIBILITY EQUATION

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Abstract

Universal Soil Loss Equation (USLE) is the most applied model for soil erosion assessment. It takes into consideration five soil erosion factors among which soil erodibilty factor (K) is a measure of inheritant reistance of soils to erosion. In the formulation of USLE model, K–factor was determined experimentally, and then the authors created USLE nomograph to facilitate the work, and in the next step, created the equation for faster K–factor determination. This paper aims to compare K–factor obtained by USLE nomograph and USLE equation. A total number of 108 soil samples have been collected in Western Serbia. For the determination of K–values, particle size distribution and soil organic matter were analyzed in the laboratory, whereas soil permeability and soil structure were assessed according to the USLE document procedure. The results have been compared using basic statistics. The average K–values obtained by nomograph and equation differ for almost 15%. There is statistically significant correlation between the two methods, but coefficient of determination amounts to 0.5802, which indicates pretty high deviation. Root Mean Square Error (RMSE) amounts to 0.008, which is quite high, whereas index of agreement amounts to 0.93, representing good agreement. These results are ambiguous. We think that 15% of absolute error is a threshold value between single samples, which occur in only 56% of the cases. Therefore, the obtained results indicate the gaps in the determination of K–factor and could contribute to further improvment of soil erodibilty determination and consequently, soil erosion assessment.

Keywords: *Soil erodibility, USLE, soil erosion.*

Introduction

The terms soil erosion and soil erodibility look very similar at first glance, but essentially they are very different. On one side, erosion is a type of land degradation that results in the loss of surface layers of the soil under the predominant action of water or wind, whereas on the other side, erodibility tells us how the characteristics of a certain soil can resist towards the action of water and wind. Soil erodibility is expressed by the K–factor. From a fundamental standpoint, soil erodibility should be viewed as the change in the soil per unit of applied external force or energy (Renard et al. 1997; Foster, 2005).

K–factor is one of the five factors of the USLE (Universal Soil Loss Equation) equation and it is the most important soil-related erosion measure. Some soils are more susceptable to erosion than others and if all other factors in the USLE equation (Wischmeier and Smith, 1978) are equal, the difference in the amount of eroded soil depends completely on K–factor. Natural predisposition of a soil to erosion is a measure that cannot be easily quantified. In the original USLE document

(Wischmeier and Smith, 1978), soil erodibility was obtained by experimental measurements, which is time consuming, tedious, and complicated for the large scale utilization.
Soil erodibility depends on silt and clay fraction, organic matter content, soil structure and permeability, humus and skeletal content, but also on the development of the root system of the plants. In general, soils erode less if the amount of silt is decreasing, regardless of whether the fractions of sand and clay increase. Also, if the organic matter content in a soil is high, then it is more resistant to erosion processes compared with the soils with low organic matter content.
The K–factor from the USLE equation is the most important soil–based erosion characteristic used in many erosion models such as RUSLE – Revised Universal Soil Loss Equation (Renard et al. 1997; Foster, 2005), SWAT – Soil and Water Assessment Tool (Arnold et al. 1998), AGNPS – Agricultural Non-Point Source Pollution Model (Cronshey and Theurer, 1998), Watem/Sedem – Water and Tillage Erosion Model and Sediment Delivery Model (Van Rompaey et al. 2001), and EPIC – Erosion Productivity Impact Calculator (Williams et al. 1983), WEPP – Water Erosion Prediction Project (Laflen et al. 1997), EUROSEM – European Soil Erosion Model (Morgan et al. 1998), and PESERA – Pan European Soil Erosion Risk Assessment (Kirkby et al. 2008) and its accuracy depends on K–determinations. Overestimation and underestimation of K–factor can lead to inappropriate conclusions which can affect and jeopardize soil conservation strategy in the long run.
This paper aims to compare the values of K–factors obtained by using the nomograph method and the nomograph equation, which were created by the same authors, but they still somewhat differ one from another, which affects erosion assessment.

Materials and Methods

Methods used for estimating soil erodibility

The nomograph for determining soil erodibility (NOM) was created by Wischmeier at al. (1971) with the help of experimental research. Using the nomograph method K–factor is obtained on the basis of the sum of the fractions of silt and very fine sand, clay fraction, organic matter content, soil structure and permeability. Soil structure and water permeability are defined by indices. Value of the structure index ranges from 1 to 4, depending on the size of soil aggregates. A value of 1 is used for very fine granural aggregates, while a value of 4 is used for blocky, platy or massive aggregates. The water permeability index has values that range from 1 to 6, and the value depends on the water permeability of a soil, where the value of 1 represents extremely high permeability, and the value of 6 extremely low water permeability.
The values of K–factor are read from the nomograph by starting from the left side of the scale which represents the sum of the fractions of silt and very fine sand and continuing to the points that represent the sand content, precentage of organic matter, structural code and water permeability code. The procedure must be performed in the specified order. The disadvantage of this method is that it does not take into account the content of organic matter which is higher that 4%. K–factor is expressed in t ha MJ^{-1} mm^{-1}, but due to robustness of the unit we will not write it in the further text.
Considering the fact that the determination of K-factor using the nomograph method is quite slow, the equation for the estimation of the K–factor (NOM1) was developed somewhat later in order to speed up the process. The equation can be used for soils whose silt and very fine sand content does not exceed 70% and it is given as:

$$K = (2.1 * M^{1.14} * (10^{-4}) * (12 - a) + 3.25 * (b - 2) + 2.5 * (c - 3))/100$$

Equation 1

where M is the paramater defined by the formula: (% of fine sand + % of silt) * (100 – % of clay), a is the precentage of organic matter, b is the structure code and c is the water permeability code.
This equation is widely applied, but it does not complitely agree with the nomograph, and its application can lead to certain deviations. The difference occurs if the soils contain a high level of organic matter, high silt content and have naturally low erodibility. Some studies in Central Europe have indicated that the equation is not valid in more than 50% of selected samples (Auerswald et al. 2014). The error that occurs is sometimes quite high and can be as much as half the value of the K-factor.

Soil sampling and analysis

A total number of 108 soil samples were collected in the area of Western Serbia in the elevation range from 350–1200 m a.s.l. Soil structure and permeability were assessed according to the procedure described by Wischmeier and Smith (1978). The particle-size distribution of the soils was determined by combining sieving and the pipette methods (Rowell, 1997), whereas the soil organic carbon was determined using the dichromate method (Rowell, 1997).

Statistical analysis

Basic statistics were used in order to compare the values of erodibility factor, namely, Root Mean Square Error (RMSE) and Index of Agreemenet (IoA). The average difference between the results was described by the RMSE as:

$$\mathrm{RMSE} = \left[n^{-1} \sum_{i=1}^{n} (K_{nom} - K_{nom1})^2 \right]^{0.5}$$

Equation 2

where n is the number of pairs of observed/measured (K_{nom}) and predicted/simulated (K_{nom1}) data.

The index of agreement was determined to further compare the obtained erodibility factor values. It is a descriptive paramater whose value ranges from 0 to 1, with the value of 1 indicating excellent agreement. The index of agreement (IoA) was calculated according to Wilmot (1982) as follows:

$$\mathrm{IoA} = 1 - \frac{\sum_{i=1}^{n} (K_{nom} - K_{nom1})^2}{\sum_{i=1}^{n} (|K_{nom}'| + |K_{nom1}'|)^2}$$

Equation 3

where $K_{nom}' = K_{nom} - \overline{K_{nom}}$, $K_{nom1}' = K_{nom1} - \overline{K_{nom1}}$, and $\overline{K_{nom}}$ and $\overline{K_{nom1}}$ are average values of predicted and observed data, respectively, and bar above the erodibility means average value.

The IoA has been applied in the literature to assess model performance (Benli et al., 2007; Todorovic et al., 2009).

Results and Discussion

The minimum value of the K–factor obtained using the nomograph method in this study is 0.006, while for the equation this value is 0.003. Also, the maximum values differ for only 0.004, which is only 6% difference. The average value of the erodibility factor for 108 soil samples obtained by using the nomograph is 0.034±0.010, whereas in the nomograph equation this value is slightly lower and it amounts to 0.029±0.012. The coefficient of variation has a lower value of 30.3% for the K–factor estimated with nomograph, which is close to normal distribution of data, whereas for the nomograph equation, coefficient of variation is higher and does not correspond to normal distribution.

Table 6 Values of basic statistics for two methods (n = 108)

K–factor	NOM	NOM1
AVG	0.034	0.029
MIN	0.006	0.003
MAX	0.065	0.069
STDEV	0.010	0.012
CV	30.26	42.32

In order to better understand the differences in K–factor values obtained by applying different methods, the methods were graphically compared. This comparison is presented in Figure 1. The values of the erodibility factor obtained by the nomograph method (NOM) are shown on the X–axis, and those obtained using the nomograph equation are on the Y–axis (NOM1). As mentioned earlier, the average value of the K–factor is slightly lower in the nomograph equation. Furthermore, the coefficient of determination (R^2) has a value of 0.5802, which represents the number of data that match the line formed using the regression equation, displayed on the graph. This coefficient indicates that although there is a statistical correlation between the two methods, a deviation occurs in 23.9% of data.

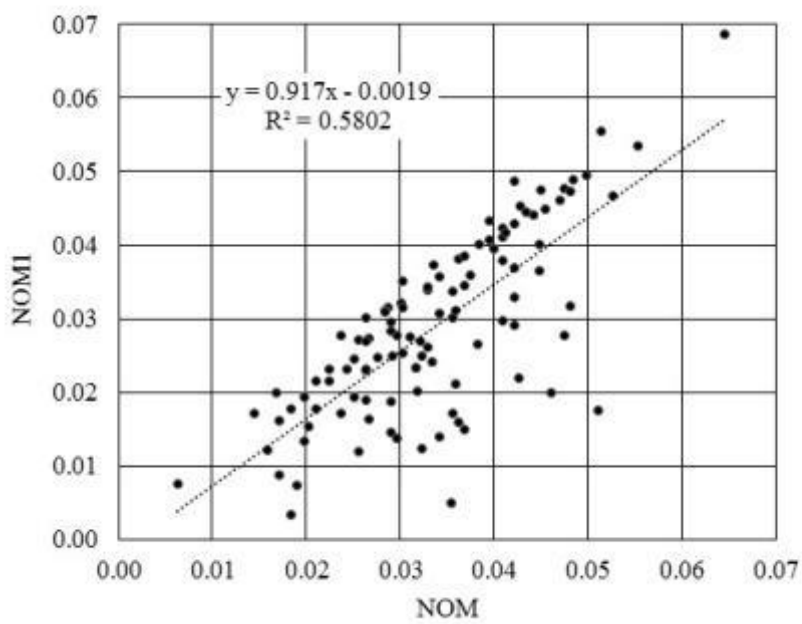

Figure 1 Correlation between K-values obtained by two different methods

It is important to emphasize that the nomograph method does not take into account soil organic matter content which exceeds 4%. It was found that the precentage of organic matter exceeds the given limit in 72 out of 108 soil samples covered by this study. Exactly this parameter can give us an insight into why the K–factor values differ so much in the applied methods. Also, we can conclude that in the 36 soil samples where the content of organic matter does not exceed 4%, there is less of a deviation in K–factor values between the two methods. In fact, there is a difference of only 1.83% in obtained K–factor values between the two methods in the case of 36 samples in which organic matter content doesn’t exceed 4%, compared to 22.58% in the rest of the samples.

The value of RMSE indicates the difference between the actual value of each point in a data set and the value of the points on the most suitable curve. RMSE has a value of 0.008 for the two methods studied in this paper, which indicates the fact that there is a difference of 23–27% between the values of the K–factor. Also, this is confirmed by the coefficient of determination. By comparing the nomograph and nomograph equation methods, it was found that the index of agreement is 0.93, which indicates good agreement and a significant correlation between the methods. Therefore, there is the ambiguity of the results reflected in the values of the root mean square error (RMSE) and the index of agreement (IoA).

Hence, one one side: a) the average K–values obtained by nomograph and derived equation are 0.034±0.010 and 0.029±0.012, which is 14.7% difference, b) Coefficient of determination (R^2) amounts to 0.5802, which indicates deviation of 23.9%, although there is statistically significant correlation between two methods, and c) RMSE is 0.008, which is 23–27% difference, whereas on the other side, a) IoA of 0.93 was observed and it implies that the covered methods match to a very notable degree. Therefore, depending on statistics, K–factor obtained by the two methods either largely coincide, or significant differences occur. We think that 15% of absolute error between separate estimations is a threshold value for good accuracy, which occur on only 56% of samples, and the best method for comparison is the one taking into consideration the comparison one to one, and not solely as datasets comparison.

Conclusion

Soil erodibility nomograph and the nomograph equation were created by the same authors. Moreover, the nomograph equation was made in order to speed up the process of determining K–factor values through the nomograph, which in the case of a large number of samples can be very tedious. Nevertheless, the values of the erodibility factor obtained by mentioned methods differ to some notable extent. The previous statement is confirmed by the average value of the K–factor, the coefficient of determination, RMSE and IoA. These results are ambiguous. Data elaboration indicate that the K–factor values obtained by the two methods largely coincide, or that significant differences occur. Assuming that all other factors in the USLE equation are equal, the difference in the amount of eroded soil material depends completely on K–factor. Therefore, we think that 15% of absolute error between K–values estimated with two methods is a threshold value for good accuracy. In fact, in our dataset which consist of 108 samples, this occurs on only 56% of samples. Knowing the importance of K–factor for erosion studies, it is crucial that soil erodibility is determined in the best possible manner in order to minimize errors in estimating the amount of eroded material. Comparison of datasets themselves only, may lead to missunderstanding of the process. The results obtained in this study imply the fact that there are gaps in determination of the erodibility factor which need to be addresed. In addition, these results could contribute to improving the estimation of soil erodibility, which may ultimately be helpful in assessing erosion intensity and contribute to its prevention.

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SUITABILITY OF SOME OAK SPECIES FOR PAPERMAKING

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Abstract

The paper qualities of oak species such as Q. robur (English oak), Q. petraea (Sessile oak) and Q. frainetto (Hungarian oak) and Q.cerris (Turkey oak) were evaluated for paper raw material availability. For this purpose, some paper properties such as between fiber dimentions and fiber dimention relationships were investigated. Schultze (1857) was used as the maceration method after the oak species were cut into pieces in the size of fine sawdust. Then, fiber dimensions such as fiber length, fiber width and cell walls were measured and evaluated from the prepared microscope slides. According to the results; Among the oak species, the longest fiber length is Q. cerris 1,337 mm and the shortest fiber length is Q. robur 1.018mm. The fiber length levels of Q. petraea and Q.frainetto species were determined as 1,103 mm and 1,062 mm, respectively. It was determined that the thickest diameter average of the oak species belonged to sessile oak (27.6 µm), while the thinnest fiber diameter was determined to belong to the stemless oak (6.3 µm). It was determined that the fiber lengths of oak species are between the maximum hardwood fiber lengths. While fiber length is an important variable that positively affects papermaking, fiber thickness and cell wall variables are also important determinants. Thus, the relationships between fiber dimentions and fiber dimention of oak species were determined separately for each species on their suitability for use in pulp production was evaluated and it was determined that they were not suitable as raw materials in papermaking.

Keywords: *Q. robur, Q. petraea, Q. Frainetto, Q.cerris, fiber morphology.*

Introduction

The most important factor determining the properties of a paper is its fiber structure. Softwoods such as pine, fir and spruce have long fibers. Paper produced from these trees has high resistance properties. Hardwoods such as oak and maple have very short fibers. Papers obtained from such woods have less resistance properties than those obtained from softwoods. However, since their surfaces are smoother, they are suitable for writing and printing. With the increasing population and the development of industry in the world, the demand for raw materials is also increasing. In order to meet the raw material needs, importance is given to the breeding of high-yield species. For this purpose, the possibilities of using oak species in the paper industry were investigated. Oak is the common name of wood-resistant forest trees with about 400 species of the type Quercus of the Fagaceae family. It has a wide distribution area in the world. Its homeland is Anatolia, Iraq, Iran, Syria, Lebanon, Afghanistan, Pakistan and Greece. Oak grows in almost every region of Turkey, from the highest mountains to the low plains. Hungarian oak is an oak species that naturally spreads in Southern Europe, Italy, the Balkans and Turkey.
The images of leaves Q. petraea, Q.robur, Q. frainetto is given in figure 1a,b and c (Öztürk 2013).

Figure1a. Q. *petraea*

Figure1b. Q. *frainetto*

Figure 1c. Q.*robur*

Figure 1d. Q. *cerris*

Q.cerris (Turkey oak) is an oak species native to Southern Europe and Anatolia. Its appearance is given in Figure 1d (URL 5).

Material and Methods

Material

The discs were taken from a height of 1.30m from the lower part of the 25 m high Oak trees. The oak samples were cut into small pieces by forming cake slices and turned into chips the size of matchwood.

Method

The fibers of the oak species were obtained using the Schultze (1857) maceration method. Preparations were prepared according to T232 cm-85 from the macerated samples, and fiber sizes were measured under a Leica 2500 DM an optical microscope. The average, maximum and minimum values of the measured fiber dimensions of each section were calculated. At least 50 measurements were made for each sample. The criteria used to determine the suitability of fiber morphological characteristics for pulp production are defined below.

Elasticity ratio:(Lumen radius/fiber width)*100
Fibring ratio:Fiber: Fiber length/fiber width
Runkel classification:Fiber width/lumen Radius
Rigidity coefficient: (Cell Wall thickness/fiber cross sectional area)*100
F-factor: (Fiber length/ Cell Wall thickness)*100
Muhlstep classification: (Cell Wall thickness area/fiber cross sectional area)*100

Results and Discussion

At least 50 measurements were made for each fiber property such as fiber length, fiber diameter, and cell wall thickness, including fiber dimensions, and their average, maximum and minimum values are given in Table 1.

Table 1. Fiber dimensions measurements of oak species woods

		Length (mm)	Width (µm)	Cell wall thickness (µm)	Lumen (µm)
Q.petraea Sessile oak	Mean	1,103	23,4	6,9	9,5
	Max.	1,513	27	8	12
	Min.	0,566	17	5	8
Q.cerris Turkey oak	Mean	1,337	25,9	7,7	10,4
	Max.	1,755	30	9	12
	Min.	0,961	21	5	9
Q.robur Pedunculate oak	Mean	1,018	22,4	6,3	9,7
	Max.	1,406	26	8	14
	Min.	0,715	18	5	6
Q.frainetto Hungarian oak	Mean	1,062	23	7,3	8,5
	Max.	1,651	27	9	12
	Min.	0,642	17	5	8

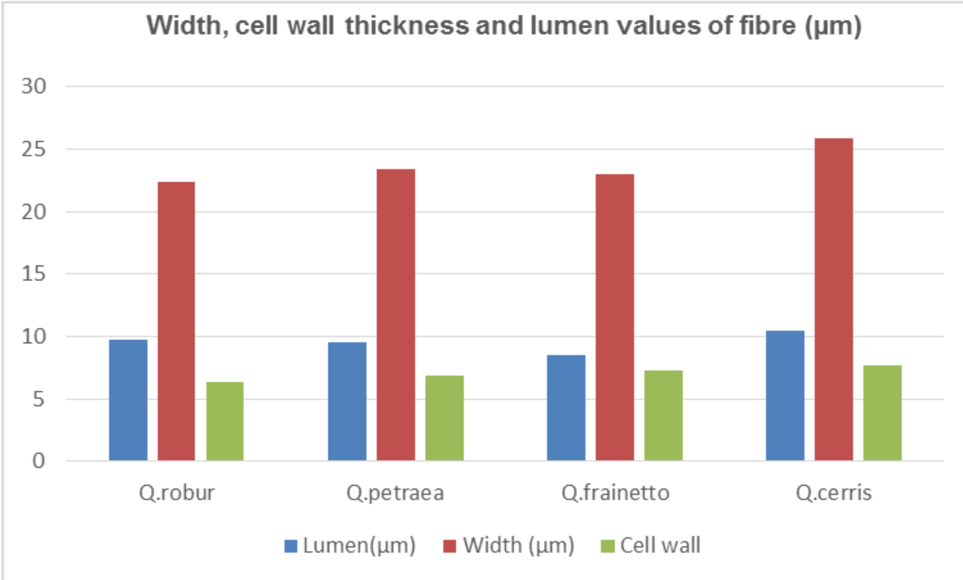

Figure 2.Width, cell wall and lumen values of oak species fibers

Among the oak species, Q.cerris were the widest diameter and cell wall thickness, while Q.robur had the narrowest diameter and cell wall thickness (Fig.2).

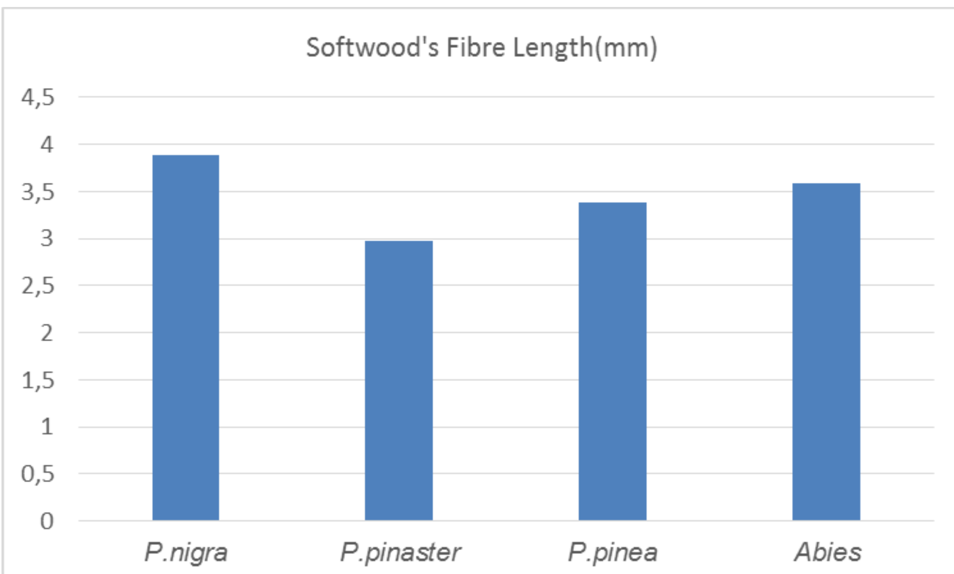

Figure 3: Comparison of softwood fibers (İstek 2008; Alkan 2003)

The fiber length of coniferous trees varies between 3-5 mm and the fiber width varies between 30-50 microns. (Akgün. 2005). According to this figure, Pinus nigra has the longest fiber length with 3,890 mm and Pinus pinaster with the shortest fiber length with 2,980 mm.
Fibers also obtained from coniferous wood (softwood) are defined as long fibers (1-2 mm) and from leafy wood (hardwood) fibers as short fibers(0.5-2 mm). Long and short fibers are used as a raw material in paper making.

Comparison of the fiber lengths of oak species both within their own species and within the hardwoods species (Ataç 2009) was given in Figure 4.

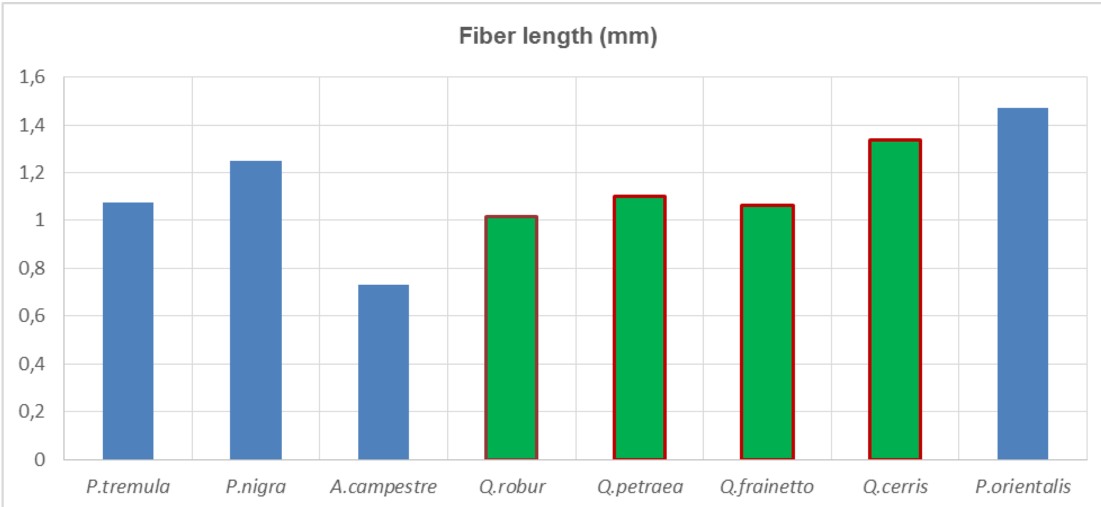

Figure 4: Comparison of oak species' fiber length

As can be seen in Figure 4, Q.robur has the shortest fiber length among oak species, 1.018 mm, and Q.cerris 1,337 mm, with the highest fiber length. Hardwods' fibers length and width are varied among the 0.8-1.5 mm and 15-30µm (Akgün, 2005).It is seen that the fiber lengths of oak species are above the average of other hardwood fibers (Fig. 4).

Among the values of hardwood fiber lengths, Platanus orientalis (1.472 mm) was the longest fiber length and Acer campestre (0.730 mm) was the shortest fiber length. It was determined that the fiber lengths of oak species take place as long fibers within the length scale of hardwood fibers (1,018mm-1,337mm).This parameter can be considered as a positive situation in terms of paper production, but it is not a sufficient variable for its use as a raw material and its usability can be interpreted by calculating its relations with other fiber sizes from its formulations.

The Runkel classification formulation, which shows the fiber sizes to be calculated and their relationships, is a criterion to be taken as a basis for evaluating the paper producibility probability. The result of this parameter is expected to be less than 1 in terms of suitability of paper production. As a result of the calculations, since the Runkel classification results are greater than 1, it can be said that oak species are not suitable to be used as raw materials in paper production (Table 2).

Conclusion

Considering the fiber morphology characteristics of oak species, their usability as raw materials in the pulp and paper industry was examined. It was determined that the maximum and minimum values of oak species are between the accepted long fiber length values for hardwood

The usability of the paper was evaluated by determining the strength properties of the paper by using the fiber dimensions such as fiber length, fiber diameter and wall thickness, and the relations between the dimensions. It was determined that the fiber lengths of oak species occured as long fibers within the length scale of hardwood fibers (1,018mm-1,337mm).

This can be considered as a positive situation in terms of paper production, but it is not a sufficient variable for its use as a raw material and its usability can be interpreted by calculating its relations with other fiber sizes from its formulations., The Runkel classification formulation, which shows the fiber sizes to be calculated and their relationships, is a criterion to be taken as a basis for evaluating the paper producibility probability. As a result of the calculations, since the Runkel classification results are greater than 1, it can be said that oak species are not suitable to be used as raw materials in paper production.

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URL 3 http://www.agaclar.org/agac.asp?id=281 Q. Cerris [4.06.2017]

URL 4 http://www.agaclar.org/agac.asp?id=290 Sessile oak [4.06.2017]

URL 5 https://7agac.cekulvakfi.org.tr/agaclar/sacli-mese-quercus-cerris-l. [8.07.2022]

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URL 7 https://www.ogm.gov.tr/ekutuphane/Yayinlar/Orman%20Atlasi.pdf Distribution Map of Oaks. Figure 2. [4.06.2017]

RAW MATERIAL CHARACTERIZATION OF WASTE PAPER

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Abstract

The use of waste paper as raw material is increasing day by day in the world and in our country. Determining the raw material characterization of waste paper is important in explaining both the raw material and the properties of the product to be produced. The raw material characterization of three types of waste paper, consisting of 1st grade 1st quality office paper, corrugated box scraps and gray cardboard determined within the scope of the study, was carried out. The analyses made in terms of raw material characterization were; fiber length and fiber width from fiber morphological characteristics; ash content (%), fines content (%), filler content (%) and SEM images. Fiber length (1.289 mm) and fiber width (20.440 µm) of 1st grade 1st quality office paper are higher than that of other papers. Corrugated box scraps have the lowest fiber length (1.124 mm) and fiber width (17.450 µm). Regarding ash content, while it was mostly found in gray cardboard (16.7%), it was found the least in corrugated box scraps (13.7%). It was determined that the amount of fines (%) was highest in 1st grade 1st quality office paper (21.25%) and the least in corrugated box scraps (7.68%). It was determined that the filler amount was highest in 1st grade 1st quality office paper (6.71%), and the least in corrugated box scraps (3.77%). No significant deformation of the fibers was observed in SEM images. 1st grade 1st quality office paper was supported by SEM images where the amount of filler was high.

Keywords: *Waste papers, Fiber morphology, Ash content, Fines content, SEM images.*

Introduction

Waste paper includes paper and cardboard products that have lost their function in the producer and consumer areas, become unusable, or are separated during production, printing and conversion stages (Akyıl 2018, Ozdemir *et al.*, 2019). The use of waste paper as raw material in paper and cardboard production is increasing day by day in the world and in our country. The use of waste paper as raw material; It ensures the sustainability of forests by preventing the cutting of trees, prevents environmental pollution by using less energy, water and chemicals compared to the production of primary paper, and has an economic added value that can be used in the production of many paper types (Kinsella 2012, Atik and Ok 2017, Bekiroglu *et al.*, 2017, Bajpai 2015). Determining the characterization of waste paper used as raw material allows us to comment on the properties of both the raw material and the product to be produced. The papers determined within the scope of this study; the 1st grade 1st quality office paper, corrugated box scraps and gray cardboard.

1st grade papers; its size is 432x559 mm and its weight is 49, 60, 75 and 90 g/m^2. It can also be classified as writing papers. Writing papers can be in bright and natural white colors and in many different colors. 1st grade papers; permanence, durability for processing, folding, binding; pen and ink writing must have sufficient hardness for erasing and typewriter. 1st grade papers are

manufactured in different types and are usually made from chemical wood pulp. In papers obtained from chemical pulp, mechanical pulp is either not found at all or in small amounts. It is divided into two as coated or uncoated, and the amount of filler varies between 5% and 25%. (Ulgen *et al.*, 2008, Paulapuro 2000).
Cardboard, includes products with a weight of 150-600 g/m^2 consisting of plantal fibers, which are thicker and have a higher stiffness than paper. Its thickness is at least 0.3 mm. They are named according to the machine from which they are made. The cartons made on the flat filter machine are single layered. Cardboard and cardboard types, which consist of many layers on top of each other, are produced in roller filter machines. Gray cardboard is the gray colored layer obtained from waste paper (Ulgen *et al.*, 2008, Tank 1998, Gul 2001).
Corrugated cardboard is a paper and cardboard product, which is formed by covering the lower and upper surfaces of one or more corrugated layers with a flat layer of Kraft liner or test liner, and is generally used in the packaging industry. Corrugated cardboard; while it is classified as single, double and triple layer, it is divided into five wave types as A, B, C, E and F. It is desirable that the corrugated cardboard has a high resistance to crushing and is durable (Kaushal *et al.*, 2015, Tank 1998). Increasing e-commerce due to the pandemic has increased the use of corrugated cardboard even more. The value of corrugated board is thought to exceed 20 billion dollars worldwide (AGED, 2022).
The use of waste paper as raw material is becoming more common day by day due to its many advantages such as not using energy, water and chemicals as much as primary papers, being harmless to the environment and providing added value in economic terms. It is necessary to know the characterization of the raw material, which is an important factor in determining the properties of the obtained papers. It is desired that the heterogeneous raw material, which comes from many different waste paper sources, produces the most suitable homogeneous pulp for paper production. In this study, the fiber morphology, ash content, % fines, filler amount and SEM images of the selected 3 types of waste paper were determined and the raw material characterization was discussed. In addition, this work was supported by the Scientific Research Projects Unit (BAP), Halkalı Kağıt Karton and, Cellulose and Paper Industry Foundation (SKSV).

Material and Methods

Samples, 1st grade 1st grade office paper, corrugated box scraps and gray chrome cardboard were obtained from Halkalı Kağıt Karton (Istanbul, Turkey). Fiber measurements were taken on a Leica DM 2500 microscope using 4x, 10x and 20x objectives. Ash determination was made according to TAPPI T211 om-12, fines determination was made according to SCAN CM 66-05 standard. SEM images were taken in FEI brand, QUANTA FEG 450 model scanning electron microscope with gold coating (Istanbul, Turkey).

Results and Discussion

Fiber length and fiber width values are shown in Table 1.

Table 1. Fiber length and fiber width of samples

Samples	Fiber length (mm)	Fiber width (μm)
1st grade 1st quality office paper	1,289	20,440
Corrugated box scraps	1,124	17,450
Gray cardboard	1,194	17,550

Fiber morphology was determined as fiber length and fiber width. As seen in Table 1, 1st grade 1st quality office paper with the highest fiber length and fiber width. The lowest one was determined as corrugated box scraps. The values for fiber-width corrugated box scraps and gray cardboard are close to each other. Fiber lengths are divided into short and long fibers in primary papers. The length of the short fibers is 1–2 mm (hardwood fiber), the length of the long fibers is 3–5 mm (softwood fiber) (Riley 2012, Sisko and Pfäffli 1995, Atik 2007). Fiber widths are 15-30 µm in softwood and 7-20 µm in hardwoods (Atik, 2007). Looking at Table 1 and the explanation above, the lengths of the fibers vary between 1-2 mm, and their widths vary in the range of 15-20 µm. In this case, it can be said that the fibers of the samples are from hardwoods.
The results of Ash content (%), Fines content (%), Sieve ash (%), filler content (%) and fiber fines (%) are given in Table 2.

Table 2. Ash content (%), Fines content (%), Sieve ash (%), filler content (%) and fiber fines (%) values of the samples

Samples	Ash Content (%) (1)	Fines content (fiber and filler) (%) (a)	Sieve ash (%) (2)	Filler content (%) (1)-(2) (b)	Fiber fines (%) (a)-(b)
1st grade 1st quality office papers	15,440	21,258	8,722	6,718	14,540
Corrugated box scraps	13,770	7,684	9,992	3,778	3,906
Gray cardboard	16,743	13,003	12,095	4,648	8,355

Looking at Table 2, subtracting (1) from (2) yields filler content. When subtracting (a) from (b), fiber fines are obtained. In Table 2, the highest content of ash (%) is gray cardboard and the lowest corrugated box scraps. While the Fines content (%) is the highest 1st grade 1st quality office paper, the lowest is in the corrugated box scraps. The filler content (%) is highest in 1st grade 1st quality office paper and the lowest in corrugated box scraps. This is supported by SEM images. The fiber fines (%) is the highest 1st grade 1st quality office paper, the lowest is in corrogated box scraps.

SEM Images

SEM images of the samples are shown in Figure 1-3.
Looking at Figure 1, it was determined that the filling materials were excessive. No significant deformation was observed in the fibers. It is seen that fine fibers are in the majority.

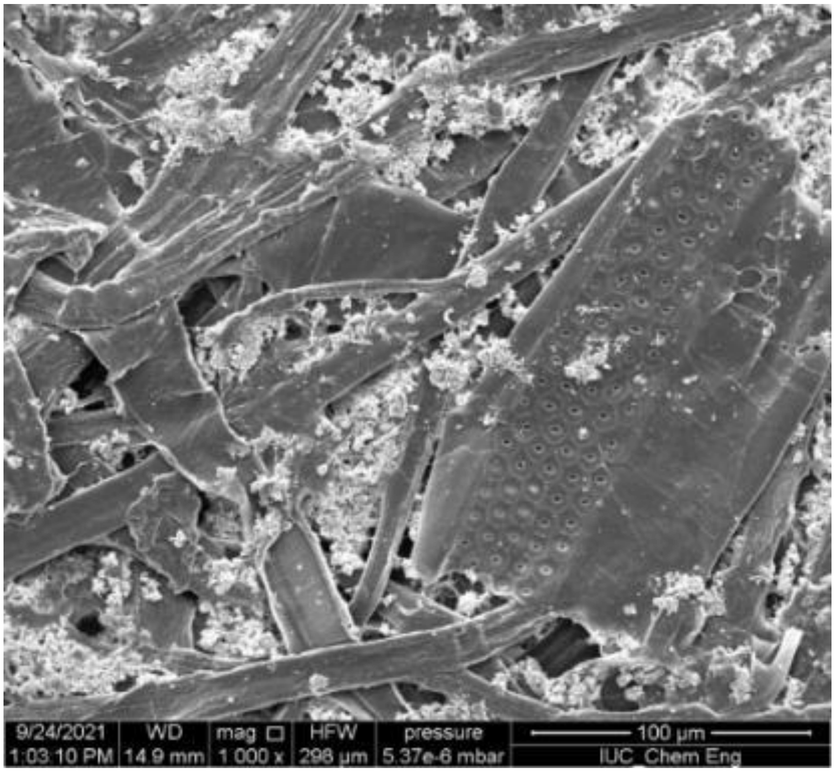

Figure 1. 1st grade 1st quality office paper

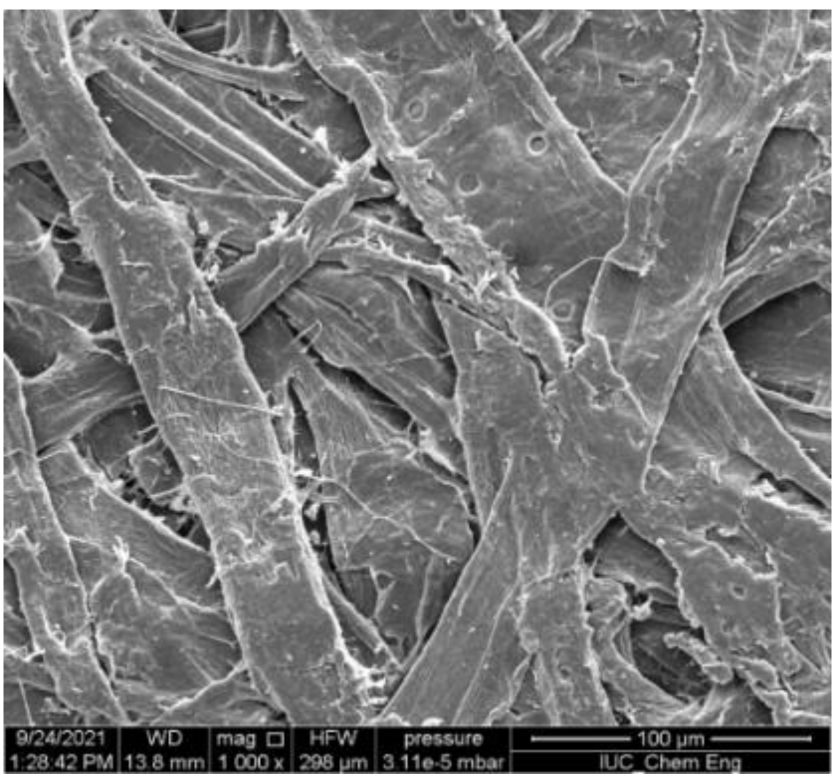

Figure 2. Corrugated box scraps

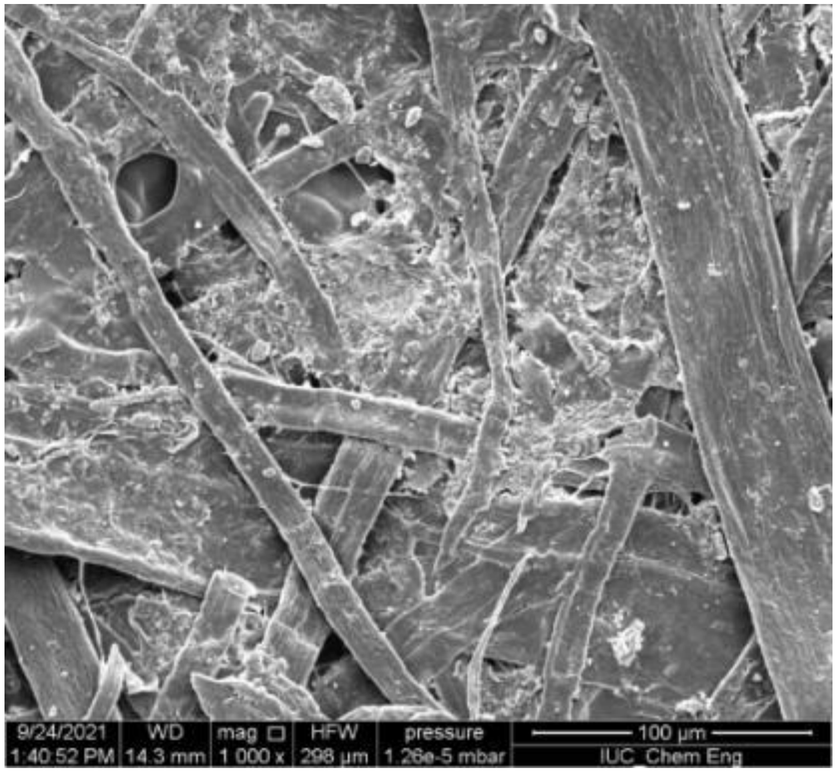

Figure 3. Gray cardboard

As seen in Figure 2, fraying was detected in the fibers. No significant deformation was observed in the fibers, but the wear of the fibers is clearly visible.
According to Figure 3, it has been determined that there are both thick fibers and thin fibers. In this case, it can be thought that there are different types of trees in it. It also contains fillers. It is a small amount compared to 1st grade 1st quality office papers.

Conclusions

The raw material characterization of three different waste papers was determined and the results are given below. It has been determined that the fiber length, fiber width, fines content (fiber and filler), filler content and fiber fines values determined in 1st grade 1st quality office paper are higher than other papers. It can be thought that the high amount of fines and filler may be due to the high amount of additives put into it or due to the application of surface treatment (sizing).
It has been determined that the fiber length, fiber width, ash content, fines content (fiber and filler), filler content and fiber fines values made in the corrugated box scraps are lower than the other papers. In SEM images, it was observed that there was a significant fraying and wearing in the fibers.

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USE OF LEMNA MINOR IN PHYTOREMEDIATION PROCESS FOR REMOVAL OF HEAVY METALS IN SECONDARY PULP

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Abstract

Chemicals, colorants and ink components from secondary fibers used as raw materials in the production of recycled paper cause heavy metals to increase in the structure of paper-cardboard.As a food packaging, it threatens human health by being exposed to heavy metal migration as a result of direct interaction with food or by contamination during the final processing of the packaging. It is aimed to decrease and removal heavy metal concentrations as result of phytoremediation process applications with the interaction of recycled pulp from waste packaging paper-based samples with *Lemna minor*. And also create a positive impact on current environment by plant production as the method. *Lemna minor* samples were obtained from Turkey's Nazlı Lake (Yedigoller National Park, Bolu) . The lead, zinc, cadmium, nickel, copper, chromium and aluminum heavy metal decrease and removal efficiencies of the recycled pulp samples were applied in batch reactor system at a temperature of approximately 27-28ºC for 7 days. For metal determination analysis, the samples were prepared by burning them gradually in a microwave device to bring them into solution. Heavy metal determinations were made with the ICP-OES device. It has been shown that the *Lemna minor* plant can be used with high efficiency in the processes of removing heavy metals and reducing heavy metal concentrations in recycled pulp. At the same time, it was created a positive effect on the environment with its plant production and appearance, without polluting the environment by decreasing and removing heavy metals pulps from waste paper, cardboard and corrugated cardboard.

Key Words: *Lemna minor, heavy metals, waste paper, recycled pulp, seconder pulp.*

Introduction

Secondary fiber is made from waste packaging papers and cardboard-based, all over the world and in EU countries, as in our country. Packages are produced using recycled paper and cardboard together with raw (primary) paper. Chemicals, colorants and ink components coming from secondary fibres in packaging making reason to increase heavy metals in paper-paperboard structure. Therefore, food packaging is exposed to human health by being exposed to heavy metal migration as a result of direct interaction with food or contamination.
Duckweeds that are spread widely in still freshwater, consists of *Lemna* L.(14 species), *Spirodela* Schleid (4 species), *Landolita* D.H.Les and.J.Crawford (1 species), *Wolffia* Horkel ex Schleid.(11 species) and *Wolffiella* Hegelm (10 species) genera of *Lemnoideae* subfamily of *Araceae* family (Daubs, 1965; Les, and Crawford, 1999; Al-Nozaily, Fet al., 2000; Les et al.,

2002; Morales et al., 2006; Cusimano, et al., 2011; The Royal Botanic Gardens, Kew Botanical Garden, and Missouri Botanical Garden, 2017).

In Turkey, 5 duckweed species belonging to 2 genera (*Spirodela* and *Lemna)* spread naturally. These species are; *Spirodela polyrhiza* (L.) Schleid., *Lemna minor* L., *L.gibba* L., *L.turionifera* Landolt., and *L.trisulca* L. *(*Ekim, 2012). Duckweeds are cosmopolitan plants that grow in stagnant and slowly flowing waters on almost every continent except the poles (Landolt, 1986).

In this study, we aimed to decrease and removal heavy metal concentrations as result of phytoremediation applications with the interaction of sekonder pulp with of *L.minor*. In this context, the aims of this study are; to provide the usage of *L.minor* during the process of removal of heavy metals in secondary pulp which are used as raw materials in recovered paper production sector. Without polluting the environment, to create a positive impact by plantation and glory caused by plantation.

Material and Method

Material

Lemna minor specimens were collected from Nazlı Lake (Yedigoller National Park, Bolu) in July 2021. Collected were identified at Istanbul University-Cerrahpaşa, Forest Faculty Herbarium (ISTO) (Uotila,1 984; Yıldırım, 2014).

Method

*Preparation of L.minor:*Provided *L.minor* are decontaminated from pollution caused by their sources by washing with pure water for a few times. Prepared of *L.minor* which wet weight is 1 kg approximately were kept for two days by putting pure water into 5 liter volumed plastic reactor containers and in this way were adapted to laboratory conditions. At every phases of this study, in order to prevent heavy metal penetration from the outer environment, plastic gloves were used during the study .During the adaptation period, it was monitored that *L.minor* that have reproduction feature reproduced at maximum level. In order to prepare for phytoremediation applications, *L.minor* were filtered with a plastic filter and experiment reactors were prepared in colored plastic containers with a wet weight of about 2% of *L.minor* and with 500 ml tap water (Mertoglu Elmas et al. 2019).

Prepared samples for heavy metal analysis

Microwave oven burning was applied to the before heavy metal analysis was performed on the ICP-OES instrument. In context of this process, 5 ml HNO_3 and 2 ml H_2O_2 were added on about 0,4-0,5 g dry plant and waste paper-based packaging samples into teflon tubes, and solutions prepared stepwise in microwave device (Berghof, İstanbul, Turkey) with the burn program, were filtered with blue band filter paper and completed to 50 ml with ultra pure water. Heavy metal quantities of prepared analysis solutions were determined by ICP-OES device.

Heavy metal concentrations of samples prepared by keeping under reactor conditions for seven days were determined in order to use at control and experiment balance as indicating I and II.

I-Seconder pulp samples in only tap water without duckweeds,

II-From tap water samples prepared for each experiment reactor and protective plastic case in only tap water.

Results and Discussion

Parameters which are effective in phytoremediation application were given in Table 1 as defined on 1.group samples. L.*minor* used in the phytoremediation application was shown in the Fig.1.

Table: 1:Parameters effective on phytoremediation application

LMB0	Initial heavy metal values of *L.minor*.
LMBİ	Final heavy metal values of *L.minor*. of which were treated phytoremediation
LMK0	Initial heavy metal values of sekonder pulp samples
LMKİ	Final heavy metal values of sekonder pulp sample which were treated phytoremediation

Figure: 1: Lemna minor

Heavy Metals

The flow chart showing the phases of phytoremediation application conducted to decrease and remove heavy metals of waste paper based packagings with *L.minor* plants were given in the Fig. 2 (Mertoglu Elmas et al., 2019).

Providing of duckweed plants from their source, washing, adapting to laboratory

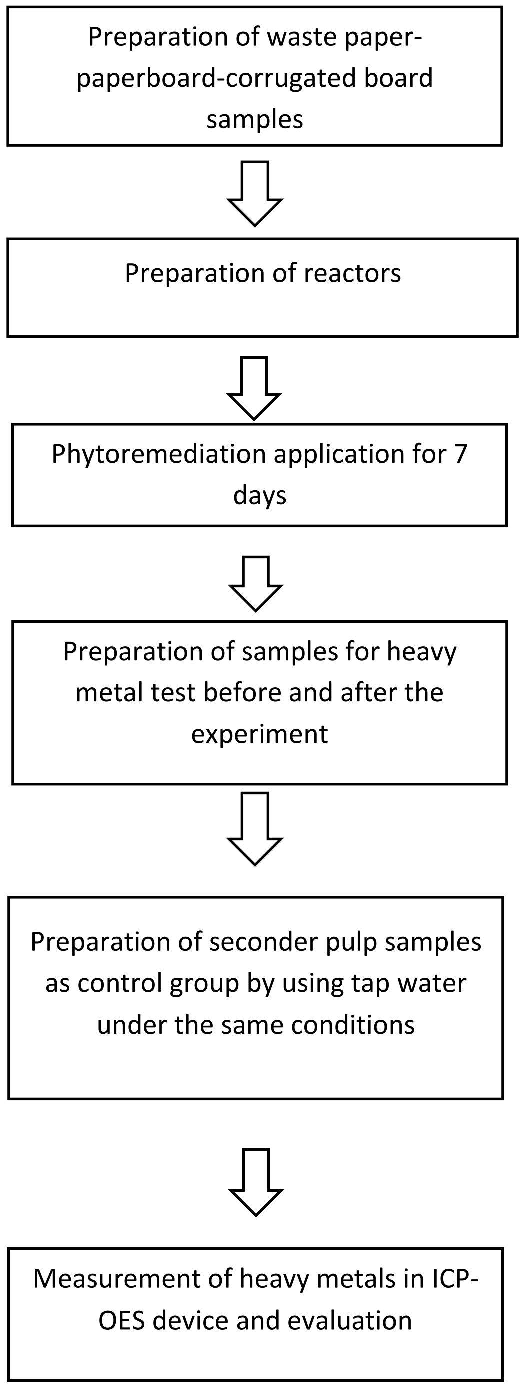

Figure 2. Flow chart of phytoremediation application

L.minor and heavy metal concentrations of seconder pulp were given in Table 2.

Table 2. Heavy metal change with the interaction of *L.minor* and waste pulp ($mgkg^{-1}$)

Samples	Pb	Cd	Ni	Zn	Cu	Cr
LMB0	6,402	0,164	1,143	29,09	4,866	2,329
LMB7	12,85	0,145	3,207	93,13	13,82	3,518
LMK0	10,18	0,157	4,96	62,43	48,14	7,79
LMK7	9,466	0,129	3,441	68,23	38,07	7,293

It was observed that as seen Fig.2 flow chart performed of phytoremediation application of seconder pulp samples with the *L.minor*, heavy metal concentrations of seconder pulp from paper based samples decreased generally and heavy metal concentrations of *L.minor* that were used increased (Table 2).

It was detected that Pb in sekonder pulp paper decreased by 7,01 % and increased by 100,0 % in *L.minor*. In waste pulp decreased by 17,8%, and Cd was not observed in the duckweeds . Cd removal in the sekonder pulp can be explained by dissolution of Cd compounds in aquatic environment.It was detected that Zn increased by 9,3 % in sekonder pulp and increased by 220,1% in the *L.minor*. I t can be concluded that the high rate of increase in the duckweeds impacted the amount of Zn in the pulp raw material and caused it to increase. It was observed that Ni concentration of sekonder pulp decreased by 30,6% and 180,5% in the *L.minor*. It was found out that Cr decreased by 6,3% in sekonder pulp and increased by 51,0% in the *L.minor*. It was observed that Cu concentration for sekonder pulp decreased by 20,9% and 184,0% in the *L.minor*. It was found out that the most Ni and the least Cr heavy metal were removed from the secondary pulp.

Conclusion

It was concluded that duckweeds plants could be used during the removal process of heavy metals in order to decrease or remove the heavy metal concentrations in sekonder pulp which are used as raw materials in especially recovered paper production sector. It was found out that paint and ink components used in coloring and printing in the pulp from corrugated board and paperboard packagings could easily dissolve in water medium.

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ANIMAL HUSBANDRY

COAT COLOR AS INDICATOR OF FERTILITY IN DAIRY COWS IN ALGERIA

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Abstract

The aim of our experiment was to estimate the correlation between the reproduction parameters of 48 dairy cows (29 Montbeliardes and 19 Normandes) and the percentage of the white coat color. Cows were imported from European countries and raised in the semi-arid region of Sétif in Algeria. Fertility parameters were estimated: the number of services per conception (SPC), calving to first service interval (FSI), calving to conception interval (CI), and the interval between calving (ICC). The percentage of white color of coat has been measured visually (C %). Statistical analysis was carried using Pearson correlation and Spearman's correlation rank when variables were not normally distributed with the SPSS package program, version 21. In Montbeliarde breed, C% was significantly correlated with ICC ($r = 0.543$, $p=0.002$) indicating that Montbéliarde cows, with high percentages of white color achieve longer ICC. In Normande cows, coat color was negatively correlated with FSI ($r = -0.565$, $p =0.012$), cows with high percentages of white color return to heat sooner after calving. The results of the current study suggest the possibility of integrating coat color into animal selection. It can be assumed that colored coats for Montbeliarde and Normande breeds appear to be related to their reproductive efficiency (interval between calving and calving to first service interval respectively).

Keywords: *Coat color, Dairy cows, Reproduction, Algeria.*

Introduction

In Algeria, milk consumption has been increasing with population growth (Makhlouf and Montaigne, 2016). To meet the growing demand for dairy products, Algeria has opted for the development of a cattle breeding policy based on the import of heifers with a high genetic potential of dairy products from Europe (Mefti Korteby et al., 2016).

The potential of milk production of cows differs among the breeds and even among individual cows within the same breed raised under various environmental conditions (Aggarwal and Upadhyay, 2012). The hair is an aspect of environmental adaptation, which reflects the response of the animals to their environments. So, coats of cattle are adapted to withstand the harsh hot climate weather. In addition, animal coat changes in response to seasonal environmental changes (Brinkmann et al., 2012). Coat color is a trait that is often hypothesized to contribute to heat tolerance in animals (Leite et al., 2018). On top of that, the genotype-environment interactions lead to differences in the expression of reproduction performance. There is a relationship between coat color and reproductive performance in dairy cattle, particularly when animals are reared under conditions of high temperatures and intense solar radiation (Becerril et al., 1994).

Heat stress can reduce the fertility of dairy cows (Ryan et al., 1992; Ealy et al., 1993). In order to minimize the effects of thermal stress on reproduction performance, animals with a coat more suited to the conditions present in North Africa can be selected (Lamari et al., 2013).

Therefore, the present study investigated the correlation between the coat color and the reproduction performance of Montbéliarde and Normande cows

Materials and methods

Information on this study was obtained in collaboration with the executives of the school farm in the semi-arid area of Sétif, Algeria from February to May 2018. The study included a total of 48 imported dairy cows of two different breeds (29 Montbeliarde and 19 Normande cows), subjected to the same breeding system and the same environmental conditions. All the cows selected were either gestating or drying up. Animals were fed twice a day a ration based on forages mostly collected from the home pasture land and purchased concentrate. Detection of estrus was made by visual observation 4 times a day for 20 minutes. The PRID protocol was preferred by the veterinarian to synchronize the estrus and artificial insemination was adopted by the manager.

All this information was collected in individual files, including data relating to the cow (identification number, breed, date of birth, etc.) and events relating to reproduction (dates of calving, dates of inseminations, number of services by conception, etc.). Four fertility parameters were estimated; the number of services per conception (SPC), calving to first service interval (FSI), calving to conception interval (CI), the interval between calving (ICC).

The percentage of the white color related to the entire body surface area was measured visually (Becerril and Wilcox, 1992). It was estimated by taking pictures of both sides of each animal. However, the tail, head, legs, and belly regions were not included in the measurement.

The mean, and standard deviation were calculated and the test for normality (Kolmogorov-Smirnov) was performed for all parameters. Correlation was used to estimate the relationship between coat color and reproduction parameters using Pearson correlation, and Spearman’s correlation rank when variables were not normally distributed. All statistical analyses were performed using the SPSS package program, version 21.

Results and discussion

Generally, the coat color varies from total white to colored with a total average of 49.79 ± 27.56% of white color but the white color was more dominant in Montbeliarde cows (51± 28%) than in Normande cows (47.89 ± 27.55 %). In fact, school farm cows were inseminated 72.62 ± 28.25 days after calving and conception was produced after 1.44 ± 0.74 attempts with high SPC (1.54 ± 0.90) in Normande cows, when compared to the SPC of Montbeliarde cows (1.34 ± 0.55). The cows of both breeds carried out the first service almost at the same time, calving to first service interval for the Normande cows was longer than the Montbéliarde cows’ interval by 5 days.

Total School farm cows (Montbeliarde and Normande) required 107.25 ± 53.18 days to achieve conception after calving. The interval between successive calving was 404.93 ± 129.55 days in the school farm, but Montbeliarde cows were characterized by a shorter CI and ICC, compared to those of Normande cows (Table 1).

Table 1: Percentage of white coat color and average reproductive performance

	Montbéliard (29)	Normand (19)	Total (48)
C%	51.03±27.99	47.89±27.55	49.79±27.56
SPC	1.34±0.55	1.54±0.90	1.44±0.74
FSI (days)	70.55±28.26	75.78±28.71	72.62±28.25
CI (days)	98.65±53.43	120.36±51.42	107.25±53.18
ICC (days)	378.34±49.05	445.52±192.87	404.93±129.55

C%: percentage of white coat color; SPC: number of services per conception; **FSI: calving to first service interval; CI: calving to conception interval; ICC: interval between calving**

The correlation results between the percentage of white color of the coat and the reproduction parameters (SPC, FSI, CI, ICC), and the total milk production are shown in table 2

The Montbeliard breed

The analysis of the correlation matrix shows that the strongest correlation recorded, r = 0.543, is that between C% and the interval between successive calving (table 2).Cows with high percentages of white color achieve longer ICC compared to other cows.

The Normand breed

According to Table 2, it appears that the percentage of white coat color and the interval between calving and first service are negatively correlated with each other (r = -0.565). Indeed, cows with high percentages of white color return to heat sooner after calving compared to other cows.

Table 2: Correlation between percentage of white coat color and reproduction parameters

	SPC	FSI	CI	ICC
Montbeliard (29)	0.311^{ns}	0.095^{ns}	0.287^{ns}	0.543^{*}
Normand (19)	0.329^{ns}	-0.565^{*}	-0.133^{ns}	-0.234^{ns}

SPC: number of services per conception; FSI: calving to first service interval; CI: calving to conception interval; ICC: interval between calving. ns: non-significant difference; *: significant difference at $p < 0.05$.

The results of the current study showed that there was a relationship between coat color and reproductive parameters. These results were similar to those obtained by Lamari et al. (2015) where they reported that Montbeliarde cows with a high percentage of white color of the coat achieved a shorter FSI**.** The results of the present study agree with the results obtained by Lamari and Saber. (2021), who reported that the colored Montbéliarde cows were characterized

by better reproductive performance (more fertile), compared to white cows (with the percentage of white coat color > 42 %) and they performed shorter successive calving intervals.
The results showed that the Normande cows with high percentages of white color return to heat sooner after calving compared to other cows. Similarly, Lamari et al. (2015) observed that the white cows resumed their cyclicity sooner than the colored cows but in Montbeliarde cows, there was a negative correlation coefficient (r = -0.359) between calving to first service interval (FSI) and percentage of white coat color (C %). Same authour,observed that , coat color was significantly correlated with FSI (r=0.674) for Fleckvieh cows during summer (Lamari et al.,2018). Nejad et al. (2016) reported that under heat stress conditions, coat color may play a role in stress severity by absorbing heat and solar radiation based on white and black colors. According to Magona et al. (2009) and Riley et al. (2012), animals with a dark coat color absorbed more heat compared to animals with light coat color. However, Bertipaglia et al. (2005) found that the white coat presents an effective barrier against thermal stress. And according to (Maia et al., 2003), the white coat offered better protection against direct solar radiation.

Conclusion

The study of relationship between hair coat color and reproduction performances of Montbeliarde and Normande breeds in the semi-arid area of Algeria shows that coat color affected reproduction parameters, including ICC for Montbeliarde breed and SPC for Normande breed. Colored hair coat cows had more efficient reproduction with relatively reduced ICC for Montbeliarde cows and a reduced SPC of Normand cows. The results suggested that hair coat color can be used as a factor to select resilient and more adaptable animals to hot climates, and it can also improve the knowledge of farmers to have the best reproductive performance under heat stress conditions.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON SOME SERUM BIOCHEMICAL PARAMETERS IN SHEEP

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Abstract

Sheep play a significant role in the Bosnian and Herzegovinian agricultural economy, particularly in mountain areas, where most are kept by the smallholder production systems. This study includes differences in values of some biochemical parameters of sheep who inhabit the Livno area and mount Vlašić of Bosnia and Herzegovina. The serum levels of total protein, albumin, globulin, urea, creatinine, glucose, aspartate aminotransferase, γ-glutamyl transferase, creatine kinase and cholesterol were determined. All analyzes were using the spectrophotometric method. Non-esterified fatty acids and β-hydroxybutyrate levels in serum were measured by Randox kit (UK). Significant differences of some serum biochemical parameters between the two areas have shown the impact of environmental factors on the sheep's metabolic status. Obtained results of β-hydroxybutyric acid, total protein, albumin, urea, aspartate aminotransferase and cholesterol were significantly decreased in the Livno area, while non-esterified fatty acids, creatinine and glucose were increased. Almost all biochemical parameters showed variations between the sampling areas, which could be caused by the influence of different climate and altitude areas.

Keywords: *sheep, biochemical parameters, blood.*

Introduction

Sheep breeding plays an important role in the Balkan area. Blood is an important and reliable medium for assessing the health status of the individual and flock animals, due to its involvement in anabolic and catabolic metabolic pathways related to various environmental conditions, productive and reproductive status, and acclimatization and adaptation processes in the certain geographical area. Metabolic homeostasis is closely related to environmental factors, such as altitude, feeding, age, sex, breed, productive and reproductive cycles, diurnal and seasonal variation. Analysis of various blood parameters in sheep flocks is an important indicator of health status. Hematological and serum biochemical analyses are widely used for the diagnosis of various animal diseases which can lead to economic losses, such as reduced quality and quantity of fur, wool, and milk production (Gemechu and Kibeb, 2017). The diseases that affect small ruminants impose severe economic impact on sheep production; they cause production losses manifested by reduced weight gain, lowered meat and milk production, and high mortality of offspring. The measurement of specific serum metabolites allows adequate assessment of main metabolic pathways associated with energy, protein, and minerals. It also provides useful information related to animal nutrition and health to optimize the productive and reproductive potential of the herd, especially in high-yield dairy herds. Recently, the utilization of blood metabolic profiling has gained significant relevance for the study and diagnosis of various metabolic and reproductive disorders. Blood parameters including glucose, non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), cholesterol, enzymes and proteins have been reviewed recently to be of great interest for metabolic profiling of dairy herds (Hussein et al., 2020). Serum biochemical parameters have common use in the clinical assessment of herd animals. Determination and monitoring of metabolic profile can be valuable parameters for presenting homeostatic mechanisms in physiological limits under different stages in animal husbandry (Kirovski et al., 2012; Hrković-Porobija et al., 2018). Climate changes negatively influence animal production and reproduction (Reynolds et al., 2010). Heard animals possess several adaptive mechanisms which are helpful for their survival in changed environmental conditions, but productive performances are compromised during the adaptation process. Among the various mechanisms, which help to maintain homeostasis in animals, physiological adaptability was considered one of the primary response mechanisms by which the heat-stressed animals survive the heat stress (Rashamol et al., 2018).

Materials and Methods

The research was conducted on a total of 89 sheep from the site of the Vlašić mountain (n = 44) and Livno fields (n = 45) in June 2018. In the area of Livno, sheep breeding has a hilly-mountainous character, with Cincar as the highest mountain (2005 m above sea level). Livno field (700 - 740 m above sea level), is an area on which several seasonal watercourses are significant from the hydrological aspect. Climatically, the sub-Mediterranean and continental zones collide here. The Vlašić plateau is located at 1000-1500 m altitude sea level, wherein the narrower area of Vlašić the climate is mountainous, while lower in the river valleys it is moderately continental. The sheep were fed by free grazing with the addition of hay and grain. Blood samples were taken from the jugular vein into BD Vacutainer® SST II gel for biochemical analyses. All biochemical analyses were performed at Norwegian University of Life Sciences, Norway within the next 24 hours and serum samples were kept at 4°C. Biochemical parameters were determined by analyzer Olympus AU400 with Beckman Coulter reagents according to the manufacturer’s protocol. Parameters for the biochemistry panel included: total protein (TP), albumin (ALB), globulin (GLO), urea (BUN), creatinine (CRE), glucose (GLU), aspartate aminotransferase (AST), γ-glutamyl transferase, (GGT), creatine kinase (CK) and cholesterol (CHO). All analyzes were using the spectrophotometric method. BHBA (β-hydroxybutyrate) and NEFA (non-esterified fatty acids) were constructed with reagents from Randox (UK).

Statistical analyses

The obtained results were statistically analyzed using Microsoft Office Excel 2010 software, module Data Analysis. The differences between the mean values of the groups were tested with the t-Test. Differences were considered statistically significant at the level of significance ($p<0.001$***).

Results and discussion

The biochemical profiles obtained from 89 animals are summarized in Table 1.
The results obtained from the Livno and the Vlašić area demonstrate that six biochemical parameters (BHBA, TP, ALB, BUN, AST, and CHO) are significantly decreased in the Livno area 0.46, 70.85, 29.43, 5.42, 133.09. and 1.72 respectively, in comparation to the Vlašić area 0.58, 75.23, 32.61, 5.78, 171.88 and 2.18, respectively. Contrary to previous, increased values in Livno area are documented for NEFA, CRE and GLU 0.50, 47.90 and 3.32 respectively, comparing to Vlašić area 0.32, 45.34 and 2.96 respectively. However, no significant differences were found for GLO, GGT, CK between research areas.

Table 1: Biochemical parameters in sheep blood

Parameters	Area Vlašić	Area Livno	p
BHBA (mmol/L)	0.58	0.46	***
NEFA (mmol/L)	0.32	0.50	***
TP (g/L)	75.23	70.85	***
ALB (g/L)	32.61	29.43	***
GLO (g/L)	42.68	41.47	ns
BUN (mmol/L)	5.78	5.42	*
CRE (µmol/L)	45.34	47.90	**
GLU (mmol/L)	2.96	3.32	***
AST (IU/L)	171.88	133.09	***
GGT (IU/L)	46.22	43.35	ns
CK (IU/L)	425.96	451.86	ns
CHO (mmol/L)	2.18	1.72	***

Abbreviations: BHBA (β-hydroxybutyrate), NEFA (non-esterified fatty acids), TP (total protein), ALB (albumin), GLO (globulin), BUN (blood urea nitrogen), CRE (creatinine), GLU (glucose), AST (aspartate aminotransferase), GGT (γ-glutamyl transferase), CK (creatine kinase), CHO (cholesterol).
*Statistical significant difference ($p<0.05$), **Statistical significant difference ($p<0.01$), *** Statistical significant difference ($p<0.001$), ns – not significant.

Biochemical serum parameters have common use in the clinical assessment of domestic animals. Determination and monitoring of metabolic profile parameters may show whether homeostatic mechanisms can maintain blood composition in physiological limits under different conditions of animal husbandry (Prodanović et al., 2012; Hrković-Porobija et al., 2018). The serum concentration of BHBA presents the magnitude of negative energy balance (NEB) and lipid

mobilization in dairy animals, hence they are diagnostic markers for subclinical and clinical ketosis (Sordillo and Raphael, 2013). NEFA reflects the magnitude of fat mobilization from fat stores in response to negative energy balance. The gradual increase of plasma NEFA during the final days of the pre-partum period may be explained by the gradual depression (Mohammadi et al., 2016). This increase is due to the required energy for parturition and milk production. Some authors reported higher BHBA levels in sheep with pregnancy toxemia as compared to lactating sheep (Marutsova and Marutsov, 2018). The glucose level in the Vlašić area was statistically significantly higher ($p<0.001$) compared with the sheep in the Livno area. The values found in both sampling areas were higher than the values given by other authors (Shek-Vugrovečki et al., 2017), which indicates that the sheep had high energy supplies obtained during the pasture period when plants are at the beginning of their vegetation (Hrković-Porobija et al., 2019). The value of cholesterol in Livno's livestock was statistically ($p> 0.001$) significantly higher than in the area of Vlašić, but also concerning the values reported by Kaneko (2008). Cholesterol values results found on the Pramenka sheep from Livno and Travnik (Hrković-Porobija, 2011) are approximate to the results of the current research. The values of total protein and albumin obtained at Dubrovnik and Lička Pramenka by Antunović et al., 2011. and Shek-Vugrovečki et al., 2017, are in compliance with results found in Livno sheep and were statistically significant ($p<0.001$). Lower values of total protein and albumin at the locality of Travnik were found by Hrković-Porobija et al., (2011).
The activity of some plasma enzymes may increase under physiological factors and the presumption is that in physiological conditions, the minimal permeability of the cell membrane might be changed reversibly. After muscle activity, transport, feed, and lodging changes or after a combination of mentioned factors, an increase in serum aminotransferases may appear. The reason for the changed enzyme activity may be due to disorders in the permeability of cell membrane, increased or decreased (Hrković- Porobija et al., 2018). The serum AST and GGT values, which were close to or above the upper physiological limits, could indicate the necessarily compensatory intensification of metabolic processes as a response to the organism, primarily the liver, to negative energy balance. Aspartate aminotransferase is an enzyme found in the liver and heart muscle. It plays an important role in the metabolism of amino acids.
The GGT concentration did not statistically differ significantly between sampling areas but was higher in value than other authors (Akgul et al., 2000), while concentration of AST was higly statistically significant ($P<0.001$) in Livno's sheep. Serum AST and ALT activities increase to variable levels during the prodromal phase of acute hepatitis and precede elevations in bilirubin. The magnitude of AST and ALT elevations do not correlate closely with the extent of liver damage. Higher activity of ALT could be a consequence of intensification in metabolic processes with an increase in age (Antunović et al., 2008). The major sources of AST in animals are the liver and skeletal muscle. The concentration of AST in tissues and blood serum is very different. In processes in which AST-rich tissues have lesions, the enzyme enters the circulation and results in an increase in AST activity in blood serum (Hrković et al., 2017). Although ALT and AST are commonly assayed together, the enzymes differ concerning protein structure and gene location, kinetic characteristics and substrate specificity, tissue distribution and its specificity. It is believed that ALT is more specific for liver disease whereas AST is more specific for the myocardium. AST and ALT may also differ with respect to the pattern of association with cardiovascular disease (Ndrepepa, 2021). The obtained urea concentrations, 5.78-5.42 mmol/L, do not differ from the given physiological values of 2.86-7.14 mmol /l (Kaneko, 2008) and are in accordance with the values we encounter during the summer sampling period (Ašimović, 2005).

The results obtained for the total protein and urea indicate higher values of our study in both study areas, while the values of creatine and albumin in the Vlašić region were lower (Comba et al., 2017). Urea concentration is used as food protein supply in sheep as an indicator of renal function. Ašimović (2005) states that the influence of the breed and breeding sites is not statistically justified. The statistically justified differences are primarily the effect of the season (summer), nor the sheep diet. Animals are fed with small amounts of creatine by consuming food containing animal tissue. Creatine is well absorbed from the intestinal tract. In this study creatinine level was in a range of 45.34-47.90 µmol/L, highy statistically significant ($p<0.01$) in Livno sheep. Creatine values at both sampling areas were lower than the values found by (Njidda et al., 2014; Sarmin et al., 2021). Interaction of urea and creatinine, the effect of the season, the effect of the nutrition as well as involvement of microorganisms of the rumen in these processes are exceptionaly important (Ašimović, 2005). Cholesterol values in our study, collected from Travnik and Livno ranged from 2.18-1.72 mmol/L, resceptively. Given values were lower comparing to results found by Sarmin et al. 2021. (46 mg/dl). In comparison with Antunović et al. 2021, the results in the area of Travnik indicate increased cholesterol levels, which is in agreement with its results. Glucose values for both areas are within the reference values. Comparing with the results of Antunović et al. 2021, glucose values were slightly lower (2.96 - 3.32 mmol / L).

Conclusion

Environmental factors are known to have a profound effect on the biochemical profiles of sheep. Therefore the establishment of biochemical reference intervals is an important tool to assess the health status of animals and understand the impact of disease on individual and population levels. Biochemical parameters showed variations between the areas with different altitudes due to the influence of different geographic areas, climate, and floristic composition, as well as the current health status of sheep herds.

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QUALITY OF RAM SPERM AS A CONDITION FOR SUCCESSFUL CONCEPTION OF SHEEP, DURING MATING SEASON AND LATE ANESTRUS SEASON

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Abstract

The area of Una-Sana Canton is a hilly area with an average altitude of around 350 m, with numerous and high quality pastures. Sheep are seasonally polyestrous animals whose reproduction is clearly biologically regulated. Seasonally reproductive activity of rams, in terms of production intensity and quality of sperm (its degree of fertilizing ability), and the intensity of sexual libido is not as strongly influenced by annual season as is the case with sheep. The aim of this paper was to examine the quality of the sperm in rams as a condition of successful conception of sheep during the periods of late anestrus (April-May) and mating season (from mid-September to mid-October). All parameters that determine fertilization capacity of ejaculate, and which are more or less related to its fertility will be examined. Our research involved 30 fully mature rams of the Pramenka breed (strain: Travnik Pramenka), with strong libido, in good health and condition. The research was conducted in three municipalities in north-western part of Bosnia and Herzegovina (Bihać, Cazin and Sanski Most), by 10 rams from each. We can conclude that the quality of ram sperm has the key importance in successful conception of sheep, as the results of examined parameters shows lower values in late anestrus than in mating season

Key words: *Mating season, Pramenka sheep, Ram sperm.*

Introduction

Sheep farming is very important branch of animal husbandry in the world, and in Bosnia and Herzegovina as well. It is predominantly present in hilly and mountainous regions, in areas with pastures, as sheep successfully use low-value fodder, and process them into products such as milk, meat, leather and wool. The number of sheep in Bosnia and Herzegovina was around 1.3 million before the war (1991), while in 1960 it was even larger and amounted 2.2 million. This indicates a significant decline in sheep population in 30 years by as much as 41%. In last two decades, the number of sheep decline even more due to migration of population from villages to towns and abroad. If we have in mind the fact that the census has not been conducted for a long time, it is not possible to determine how many sheep there are in Bosnia and Herzegovina today.

Sheep are seasonally polyestrous animals whose reproduction is clearly biologically regulated. Seasonal sexual activity of rams is similar to sexual activity of sheep, and has minor oscillations. In the period outside of mating season, we can notice the lower production of sperm, as well as reduced desire to mate. Diet, housing, temperature, and especially the length of daylight have a direct impact on the production of ram sperm. High temperature (above 27°C) affects the quality of ram semen so that it leads to summer infertility. For this reason, breeding rams are subject to a special regime of keeping, feeding and rhythm of daylight (photoperiod) in order to give the highest quality sperm for insemination of sheep.

Materials and methods

Our research involved 30 fully mature rams of the Pramenka breed (strain: Travnik Pramenka), with strong libido, in good health and condition. Examinations were performed in both mating season and late anestrus season. The samples of sperm were taken from 10 rams in each municipality (Bihac, Cazin and Sanski Most) during mating season (September to mid-October 2017) using an artificial vagina. The ejaculates were taken from the same rams again in late anestrus season (May-June 2017). All rams were aged 1-3 years, with good general health and condition. The rams were separated from the sheep for 2 days (48 hours) prior to sperm collection, in order to give the highest quality ejaculates.

Picture 1. Native sperm samples in graduated tubes (Photo: Husak, 2017)

The samples were taken in graduated tubes, and the amount of ejaculate was measured in each sample. After that, the samples were divided and measurements of the following parameters was performed.

a) Macroscopic (ejaculate volume, ejaculate color, density, odor),

b) Microscopic (percentage of progressive movability, total number of sperm in ejaculate, number of live and dead sperm, presence of other substances – blood, pus, impurities)

c) Physicochemical (proteins, fructose, calcium, citric acid, Mg, pH score).

The same parameters were examined at the samples taken in late anestrus season. Evaluation of spermatozoa movability was performed using light microscope at magnification from 100 to 400 times on preheated slides without glass that allowed the spermatozoa to show its movability. Progressive movability is expressed as percentage of movable spermatozoa out of total number of spermatozoa in visual field. The number of living, dead and morphologically abnormal spermatozoa was determined by the Blomm spermatozoon staining method. A small drop of native semen was on a clean glass slide, preheated to 370°C, followed by twice as large drop of 5% aqueous nigrosine solution (black histological color) and a three times larger drop of 10% eosin solution (red histological color). Then, a thin smear is made and covered with glass. This preparation is passed over the burner to dry and observed under the light microscope. Spermatozoas whose heads turn red were dead before the preparation was made, because its cell membrane leaked a large molecules of eosine into the cytoplasm. Cell membranes are

semipermeable and non-permeable for large molecules of eosine. Sperm concentration was measured using hemocytometer. A light microscope, an erythocyte counting chamber (hemocytometer), an erythocyte melanger and a 3% aqueous NaCl solution were used for this method. A hemocytometer was placed under the microscope, and a small counting grid (chamber) was found at low magnification. Native semen is then drawn into the melanger to the 0.5 mark, and a 3% NaCl to the 101 mark. This is how the dilution of 1:200 was made.
The ends of melanger are then closed by your thumb and finger, and shaken several times.
First drop from melanger capillary is dropped and the rest is inserted between the cover plate and the glass slide of the hemocytometer.
The microscope lens is adjusted to medium magnification and the spermatozoa placed in the 5 middle (80 small) squares of the grid are counted.
The protein content in sperm was determined according to the Kjeldahl method, which is based on the destruction of organic substances by heating with sulfuric acid, whereby all protein and non-protein nitrogen (except nitrates and nitrites) are converted into ammonium sulfate. The addition of sodium hydroxide releases ammonia, which is predistilled into a certain amount of boric acid, and the resulting sodium borate is treated with a solution of 0.02 mol/l sulfuric acid. The amount of protein is expressed as a percentage.
Magnesium was determined by titration with a standard EDTA solution with Murexide as an indicator of the pH range.
Calcium was determined using flame atomic absorption spectrometry (FAAS).
Citric acid was determined by a photometric test of semen quantification, and to get the total amount of citric acid, we multiply the result by the volume of sperm. We read the results of the samples and the standard at a wavelength of 450 nm.
Fructose was determined by a photometric test in sperm. Fructose reacts in the presence of HCl under heat with indole to produce a colored complex that absorbs at a wavelength of 450-492 nm. To get the total amount of fructose, multiply the result with the total volume of the seed sample.
Data collected during the research were statistically proccessed using the statistical program SPSS 24.0 (IBM Somers, NY, USA) and presented using descriptive statistic parameters (mean value, standard deviation, standard error, minimum and maximum values, and coefficient of variation). The test for statistical analysis of obtained data was selected on the basis of normal distribution of the obtained results. The statistical significance of the differences between the obtained values for examined parameters was tested by ANOVA method and linear correlation (Pearson). As statistically significant were taken differences at levels $P < 0.05$, $P < 0.01$ i $P < 0.001$.

Results and Discussion

Ruminant ejaculate, in our case rams, is composed of spermatozoa and sperm plasm, and fertilization capability of the sperm depends on its quality. For these reasons, in this paper we have conducted examinations of chemical composition of sperm and evaluation of native sperm quality in both normal mating season as well as in anestrus seasion, in order to determine differences between them. The obtained results are presented in tables and graphs through the basic parameters we examined.

Table 1. Chemical composition of ram sperm in mating season – municipality Bihać

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	5.1	244	195	5	6.04
2	5.0	256	255	6	6.02
3	5.1	252	110	7	6.04
4	4.9	259	220	6	5.05
5	5.3	247	145	6	6.04
6	5.1	265	155	4	6.02
7	4.8	240	265	6	5.07
8	5.1	251	175	5	5.05
9	4.8	253	125	8	6.03
10	5.2	249	150	7	6.00
Average	5.04	251.6	179.5	6.0	5.73

Table 2. Chemical composition of ram sperm in mating season – municipality Cazin

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	5.6	251	190	6	6.01
2	4.9	254	195	4	5.09
3	5.2	252	245	5	5.09
4	5.2	252	220	6	5.09
5	5.0	256	165	7	6.05
6	5.0	255	200	6	6.00
7	5.1	247	145	5	6.08
8	5.4	246	185	6	6.09
9	4.9	250	125	6	6.00
10	5.0	251	255	6	5.09
Average	4.63	251.4	192.5	5.7	5.65

Table 3. Chemical composition of ram sperm in mating season – municipality Sanski Most

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	5.2	257	240	4	5.00
2	5.4	249	150	6	6.00
3	4.9	244	235	6	6.01
4	5.0	251	240	5	6.02
5	5.3	255	115	8	6.00

6	5.1	250	200	6	5.09
7	4.9	244	195	7	6.01
8	5.0	250	255	6	6.00
9	5.3	256	275	5	5.05
10	5.0	251	210	7	6.04
Average	5.11	250.7	211.5	6.0	5.72

Table 4. Average results of chemical composition of ram sperm in mating season (Bihać, Cazin, Sanski Most)

Parameter / Municipality	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
Bihać	5.04	251.6	179.5	6.0	5.73
Cazin	4.63	251.4	192.5	5.7	5.65
Sanski Most	5.11	250.7	211.5	6.0	5.72
Average	4.92	251.2	194.5	5.9	5.70

From the data shown in table 4, it can be noticed that the lowest average value of proteins was found in sperm samples from Cazin (4.63 g/100 ml), while sperm samples from Bihać had 5.04 g of protein per 100 ml, and the highest value of proteins was found in samples from Sanski Most (5.11 g/100 ml). The average value of fructose in sperm samples during mating season is almost the same in each municpality. The lowest was found in sperm samples from Sanski Most (250.7 mg/100 ml), then in Cazin (251.4 mg/100 ml), while the highest value of fructose was found in Bihać (251.6 mg/100 ml). When it comes to average value of citric acid in 100 ml of sperm samples, the lowes amount was found in Bihać with 179.5 mg/100 ml, followed by Cazin with 192.5 mg/100 ml, and the highest value of citric acid was found in samples from Sanski Most (211.5 mg/100 ml). The lowest average value of magnesium was found in sperm samples from Cazin (5.7 mg/100 ml), while the samples from Bihać and Sanski Most had exactly the same value of 6.0 mg/100 ml. The lowest average value of calcium was in sperm samples was also found in Cazin (5.65 mg/100 ml), while the samples from Sanski Most and Bihać were almost the same, with 5.72 mg/100 ml and 5.73 mg/100 ml, respectively.

Table 5. Chemical composition of ram sperm during late anestrus season – municipality Bihać

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	4.9	252	240	5	5.05
2	5.2	249	200	7	5.10
3	5.1	240	242	6	6.00
4	4.7	255	252	6	5.90
5	5.1	246	135	7	5.00
6	5.2	235	205	7	6.00
7	4.3	222	215	6	5.05
8	4.8	241	262	6	6.00

9	5.1	256	265	5	6.05
10	5.0	245	217	6	5.04
Average	4.14	220.6	223.3	6.1	5.51

Table 6. Chemical composition of ram sperm during late anestrus season – municipality Cazin

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	5.3	249	245	5	6.00
2	4.8	257	250	8	5.00
3	4.3	241	235	5	6.02
4	5.1	238	220	6	6.01
5	5.3	252	118	7	5.05
6	4.7	245	230	7	5.09
7	4.6	243	180	6	6.00
8	5.1	250	250	5	6.05
9	5.4	251	262	7	5.01
10	4.9	249	215	6	5.04
Average	4.95	247.5	220.5	6.2	5.52

Table 7. Chemical composition of ram sperm during late anestrus season – municipality Sanski Most

Parameter / Number of ram	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
1	5.0	249	220	6	5.00
2	4.1	250	180	4	6.01
3	4.5	241	205	4	5.01
4	5.1	251	240	5	5.09
5	4.9	244	118	7	5.05
6	4.6	252	190	6	5.00
7	4.9	240	205	6	6.00
8	5.0	250	230	5	6.01
9	5.1	255	250	7	5.02
10	4.8	245	245	4	6.02
Average	4.8	247.7	208.3	5.4	5.42

Table 8. Average results of chemical composition of ram sperm during late anestrus season (Bihać, Cazin, Sanski Most)

Parameter / Municipality	Proteins (g/100 ml)	Fructose (mg/100 ml)	Citric acid (mg/100 ml)	Mg (mg/100 ml)	Ca (mg/100 ml)
Bihać	4.14	220.6	223.3	6.1	5.51

Cazin	4.95	247.5	220.5	6.2	5.52
Sanski Most	4.80	247.7	208.3	5.4	5.42
Average	4.63	238.6	217.3	5.9	5.48

When it comes to chemical composition of sperm samples during late anestrus season, we can see from the data shown in table 8, that the lowest average value of proteins was found in samples from Bihać (4.14 g/100 ml), while the slightly higher values were found in Sanski Most (4.80 g/100 ml) and Cazin (4.95 g/100 ml). The lowest average amount of fructose in sperm samples was also found in Bihać (220.6 g/100 ml), while the samples from Cazin and Sanski Most were almost the same, with 247.5 mg/100 ml and 247.7 mg/100 ml, respectively.
When we analyze the average amount of citric acid in sperm samples, we can see that the lowest amount was found in Sanski Most (208.3 mg/100 ml), with slightly higher value found in Cazin (220.5 mg/100 ml), and the highest value in Bihać (223.3 mg/100 ml). The lowest average value of magnesium was in sperm samples was found in Sanski Most (5.4 mg/100 ml), while the samples from Bihać and Cazin were almost the same, with 6.1 mg/100 ml and 6.2 mg/100 ml, respectively. The lowest average value of calcium in sperm samples was again found in Sanski Most (5.42 mg/100 ml), while the samples from Bihać and Cazin were almost the same, with 5.51 mg/100 ml and 5.52 mg/100 ml, respectively.

Table 9. Statistical analysis of all chemical parameters of sperm in mating season and late anestrus season

Examined parameters	Bihać	Cazin	Sanski Most	ANOVA
Proteins	4.99 ± 0.23	5.04 ± 0.30	4.95 ± 0.30	*P* > 0.05
Fructose	248 ± 9.47	249 ± 4.9	249 ± 4.8	*P* > 0.05
Citric acid	201 ± 50	206.5 ± 43	210 ± 43	*P* > 0.05
Mg	6.0 ± 0.9	6.0 ± 0.9	5.7 ± 1.17	*P* > 0.05
Ca	5.62 ± 0.48	5.6 ± 0.49	5.57 ± 0.49	*P* > 0.05

As it can be seen from the results of statistical analysis of chemical parameters in table 9, the highest average value of proteins in sperm samples was found in Cazin of 5.04, with standard deviation (SD) of 0.30, followed by Bihać with 4.99 ± 0.23 and Sanski Most with 4.95 ± 0.30 (P > 0.05). For examined values of fructose in sperm samples, we can say that they are very similar in each location, with the highest mean value found in Cazin (249 ± 4.9), followed by Sanski Most 249 ± 4.8, while the lowest mean value was found in 248 ± 9.47 (P > 0.05). The highest mean value of citric acid was found in samples from Sanski Most (210 ± 43), then in Cazin with 206.5 ± 43, while the lowest average amount of citric acid was found in Bihać with 201 ± 50 (P > 0.05). When it comes to amount of Mg found in sperm samples, the highest value was found in Bihać and Cazin with exactly the same value of 6 ± 0.9, while in Sanski Most it was 5.7 ± 1.17 (P > 0.05). The highest mean value of Ca was found in sperm samples from Bihać with 5.62 ± 0.48, followed by Cazin (5.6 ± 0.49), and the lowest average value was found in Sanski Most 5.57 ± 0.49 (P > 0.05). Statistical analysis have shown that there are no significant differences in examined parameters between the mating season and late anestrus season (P > 0.05), as well as highly significant differences between the localities.

Table 10. Statistical analysis of parameters of sperm quality between the mating season and late anestrus season

Examined parameters	Mating season	Late anestrus	ANOVA
Sperm volume	1.18 ± 0.29	0.78 ± 0.21	$P \leq 0.01$
Number of spermatozoa in 1 ml	2.44 ± 0.74	1.52 ± 0.39	$P \leq 0.01$
Total number of spermatozoa	2.98 ± 1.03	2.10 ± 0.53	$P \leq 0.01$
Percentage of sperm movability	79.3 ± 6.31	59.3 ± 7.70	$P \leq 0.01$
Sperm pH	6.3 ± 0.42	6.0 ± 0.28	$P \leq 0.05$
Percentage of dead and pathologically changed spermatozoa	20.7 ± 6.3	40.6 ± 7.7	$P \leq 0.01$

Table 11. Conception values after natural insemination of Pramenka sheep in examined municpalities during normal mating season

Municipality	Conception value		
	Inseminated sheep (n)	Lamb born (n)	Conception (%)
Bihać	30	28	93.3
Cazin	30	26	86.7
Sanski Most	30	27	90.0
Average	30	27	90.0

The results from table 11 clearly shows that statisfactory level of conception has been achieved in all municipalities involved. The highest conception success was recoreded in Bihać, with 93.3%, followed with Sanski Most with 90.0% and Cazin with 86.6%. There were no statistically significant differences between the municipalities in conception of sheep during normal mating season (P > 0.05). Testosterone, involved in many male reproductive processes, fluctuates from season to season. Short days (or shortening the length od daylight) trigger testicular development by stimulating the secretion of FSH and ICSH, which in turn induces the testosterone secretion. Long days (or increasing the day length) induces testicular regretion by inhibiting gonadotropic secretion and thus testosterone secretion (Langford et al, 1987). The sperm plasm contains Na, K, Ca, Zn, Mg, Fe, chlorides, bicarbonates, fructose, citric acid, alanine, glycine, tyrosine, vitamins A, D, E, B complex, C, then prostaglandins, various enzimes, salts, etc. (Podžo, 1999). Our research showed that there is significant difference in sperm quality between the mating season and late anestrus season in all examined parameters except for Mg. We also found that location of the rams did not influence the sperm quality in neither mating season nor late anestrus season, which means there are no significant differences between the municipalities involved in the research. Unlike the other parameters, citric acid in semen had a higher value in anestrus season (217.3 mg/100 ml) than in mating season when averaged at 194.5 mg/100 ml. Stančić (2014) states that the average value of citric acid in sperm samples in mating season was 140.0 mg/100 ml, which is lower than the value obtained in our study. The average value of Mg determined in our research was 5.9 mg/100 ml in mating season and anestrus season, while

Stančić (2014) states the average value of 8.0 mg/100 ml. Also, in the paper of Stančić (2014), we found that the value of Ca is 11.0 mg/100 ml, which is significantly higher than our obtained results. The concentration of sperm is a function of several parameters that include the degree of sexual preparation of the ram, the age of the ram, the season, the health condition, the nutrition status, the frequency of sperm intake, and production capability of the ram. Since it is well known that there is direct correlation between the quantity of sperm and sheep fertility results, it is important that the sperm concentration in the ejaculate is determined as accurately as possible, taking into account all factors that may affect the result. For the total nuber of spermatozoa in the ejaculate, we can see that the average value was higher in mating season (2.98×10^9) than in anestrus season (2.10×10^9). According to research by Stančić (2014), total number of spermatozoa in the ejaculate was around 2.10×10^9, which is consistent with our results. Oberst et al. (2011) have found the largest concentration of spermatozoa in the ejaculate is determined in autumn, during normal mating season. The number of spermatozoa in 1 ml of semen, which was established on the basis of our research in the normal mating season, averaged 2.44 x 10^9, and in the late anestrus season 1.43 x 10^9. Our results are comparable to those obtained by Deldar et al. (2007) and Maghaddam et al. (2012), with maximum sperm concentration values in autumn and reduced values during winter.

Higher values than ours are found in the works of Karagiannidis et al. (2000) where they amount to 3.33-4.44 x 10^9 and in Kafi et al. (2004) 3.93-4.90 x 10^9.Statistical analysis showed highly significant difference ($P \leq 0.01$). Statistical analysis for the impact between the three localities did not show significant difference ($P > 0.05$).

Conclusions

Based on the results obtained in this paper, as well as on the examinations of sperm quality of Pramenka breed rams on three localities between the normal mating season and late anestrus season, and the results of conception and lambing in mating season, the following conclusions can be drawn:

When it comes to chemical analysis of the sperm, it can be concluded that all the examined parameters except for Mg, have statistically significant difference between the seasons, and no significant difference between the locations. As expected, the volume of the ejaculate was higher in mating season (1.184 ml) than in late anestrus (0.76 ml), just like the number of spermatozoa in 1 ml of sperm, where we found 2.44×10^9 , while in late anestrus it was 1.43×10^9. A very highly significant difference was found in the total number of spermatozoa in the ejaculate between the season, but no significance between the locations.

The percentage of movable spermatozoa was 79.3% in mating season, compared to 59.3% in late anestrus, which is in correlation with dead and patologically changed spermatozoa, where we found 20.7% in mating season and 40.6% in late anestrus.

Again, the highly significant difference was found between the seasons, and no statistically significant difference between the locations. The value of conception after natural insemination during mating season was satisfactory and amounted 90% on average. Having in mind the results of all examined parameters of sperm quality in mating season and late anestrus season, brings us to conclusion that the sperm quality is the key reason for successfull conception of sheep.

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EXTERIOR MEASURES AND WOOL QUALITY IN SHEEP BREEDED IN THREE LOCATIONS IN BOSNIA AND HERZEGOVINA

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Abstract

The aim of this study was to measure the basic external characteristics of Pramenka sheep: height at withers, the crosses, carcass length, chest width, chest depth, chest circumference, breast circumference carcass, shin circumference and body weight, as well as to determine the basic parameters of wool quality: strand length, fine fiber fineness, strength, twisting ability, tensile strength, in order to compare the measured values to assess the impact of growing areas on them. Domestic autochthonous strains of Pramenka (Kupres, Travnik) were used for research. There were 36 sheep in the group animals. Measurements were performed on long-term purebred herds of Pramenka on three private farms in the Una-Sana Canton, Cazin, in Central Bosnian Canton, Travnik, and Kupres, Livno Canton. Based on the presented average values of external characteristics of Pramenka sheep and their variations for all examined localities, we can conclude the following: that sheep are longer in relation to their height and that Pramenka is of medium physical development, that differences in physical measures in and the origin of certain breeds-strains of sheep of the Pramenka breed (Kupres strain, Vlašić strain), as well as the quality of pasture areas and unequal access to food. The measured parameters of wool quality indicate that the quality of wool, in almost all examined parameters, is poor, regardless of the location of sheep breeding. Comparing our results with the results of other authors who examined the exterior of other strains of Pramenka (from region in Croatia: Rab, Lika, Pag, Istria) in our wider environment, we conclude that Vlašić Pramenka is the largest strain of Pramenka in this area.

Key words: *Sheep, External traits, Quality, Wool.*

Introduction

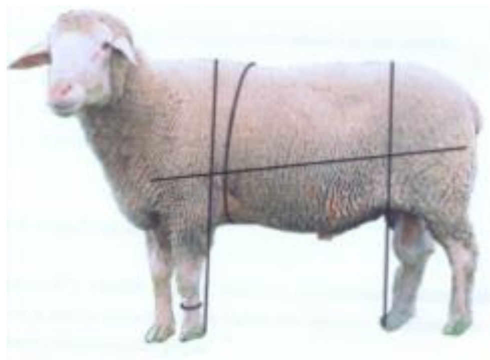
Sheep farming in Bosnia and Herzegovina, along with cattle breeding, is one of the main branches of livestock production. 1990 in Bosnia and Herzegovina, the number of sheep was about 1.3 million, and in 1960 it was even larger and amounted to about 2.2 million. This indicates to the fact of a significant decline in 30 years, even by 41%. In the last period the number of sheep suffered even bigger fall, and since the list has not been made for a long time, it is not possible to say with certainty how many sheep there are today in BiH.
The characteristics of sheep breading in developed countries is that with intensification of agriculture, the number decreases, but therefore increases production. Although the number of sheep in BiH has decreased, however, there was no significant increase in production.

From the earliest times natural meadows have been most successfully exploited with sheep. They are together the greatest extent absolute sources of food for sheep, in relation to other species of domestic animals. That is why hill and mountain areas, economically speaking, can be most successfully used by sheep breading. The existing population of sheep in BiH in a high percentage is Pramenka with a large number of strains (around 80%), and in smaller number are represented by various types of crossbreeds (around 20%). Production capabilities of domestic Pramenka are low, which implies a small body weight of adults, low production of meat and milk, and low yield of unwashed coarse wool.
Exterier presents external appearance, that is, development and connection of individual parts of the body, then the size of the whole body and its overgrowth and the color of the wool in sheep, as well as everything else that can be seen on them and can be measured and evalueted (horns, hoovs, etc.). Exterior assessment is of particular point when it comes to breeding choice (Krajinović *et al*., 2004). Nikolić *et al.* (1985) a good assessment of the exterior is considered to be a powerful means which a reliable picrture of their breeding value can be created. The exterior of domestic animals can be assessed in three ways, and those are: by measuring the development of individual parts of the body and determining the body measurements of the animal with the help of special accessories for this purpose, by assessing the appearance of the said parts by photographing the animals. The animals should stand on a flat and firm surface during the measurement. Body dimensions are taken in the following order: hight, lenght, depth, width and volume. The measurement is performed on the left side of the animal. The measurement of domestic animals is of special importance because only by measuring can reliable and accurate data on the development and relations of individual parts of the body and the animal as a whole be established. The data obtained by measurements are entered in the appropriate registers. Based on the data obtained by measuring animals, it is possible to monitor their growth from birth to full physical development.
A large number of authors have investigated the exterior characteristic of some strains of Pramenka and crossbreed. The works of following authors stand out in paricular: Brinzej *et al.* (1991), Čaušević *et al*. (1991). There are a large number of strains of Pramenka that different from by production characteristics, which is the result of action of existing enviromental factors. The division into strains was performed according to the area of cultivation and variability at external and production characteristics. The most famous strains are: from Vlašić region (Dupska, Travnik), Kupres strain, Privor strain, Duvanj strain, Glamoč strain, Gatac strain, Ključ strain, Stolac strain, Podveleš strain and others (Brinzej *et al.*, 1991). The hull is rectangular in shape, with lenght of the hull being greater in relation to the height of ridge. The height of the ridge in sheep ranges on average from 63.5-66.9 cm, and lenght of the hull from 67.7-69.8 cm. The depth of the chest is 28.4-36.5 cm, the width of the chest is 18.8-23.5 cm, while the circumference of the chest is 67.0-94.5 cm. The body weight of sheep ranges from 20.0-50.0 kg, and rams up to 70.0 kg, noting that the upper limits of variability may be higher (Telalbašić *et al.*, 1979; Pavić *et al*., 2005; Antunović *et al*., 2001; Antunović *et al*., 2007; Mioč *et al*., 2006; Vilić, 2013). The story of sheep and wool begins in Asia 10,000 years ago during the Stone Age. The people of Mesopotamia at the time exploited sheep and their products to meet three basic needs: food, clothing, and shelter. From then until today, sheep are considered very efficient, the least demanding of all domestic animals. Due to the fact that sheep are fed various, indigestible nutrients, they convert food into proteins for human consumption (meat, wool, milk) (Jovanović, 1988). One of the most important products of sheep is wool, which is obtained from domestic breeds, and consists of wool fibers folded into strands. Wool in the narrow sense means sheep's

hair, which does not separate after shearing and has the ability to spin and roll (Nikolić and Simović, 1985). For many years, sheep's wool from the territory of Bosnia and Herzegovina has not been industrially processed and has no economic importance. Wool is sheared primarily for the welfare of the animal and not for obtaining useful textile raw material. The reason for this lies in the fact that the wool of indigenous breeds-Pramenka and its strains are considered coarse wool that are not suitable for making fine and prized clothing products. In addition, wool is still an irreplaceable textile raw material in some countries due to its chemical and physical properties. The world produces over 2.5 million tons of medium fine and fine wool. The wool of our sheep breeds belongs to the roughest assortment and is unsuitable for processing. Wool has the natural property of regulating temperature, the inner hair of wool can absorb water vapor, while the outer surface gets steam (Jovanović, 1988). Wool, as the noblest textile raw material, is mostly used for the production of men's suits and women's costumes and dresses. It is also often used, alone or in a mixture with other fibers, for the production of knitted clothes, carpets and rugs. Wool can be used in a mixture with almost all textile fibers (Brinzej *et al.*, 1991).

Material and Method

The experiments were performed on long-term purebred heard of Pramenka on private farms in Una-Sana Canton, Cazin, as well as one on private farm in Central Bosnia (Vlašić) and one in Kupres (Livno Canton). The results of measurements and analysis of external properties of sheep of the examined breed using standard methods and methods of measurements are presented. To assess the body of an animal, each part of the body is assessed. In this paper, measurements of individual body parts were performed to assess the exterior using a Lydthin rod and tape. A total 108 heads were measured at the mentioned 3 locations. The following measurements were made: height of the ridge, (from the ground to the highest point), trunk lenght (oblique distance from another edge of the shoulder-scapular joint to the posterior point of the static bone), chest widht (measured just behind the shoulder blade), chest depth (vertical distance from the top of the ridge to the lower edge of the sternum), the circumference of the breasts, the circumference of the carcass behind the shoulder blades (tape), the circumference of the shin, measured at the thinnest point of the shin (tape), body weight (weighing the body of the animal with a livestock scale). In addition to external measures, the quality of wool in the examined sheep was determined, and the following physical and mechanical properties of wool were analyzed: length, elasticity, stretch ability, twisting ability and flexibility. A total of 50 samples from each farm were analyzed. These researches were conducted in the laboratories of the Biotechnical Faculty. For these researches, the breed of Pramenka sheep was used, on three farms, in Cazin, Vlašić and Kupres. The sheep were kept in appropriate conditions and fed classically, in the summer they stayed outdoors and fed on pastures, and in the winter they were kept in facilities and fed with quality bulky food and, if necessary, concentrated nutrients.



Picture 1. Marked places where body measurements are taken in sheep

Picture 2. Taking and preparing a wool sample or analysis

A **B**

Picture 3. Used apparatus for determining the quality of wool A-lanomether, B-Spectrophotometer

Results and Discussion

Table 1. Values of exterior measures of examined sheep from 3 localities.

Locality	Body weight (kg)	Shin circumference (cm)	Chest width (cm)	Chest circumference (cm)	Chest depth (cm)	Chest length (cm)	Cross height (cm)	Reef height (cm)
Cazin	65.27	8.47	22.73	90.60	36.88	71.63	67.86	67.89
Vlašić	65.44	9.16	24.77	93.20	39.67	74.98	70.26	70.63
Kupres	57.95	8.27	21.62	86.43	33.74	69.39	69.39	64.56

From Table 1 it can be seen that the highest weight had sheep from Vlasic (65.44 kg), slightly lighter were sheep from the locality Cazin (65.27 kg), and the lightest were sheep from the locality Kupres (57.95 kg). The same regularity is observed in other measured parameters, so it can be concluded that sheep from the locality Vlašić are the largest breed of analyzed sheep. Based on the presented results in the mentioned areas of research, we can conclude that the differences in body measures in the examined areas are greatly influenced by the origin of certain breeds - strains of sheep breed Pramenka (Kupres strain, Vlašić strain) as well as the quality of pastures and unequal access to food. In the examinations, Omanović (2006), states similar results for the Kupres pramenka. By comparing our results with the results of other authors who examined the exterior of other strains of pramenka (from region in Croatia: Rab, Lika, Pag, Istria) in our wider environment, we conclude that Vlašić pramenka is the largest strain of pramenka in this area.

Table 2. Strand length and wool fiber thickness

Farm	Number of samples	Strand length (cm)	Fineness of wool fibres (µm)
Cazin	50	12.17	38.9
Vlašić	50	12.42	38.6
Kupres	50	12.01	38.7
Average	50	12.25	38.8

The length of the fibres was the highest in sheep from the locality Vlašić (12.42 cm), then in those from the locality Cazin (12.176 cm), and the lowest in sheep from the locality Kupres (12.01 cm). Regarding the fineness of the fibres, the finest wool was measured in sheep from the locality Vlašić (38.6 µm), then in sheep from the locality Kupres (38.7 µm), and the coarsest wool was measured in sheep from the locality Cazin (38.9 µm).

Table 3. Values of 3 measured parameters (strength, extensibility, twisting ability) in sheep on three farms

Farm	Strength	Extensibility	Twiating ability
Cazin	Good	No	No
Vlašić	Good	No	Weak
Kupres	Good	No	Weak
Average	Good	No	No/weak

From the results shown in Table 3, it can be seen that the strength is good in all samples, that there is no extensibility while the twisting ability was absent or was weak. These results are characteristic of wool fibers in Pramenko sheep and completely agree with the results of numerous authors (Markotić and Čaušević, 1975; Čaušević and Parker, 1991; Krajinović *et al.*, 2004; Mioč *et al.*, 2000).

Conclusion

Based on the presented average values of external properties of Pramenka sheep and their variations for all examined localities, we can conclude the following:
a) That sheep are longer in relation to their height and that Pramenka is of medium physical development.
b) That the differences in body measures in the examined areas are greatly influenced by the origin of certain breeds - sheep strains of the Pramenka breed (Kupres strain, Vlašić strain), as well as the quality of pasture areas and unequal access to nutrition.
c) By comparing our results with the results of other authors who examined the exterior of other strains of pramenka (from region in Croatia: Rab, Lika, Pag, Istria) in our wider environment, we conclude that Vlašić pramenka is the largest strain of pramenka in this area.
Examining some of the characteristics of wool fibers in Pramenka sheep, on three farms, the following conclusions can be drawn: the average length of the strands was 12, 25 cm, and the average fineness of the wool fibers was 38.8 micrometers, after the extensibility test it was shown that there was no extensibility, the twisting test showed that there is no or weak twisting ability. In general, it can be concluded that the quality of Pramenka wool fibers is poor.

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THE EFFECT OF AMBIENT TEMPERATURE ON EATING TIME AND RUMINATION TIME AND MILK YIELD AND CHEMICAL COMPOSITION OF MILK IN LACTATING COWS

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Abstract

The paper presents the results of research on the influence of ambient temperature in different periods of the year on eating time, rumination time, and milk yield and chemical composition of milk in Simental cows in the late lactation (over 150 days). Collars with sound-detecting sensors (GEA CowScout Neck) were used to monitor eating time and rumination time once per day. Ambient temperature was registered using data logger Testo 174T. The air temperature was measured every hour. It was noticed that different ambient temperatures affect the eating time ($p<0.01$), rumination time ($p<0.01$), milk yield ($p<0.05$) as well as milk fat content ($p<0.01$). The average ambient temperature of 11.88 ºC had a positive effect on eating time (302.4 min/day), rumination time (379.8 min/day), milk yield (28.55 kg/day), and milk fat content (4.42%), compared to the average ambient temperature of 25.35 ºC, where the observed eating time was 266.4 min/day, rumination time 345.6 min/day, milk yield 26.42 kg/day and milk fat content 4.12%. An increased chewing time was achieved at an adequate ambient temperature, as well as higher milk yield and improved milk composition.

Keywords: *dairy cattle, production performances, chewing activity, environment temperature*

Introduction

The collar with a unique motion sensor represents an effective system for monitoring chewing activity in cows. The basic principle consists in registering sound signals from a microphone that is in contact with the cow's neck in order to measure the chewing time (Bar and Solomon, 2010). The feeding behavior of dairy cows has been previously described (Campling and Morgan, 1981; Beauchemin, 1991; Albright, 1993), but modern automation of equipment for feeding and recording feeding behavior in modern dairy allows systematic insight and monitoring of the total chewing activity of cows, as and registering other types of activities. Also, earlier studies provided the foundation for our understanding of the mechanics of chewing, the physiological role of chewing for cows, and how chewing activity is affected by changes in the chemical composition and physical characteristics of the ration (Welch, 1982; De Boever et al., 1990). Lactating cows spend about 4.5 hours/day eating (range: 2.4–8.5 hours/day) and 7 hours/day ruminating (range: 2.5–10.5 hours/day), with a maximum total chewing time of 16 hours/day (Beauchemin, 2018). Peak chewing activity during consumption usually occurs after meal distribution (King et al., 2016) or during the subsequent redistribution of bulk portions of the meal during the day. Therefore, more frequent distribution of a total mixed ration (TMR) tends to promote consumption activity and a more even distribution of food consumption time throughout the day, although dry matter (DM) intake is not necessarily increased (Miller-Cushon and DeVries, 2017). Competition between cows during TMR distribution increases the rate of consumption (kg DM/min) and decreases the total time spent consuming during the day (Proudfoot et al., 2009). DM intake also decreases with competition at the manger or limited time to access food (Munksgaard et al., 2005). Cows modify their feeding behavior to consume feed in a shorter period when needed, which indirectly enables them to compete more efficiently for feed and maintain adequate DM intake (Crossley et al., 2017). Rumination time can also be an indicator to determine the level of heat stress in dairy cattle. Acatincai et al. (2009) concluded that when the temperature exceeds the upper limit of thermal comfort of a particular breed, the rumination process is severely affected. Temperatures beyond 27–28 °C reduce the overall rumination process, including the frequency and duration of this activity. Muschner-Siemens et al. (2020) observed that rumination time is affected by several individual cow factors even in moderate climates, indicating that the parameter could be used to evaluate effect of climate change in dairy cows. The purpose of the conducted experiment was to determine how different ambient temperatures affect eating time and rumination time, as well as the milk yield and chemical composition of milk.

Material and Methods

The experiment was carried out on the farm of dairy cows of the Simmental breed “DMN” Malo Crnić́e, Požarevac. The research included determining the effects of different ambient temperatures on eating time and rumination time, as well as on the milk yeild and its chemical composition. The experiment consisted of two experimental periods in different periods of the year. The total mixed rations (TMR) used were uniform in terms of chemical composition for both experimental groups of cows. The composition and nutritional value of the total mixed ration are given in Table 1. During the experimental periods, the total mixed rations for lactating cows consisted of identical nutrients.

Table 1. The composition and nutritional value of TMR

Item	Total mixed ration, kg
Alfalfa hay	3
Corn silage	25
Straw	0.5
Concentrate 21,6% CP	8.9
Dry matter, DM, kg	19.16
In DM of ration	
Crude protein, %	15.31
Neutral detergent fibers, %	34.42
Acid detergent fibers, %	21.20
Starch, %	19.85
Ca, %	0.83
P, %	0.30
NEL, MJ kg^{-1}	6.71

In addition to TMR, both groups of cows received 3 kg of concentrate with 12% CP during milking (3 times per 1 kg of concentrate). The cows were kept in a facility with free-stall system. The research consisted of two experimental parts of 30 days each in different periods of the year. During both experimental periods, an identical TMR was used in the diet of dairy cows, the number of cows in the group was 35, with an average age of 40 months and a lactation phase of 180 days. For both groups of cows, based on the average milk yield, a ration was formulated to produce 29 kg of milk with 3.6% milk fat and 3.2% protein.
BVL mixer trailers with a volume of 8 m^3 were used for the preparation and distribution of meals. Food was distributed to the cows once a day at 8 am.
A Testo 147T data logger was installed in the stall where the cows stayed during the experiment. Air temperature was measured every hour. The average ambient temperature measurement values are giving in table 2.

Table 2. Average ambient temperatures in the experimental periods

T1	T2	Significance
11.88 °C ± *4.33* °C	25.35 °C ± *5.13* °C	**

T1 - lower ambient temperatures
T2 - higher ambient temperatures
± - standard deviation
** - $p<0.01$

All cows participating in the experiment were equipped with GEA CowScout Neck sensor collars that monitored eating time and rumination time which were registered using GEA DairyRobot R9500 software. Milk yield was recorded daily, while milk fat and protein content were determined at 10-day intervals. To analyze the statistical significance of the differences for the determined values of the observed parameters - ambient temperature, eating time, rumination time, amount of produced milk and its chemical composition, the t-test was used, at the level of significance $p<0.01$ and $p<0.05$, and as a parameter of descriptive statistics and standard deviation.

Results and Discussion

By analyzing the average daily temperatures in different periods of the year, it was determined that there was a very significant difference between the two observed periods. These differences had an effect on eating time and rumination time, which is shown in Table 3.

Table 3. Eating time and rumination time in cows in different ambient temperature

	T1	T2	Significance
Eating time (min/day)	302.4±*0.65*	266.4±*0.55*	**
Rumination time (min/day)	379.8±*0.48*	345.6±*0.46*	**

T1 - lower ambient temperatures

T2 - higher ambient temperatures
± - standard deviation
** - $p<0.01$

Statistical processing of the data revealed that the eating time of the observed groups of cows differs statistically very significantly, which can also be concluded for the rumination time. The obtained values for chewing activity during rumination in lactating cows are lower compared to the results reported by Bar and Solomon (2010), where the average rumination time during lactation was determined to be 478 min/day. In a study involving 515 dairy cows in 7 experiments with more than 1.2 million cow manger visits, the cows consumed an average of 7.7 meals per day, with a time interval between meals of at least 29 minutes (De Mol et al., 2016).
When temperature exceeds the threshold values, a 1 °C increase in daily mean temperature reduced rumination time by 5.12 min per day and reduced rumination efficiency by 0.07 kg DM per cow per hour (Ji et al., 2020). In another study, total rumination time, day rumination and night rumination time were reduced with high temperature in early, mid and late lactation in dairy cows (Abeni and Galli, 2017). More interestingly, heat stress reduced rumination time by 22.9% even in dried-off dairy cows leading to reduced degradability of the feed consumed (Maia et al., 2020). Different ambient temperatures had an impact on the milk yield as well as the chemical composition of milk. Milk yield, milk fat and protein content in milk are given in Table 4.

Table 4. Impact of different ambient temperature on milk yield, and milk fat and protein content

	T1	T2	Significance
Milk yield, kg/day	28.55±1.95	26.42±1.72	*
Milk fat content, %	4.42±0.04	4.12±0.07	**
Milk protein content, %	3.83±0.03	3.79±0.02	ns

T1- lower ambient temperatures
T2 - higher ambient temperatures
± - standard deviation
** - $p<0,01$
*- $p<0,05$
ns - not statistically significant

By comparing the data, it was determined that the milk yield of the two observed groups of cows is statistically significantly different. Daily milk yield in the group of cows in the period of the year with lower ambient temperature was higher ($p<0.05$) compared to the group of cows in the period of the year with higher ambient temperature. Also, the amount of milk fat in this group of cows was statistically significantly higher ($p<0.01$). The milk protein content was not statistically different in the observed groups. Insufficient rumination in lactating cows can lead to a number of negative effects on health as well as production performance. The most common problem that occurs in such situations is SARA (subacute ruminal acidosis). This metabolic disorder is characterized by clinical signs: anorexia, diarrhea, poor body condition, liver abscesses, poor rumen activity and reduced milk production (Dirksen, 1985; Aschenbach et al., 2011).

Conclusion

Chewing activity during consumption and rumination is important for ensuring high levels of feed intake and efficient digestive function in high-producing dairy cows. This is necessary from the perspective of ensuring normal function of the rumen, efficient utilization of consumed food and optimal production. Based on the results of this experiment, it can be concluded that different ambient temperatures affect eating time and rumination time, as well as milk yield and milk fat content. An ambient temperature of 11.88°C was found to have a beneficial effect on chewing activity, milk yield and milk fat content compared to the temperature of 25.35°C. In order to achieve optimal production results, it is necessary to pay more attention to factors affecting the ambient temperature on dairy farms. Also in periods with higher air temperature values, it is necessary to adjust the composition of meals and the feeding technology of productive animals to the possibilities of food consumption.

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INFLUENCE OF STIMULATIVE FEEDING MEASURES ON SPRING DEVELOPMENT OF BEE SOCIETIES IN DB AND LR HONEYCOMBS

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Abstract

In order for the bee colonies to make the most of the main pasture, and primarily black locust, it is very important that they are in good condition. It is necessary for the societies to reach their maximum strength at the beginning of the main pasture, and not for it to serve them for further development. The beekeeper himself plays a crucial role in the accelerated spring development of bee colonies. The aim of this study was to determine the effect of different ways of spring feeding on the strength of bee colonies and the food supply in them. The experiment was performed with companies that were in Db (Dadant-Blatt) and Lr (Langstroth-Ruth) hives. Four groups of companies were tested, and there were five companies in each group. The first group was supplemented with sugar syrup, enriched with vitamin-mineral complex - Foprsapine (10 drops per liter of syrup). The second group of societies was fed with honey dissolved in water (ratio of honey and water 1:1). The third group of the companies was fed with sugar dough (energy cake without additives). The fourth group of companies was supplemented with sugar syrup (the ratio of water and sugar was 1:1). At the beginning of the experiment, the societies in Lr hives had an average of 4.5 frames with bees, and 2.5 frames with a brood. In the Db hives, the societies occupied five frames with bees, and had 2.7 frames with a brood. At the end of the experiment Lr societies had 8.7 frames with bees, and 4.5 frames with brood, while Db societies had nine frames with bees, and 4.7 frames with brood. The experiment was performed at the apiary of the Institute for Forage Crops in Kruševac.

Key words: *honey bee, spring development, stimulating feeding, Db and Lr hive.*

Introduction

Spring development is a very important aspect of the activity of bees and the beekeeper himself. During the spring, bee colonies prepare to reach their maximum strength. To reach the full potential of honey production, societies must produce a large number of young worker bees. In order to achieve this, it is necessary to add nutrients that stimulate the performance of the young brood, and thus increase the strength of the company. The main foods for the honey bee (*Apis mellifera* L.) are pollen and honey. Pollen is the main source of protein, fat, vitamins and minerals in bee nutrition. On the other hand, honey is rich in carbohydrates, primarily fructose and glucose and to some extent sucrose. Complete nutrition with these substances contributes to the maximum development and work of the hypopharyngeal glands in young bees. The number of broods and bees in the spring depends on a large number of factors; racial affiliation (Kulinčević 1997), strength of society during wintering (Jevtić et al., 2005), quality and quantity

of food left for the winter (Mladenović et al., 2002a), stimulative supplementation (Mladenović et al., 2002b), application of vitamin complex in supplements (Mladenović et al., 2003) and others. There is also a large number of negative factors, among which the great use of pesticides, unfavorable weather conditions, the presence of diseases and parasites, etc. stand out. (Calovi et al., 2021).
The aim of this study was to determine how stimulating supplementation affects spring development and food stocks in societies before the start of the main (black locust) pasture.

Material and methods

The experiment was performed at the apiary of the Institute for Forage Plants in Kruševac in the period from 1 April to 5 May 2018. The study included five groups (treatments) with three societies in LR (Langstroth Root) and the same number in DB (Dadant – Blatt) hives. At the beginning and at the end of the experiment, all societies were inspected and their strength (quantity of bees and area with brood) and food supply (area with honey and pollen) were determined. The number of bees per company was determined visually based on the number of bees occupying the frames. This feature is expressed in the parts of the frame that are occupied by bees (1/10). By summing the amount of bees on the frames, the total amount of bees per company was obtained. The area under the brood was also assessed by a detailed survey of each frame with the brood open and closed in the hive. The results are also expressed in parts of the frame (1/10). The surface of honey and pollen was determined in the same way and at the same time as the two previous characteristics. Experimental groups of the society have been stimulated to faster spring development by various methods. The companies from the first group were supplemented with sugar syrup (water / sugar ratio was 1:1) to which 10 drops of forsapine were added. The second group of societies was fed with honey dissolved in water (water / honey ratio was 1:1). During the experiment, 2 sugar cakes (energy cakes without protein and vitamin supplements) were added to the third group of companies. The fourth group of companies was fed only with sugar syrup (water-sugar ratio 1:1). The fifth group were control societies that were not additionally stimulated (fed). To the companies to which sugar syrup or honey was added, a total of 2-2.5 kilograms was added in 15 equal portions (150 grams of honey or sugar per feeding). During the spring development, all societies were subjected to the same apitechnical measures: unfolding the honey, rotating the frames, expanding the brood, adding honeycomb bases, etc. In order to determine the real impact of the applied measures, the ratio between the values obtained at the end of the experiment and at the initial state of the companies was calculated. This was done because the companies at the beginning of the experiment differed in certain characteristics.
All traits were statistically processed by the method of one way analysis of ANOVA variance according to a completely random plan. The data program STATISTICA - 7.1 was used for data processing (StatSoft, 2006). Differences between mean treatment values were tested by LSD test at the significance level of 95% ($p<0.05$).

Results and discussion

At the beginning of the experiment, all societies had an average of 4.41 frames with bees in LR and 5,014 frames in DB hives (Table 1). The most bees in LR hives had societies in control treatment, and the least in forsapine treatments. The same situation was repeated in DB hives.

The area with the brood in the experimental societies at the beginning of the experiment was relatively uniform in both the LR and the DB hives. The number of bees was somewhat smaller, and the area with brood was slightly larger compared to the long-term average in this area (Jevtić et al., 2012).
The stock of honey in LR companies averaged 2.8 frames, the most in companies stimulated with syrup, and the least in companies with cakes. In the DB hives, the companies had an average of 2.5 frames with honey, the most in the companies stimulated with cakes, and the least in the companies stimulated with syrup. Honey stocks were lower, and pollen slightly higher than the multi-year average (Jevtić et al., 2012).
The selected societies were very uniform in strength (number of bees and brood area) and very low coefficients of variation (CV) for these traits speak in favor of this claim (Table 1). Somewhat more significant differences between the companies at the beginning of the experiment were found in food stocks. The coefficients of variation for these traits are in LR societies 15% - honey and 22% - pollen, and in LR societies 18% - honey and 15% - pollen. The CV for the area with honey and pollen was slightly higher in relation to the coefficients for the amount of bees and the area with brood also in DB hives.

Table 1. Strength and food supply of companies in LR and DB hives at the beginning of the experiment (unit is the number of hive frames possessed by bees, brood, honey and pollen)

hive	LR				DB			
treatment - features	bees	brood	honey	pollen	bees	brood	honey	pollen
Forsapine	4.23	2.47	2.90	0.80	4.50	2.80	2.12	1.02
honey	4.43	2.47	3.13	0.63	5.17	2.83	2.58	0.96
energy cake	4.50	2.57	2.33	0.77	4.83	2.67	3.15	1.12
syrup	4.27	2.47	3.20	0.43	5.10	2.60	1.92	0.88
control	4.60	2.47	2.37	0.70	5.47	2.53	2.52	1.32
average	**4.407**	**2.487**	**2.787**	**0.667**	**5.014**	**2.686**	**2.458**	**1.06**
standard deviation	0.155	0.045	0.413	0.147	0.367	0.161	0.475	0.175
CV	3.51	1.81	14.82	22.04	7.01	5.86	18.22	14.99

After feeding, the companies were re-examined and their strength and food supply were established (Table 2). In LR hives, companies occupied an average of 8.3 frames, the most those stimulated with sugar syrup, and the least companies stimulated with sugar cakes. An average of 8.9 frames with bees were recorded in DB hives the most in companies fed with honey, and the least in companies with sugar cakes, as it was the case with LR hives. The most broods at the end of the experiment were those fed with sugar syrup, regardless of which hive they were in. With the similar way of stimulating the companies Andjelković et al. (2011) obtained the best results for the amount of bees in societies fed oligovite (9.53) and honey (9.3 frames). In their research, the brood area was the largest in societies stimulated with oligovite and forsapine (5.03 frames).
The companies fed with honey and sugar syrup had the most honey at the end of the experiment in LR hives. In DB hives, companies fed on energy cake had the most honey, and companies fed on syrup had the least. Cake-fed societies had the most honey at the end of the experiment in the research by Andjelković et al. (2011). However, in their research, these societies had the most honey at the beginning of the experiment, which was not the case with our investigation. The amount of pollen was significantly higher in the societies located in DB hives, on average 1.5 frames, compared to 0.7 frames in LR hives.

The coefficient of variation shows that the societies differed in relation to the survey before the beginning of the trial. This time it is more than 10% for most features. The greatest variation between treatments was found in the area with pollen in LR hives and was 30.2%, while at the beginning of the experiment it was 22%. The largest difference was found in the area with litter in LR hives, which was only 1.8% at the beginning of the experiment, and 9.8% at the end. Stimulating supplementation in DB hives led to the unification of societies in the number of bees, because the CV was 7.1% at the beginning, and 4.6% at the end of the experiment (Table 2).

Table 2. Strength and food supply of companies in LR and DB hives at the end of the experiment

hive	LR				DB			
treatment - features	bees	brood	honey	pollen	bees	brood	honey	pollen
Forsapine	8.27	4.07	2.97	0.93	9.03	4.70	2.55	1.52
honey	9.17	4.70	3.10	0.53	9.33	4.93	3.05	1.66
energy cake	6.60	4.30	2.53	0.50	8.23	4.23	3.38	1.49
syrup	9.60	5.20	3.10	0.57	8.87	4.93	2.28	1.36
control	7.87	4.30	1.93	0.87	9.10	4.53	2.95	1.62
average	**8.300**	**4.513**	**2.727**	**0.673**	**8.912**	**4.664**	**2.842**	**1.530**
standard deviation	1.175	0.445	0.503	0.203	0.416	0.295	0.432	0.118
CV	14.16	9.86	18.45	30.16	4.56	6.19	14.43	11.46

In order to determine as precisely as possible how much the treatment affected the increase in strength and increase or decrease in food stocks, the ratio between the values obtained at the end of the examination and the values obtained at the beginning of the experiment was calculated (Table 3). Supplementation with syrup and honey had the greatest impact on the strength of societies in LR hives. Societies in control treatment (without stimulation) had the lowest intensity of development (quantity of bees and area with brood). Measures to stimulate development have almost not affected the food supply. The surface with honey decreased slightly (2.4%), and the surface with pollen slightly increased (4.4%). In societies in DB hives, the greatest impact on the amount of bees was exerted by forsapine supplementation (increase of 96%) compared to the control (64%). However, in these hives, forsapine supplementation had the least effect on the increase in brood area by only 65%, which is less than the control treatment (69%). The biggest influence on the increase in brood had the treatment with sugar syrup (86%) and the treatment with honey (82%).

In the society in DB hives, the amount of bees increased slightly less (75%) compared to the increase in LR hives (89%). The level of brood development was very similar in both types of hives. Unlike companies in LR hives, where the amount of honey decreased in companies in DB hives, it increased by 15%. An even greater increase was achieved in the area with pollen, which at the end of the experiment increased by 42% compared to the initial state. In these hives, the largest increase was obtained in the treatment with honey (65%) and sugar syrup (51%). Mirjanic et al. (2022) found that the amount of pollen collected in the spring ranged from 0.74 dm^2 (supplementation with acid-inverted syrup with the addition of brewer's yeast) to 1.39 dm^2 (enzyme-inverted syrup with the addition of brewer's yeast).

The resulting differences for the amount of bees in LR hives were statistically significant. Syrup-fed societies differed significantly from the societies fed with sugar cake. In DB hives, statistically significant differences occurred in the pollen area per society. The results obtained in the treatment with honey were statistically significantly different from the control treatment (Table 3).

Table 3. Analysis of variance for the relationship between the first and second review (Table 2 / Table 1) of the observed characteristics of the companies in LR and DB hives.

hive	LR				DB			
treatment - features	bees	brood	honey	pollen	bees	brood	honey	pollen
Forsapine	1.90 ^{ab}	1.65	1.02	1.16	1.96	1.65	1.19	1.44^{ab}
honey	2.07 ^{ab}	1.90	0.99	0.84	1.77	1.78	1.17	1.65^{a}
energy cake	1.47^{b}	1.67	1.09	0.65	1.68	1.68	1.07	1.30^{ab}
syrup	2.25^{a}	2.11	0.97	1.33	1.71	1.86	1.17	1.51^{ab}
control	1.71 ^{ab}	1.47	0.81	1.24	1.64	1.69	1.16	1.21^{b}
average	**1.890**	**1.760**	**0.976**	**1.044**	**1.752**	**1.728**	**1.152**	**1.422**

Conclusion

After stimulating the companies for faster spring development in LR and DB hives, several conclusions can be drawn:

Stimulating feeding in both types of hives led to an increase in most of the observed traits.

Sugar syrup and honey supplementation had the greatest impact on the increase in the number of bees and the area with brood. Societies stimulated with sugar syrup gave 125% more bees and 111% more honey compared to the initial state.

The stock of food in LR hives did not change significantly during the experiment, regardless of what the companies were fed.

Forsapin treatment had the greatest impact on the number of bees (96% increase) and the area with honey (DB%) on the societies in DB hives.

The greatest impact on the brood had the treatment with sugar syrup (increase of 86%), and on the surface with pollen treatment with honey (increase of 65%).

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HUMAN-ANIMAL RELATIONSHIP AS A FACTOR OF CALF WELFARE IN THE FIRST MONTH OF LIFE

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Abstract

Contact with a breeder is extremely important for the welfare of calves in the first month of life. In the intensive way of raising cattle, it is increasingly difficult to establish a good relationship between breeders and animals. The authors defined 12 criteria for assessing animal welfare, which they classify into four groups, one of which is good behaviour in terms of social and other forms of behaviour and a good human-animal relationship. This implies the absence of fear because fear is an important animal welfare problem. The attitude of farmers towards calves in the first month of life was examined on two farms with an intensive production system. The relationship of humanstocalves was assessed using the test of approach and touch. Farmers competence assessments on the surveyed farms were satisfactory. It is characteristic of both farms that breeders who handle calves do not have a formal education in the field in which they work. Accordingly, their knowledge and skills are based on many years of work experience.The approach and touch test indicated a positive relationship between breeders and calves. The largest number of calves allowed approaching 1 or 2 steps, and a significant number also allowed touch, while a negligible number of calves avoided eye contact, as the most unfavourable type of contact.

Key words:*human-animal relationship, calf welfare, approach and touch test.*

Introduction

A good human-animal relationship is essential for the welfare of calves. This is very difficult to achieve and, at the same time, meet all the conditions of the intensive production system. To improve the human-animal relationship, the behaviour of breeders must change comprehensively. The personal attitude of the individual is not enough (Burton et al., 2012). This fact is confirmed by the conclusions of a group of authors (Elingsen et al., 2014) who evaluated the attitude of farmers towards calves on farms in Australia. The behaviour of the workers caused a compatible reaction in the calves. The calves reacted to the positive behaviour with positive behaviour. Calves with more contact with humans, according to Lensink et al. (2001), are less afraid of people and do not withdraw in contact with strangers, they have more confidence, easier to "manage" and are less stressed than calves who are in minimal contact with people. The results obtained by Lürzel et al (2015) are in agreement with the present study. Female calves who were pet for 42 minutes longer than the usual treatment and who were "spoken to" manifested less avoiding behaviour to people after treatment, had a less negative reaction to weaning, higher growth, and later higher milk production. However, Schütz et al. (2012) find that pet calves respond better to humans and do not avoid contact with humans, but

with no reliable evidence that a positive relationship with humans also influences the provocation of positive emotions in calves. Depending on the treatment, they reacted positively or negatively to known persons, but they had uniform reactions to strangers.
The key factor in the success of raising calves is the workers in charge of their nutrition and care. An unprofessional and incompetent workforce can jeopardize everything that is achieved by applying the latest technology. Also, the professional and competent activity of employees can compensate for some technological shortcomings. The attitude of workers towards calves affects the overall assessment of the quality of welfare of calves. Broom (2004) states that neglect, calculated, accidental or ignorant, is a possible cause of animal welfare problems. Hristov et al. (2011) have determined that the welfare of calves is endangered by the delayed reaction of workers, especially in the medical treatment of calves and the necessary dietary corrections.
An assessment of the relationship between farmers and calves can be obtained by performing a proximity and contact test. In the worst case, calves avoid eye contact. At best, calves also allow physical contact. Meagher et al. (2016) present results on the reliability of the proximity and contact test. They believe that a positive test is not reliable enough (0.22), but that the frequency of negative reactions is moderately reliable (0.55). The proximity and contact test, in addition to analysing the human-animal relationship, is also used to determine early onthe incidence of diseases in calves. Diseased calves are less prone to exploratory behaviour and less likely to approach strangers (Cramer et al., 2015).

Material and method

The manifestation of the basic physiological forms of behaviour of Holstein Friesian calves in the intensive system of production, in the period from birth to 30 days of age, was observed on two farms (marked as farm A and farm B). The established rearing technology differed to some extent. Calves were separated from their mothers immediately after birth. For the first 7 days of life, they were housed in a maternity ward, tied to a bed on farm A and in an individual box, on farm B. From days 8 to 30, calves were housed in group boxes.
The attitude of humans towards calves was assessed by the proximity and contact test on a scale: avoidance of eye contact; allowing visual contact but avoiding proximity; allowing approaching by 1 step; allowing approaching by 2 steps, but without contact; possible contact.
Considering that the competence of farmers is one of the most important factors and impacts on the welfare of calves and the manifestation of positive forms of behaviour, the competence of workers who were in direct contact with calves was assessed on the mentioned farms. Knowledge, skills, abilities, level of education, work experience, training and coaching of workers were analysed. But an important part of the farmers assessment was also their attitudes, reaction time and making unnecessary noise.

Results and discussion

Raising offspring is of key importance for the entire livestock production. Due to its sensitivity, this category of animals requires extreme care and attention. Therefore, the attitude of farmers towards calves in the first month of life has great consequences on the quality of welfare of calves and the success of production in cattle breeding.

Proximity and contact test

The results of the proximity and contact test on farms A and B at different ages of calves, by months and seasons, during the study period are given in the following table.

Table 1: Results of the proximity and contact test

Test of proximity and contact	**Farm A**	**Farm B**
Avoiding contact	40	18
Visual contact	175	131
Approaching by 1 step	849	648
Approaching by 2 steps	1165	1310
Allowing contact	751	753

Analysing the presented data, it can be seen that the situation on farms A and B is similar to each other and is relatively favourable on both. The majority of calves allowed approaching by 1 or 2 steps, and a significant number also allowed contact, while a negligible number of calves avoided eye contact, as the most unfavourable type of contact. This indicates a positive relationship between breeders and calves, and an improvement can be expected in reducing the number of calves that do not allow approach and contact and increasing the number of those that allow contact. Every contact, physical and visual, was avoided by the small number of calves on both farms during the research, 40 and 18on farms Aand B, respectively. Also, a small number of calves allowed only eye contact. The number of calves allowing one-step proximity on both farms is 849 and 648, on farms Aand B, respectively, while the majority of calves allowed 2-step proximity (1165 and 1310, on farms and B, respectively). The number of calves that allowed workers to touch and pet them was very similar on both farms (751 and 753).
A significant contribution to the quality of welfare of calves on farms is given by the relationship between people who come into direct contact with calves. This relationship must be good and friendly because calves "reciprocate" in a way similar to the way people treat them. Friendly, kind, patient behaviour of people leads to lively and positive behaviour of calves, friendly and full of trust. On the other hand, nervous and aggressive behaviour leads to fear, tension, and anxiety in calves (Lensink et al., 2001; Lürzel et al., 2015).

Competencies of farmers

The choice of workers to be engaged in raising offspring is the result of careful monitoring of the quality of their work over a long period. It is necessary for the workers dealing with rearing offspring to have a certain level of knowledge and skills necessary for working with calves, to be educated and trained, and to be ready to react timely to some extraordinary events. Above all, they must have a positive attitude towards the animals they take care of and be conscientious and responsible. Average grades (from 1 to 5, where 1 is the worst and 5 isthe best score) for the expertise and competence of workers in maternity and calf nurseries, are given in Table 2.

Table 2: Average assessment of the competence of workers on farms A and B

Competencies of farmers	Farm A	Farm B
Knowledge	3.33	3.50
Skills	3.17	4.00
Capabilities	4.17	4.17
Attitudes	2.83	3.83
Level of education	3.00	3.00
Work experience	5.00	4.17
Training	4.67	4.33
Coaching	3.50	3.50
Response time	3.83	4.00
Making unnecessary noise	2.83	4.00
Average score	3.63	3.85

The competencies of the workers on the surveyed farms are satisfactory, but with a lot of room for improvement. On farm B, the overall score was slightly higher (3.85) compared to farm A (3.63). It is characteristic of both farms that the workers donot have a formal education in the field in which they work. Accordingly, their knowledge and skills are based on many years of work experience. In addition, there was no special training for farmers on the farms, but the necessary knowledge and skills were acquired along with the work, and most often the "coaches" were farmers with the most experience. That is why it is necessary to improve their knowledge and skills. At the same time, the personal attitude of farmers towards animals needs to be improved. This is especially important for Farm A. Although the workers on this farm have little more experience and training, the better assessment of the workers on Farm B has been influenced by personal attitudes and commitment. The obtained results are in accordance with the research of numerous authors (Lensink et al., 2001; Burton et al., 2012; Elingsen et al., 2014; Lürzel et al., 2015).

The overall assessment of the quality of welfare of calves in the first month of life includes an assessment of the competence and attitude of workers, which is shown in Table 3.

Table 3: Assessment of welfare indicators (human-dependent items are coloured)

Indicator	Farm A	Farm B
Assessment of planning, organization and implementation of welfare protection	1.00 - 1	1.00 - 1
Employee Welfare Awareness Assessment *	2.75 - 3	3.00 - 3
Competences of employees regarding welfare protection*	2.78 - 3	3.22 - 3
Relation of breeders to the needs of animals *	2.67 - 3	3.00 – 3
Assessment of monitoring and inspection of animals and equipment *	4.62 - 5	4.62 – 5
Treatment of animals *	2.67 - 3	2.67 – 3
Feeding and watering*	3.73 - 4	3.73 – 4
Housing conditions	2.70 - 3	3.00 – 3
Microclimatic conditions	2.25 - 2	2.12 – 2

Hygienic conditions in the facility *	2.67 - 3	2.55 – 3
Animal body hygiene and care *	3.00 - 3	3.00 – 3
Reproduction	3.00 - 3	3.00 – 3
Productivity	3.33 - 3	3.22 – 3
Behaviour	3.45 - 4	3.18 – 3
Health	3.33 - 3	3.33 – 3
Average score	2.93 - 3	2.98 -3

The assessment of biosecurity indicators was higher on farm B. One of the reasons is the better attitude of workers toward the needs of animals, which agrees with the statements of Hristov et al. (2011).
Employees should be trained in the importance of all aspects of welfare and biosecurity on farms, and certain written procedures and protocols should be adopted accordingly. Raising the awareness of breeders about the importance of respecting the principles of welfare would also improve their treatment of animals, care for hygienic conditions in facilities and hygiene of animal bodies.

Conclusion

The proximity and contact test in calves on farms A and B showed that calves did not show many negative feelings such as fear or threat during the study period. Only 40 calves on farm A and 24 calves on farm B avoided any contact.
Employed farmers on both observed farms did not have formal education in the field of animal husbandry, nor organized education and training. They acquired the necessary knowledge and skills by working with more experienced colleagues. The response time of farmers to any problems related to the health, nutrition and care of calves was satisfactory, slightly better on farm B than on farm A. On farm B, more positive attitudes of employees towards calves were recorded.
It is recommended that continuous training of breeders be carried out to improve the quality of work and the relationship with calves. This has far-reaching consequences for the health and emotional state and behaviour of calves. Raising the awareness of breeders about the importance of respecting the principle of welfare would also improve their treatment of animals, care for hygienic conditions in facilities and hygiene of animal bodies.

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NUTRITIVE VALUE AND THE POSSIBILITY OF USING APPLE POMACE IN THE NUTRITION OF DAIRY COWS

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Abstract

The aim of this study is to analyze the nutritional value of apple pomace and to review the possibility of its use as a feed in the diet of dairy cows. Apple pomace is a by-product of apple juice production, making up approximately 20-30% of processed apples. Several million tonnes of this by-product are generated annually in the world. Despite increasingly strict legal regulations in biodegradable waste management, large amounts of apple pomace are still disposed of in landfills, posing a serious environmental issue and requiring new ways of its treatment or further use. Apple pomace is a rich source of carbohydrates - dietary fiber and sugars, also containing lower amounts of proteins and fat, minerals and vitamins, which makes it a potential feedstuff in the diet of farm animals. It is considered to be a valuable energy feed for ruminants and can replace conventional feedstuffs such as maize and other cereals. Due to its high moisture content, fresh apple pomace is susceptible to spoilage, thus, it has been mostly used in dry or ensiled form when it comes to the nutrition of dairy cows. In the conditions of unstable supply and prices of conventional feedstuffs, introduction of alternative feedstuffs is gaining importance. Therefore, further study of apple pomace, with a focus on its content and form (fresh, dry or ensiled) is needed, in order to examine the impact on production performance, product quality and the health of dairy cows and other species and categories of farm animals.

Keywords: *Apple pomace, Nutritive value, Animal feeding, Dairy cattle.*

Introduction

Reduction of food waste and byproducts in the food industry and agriculture is one of the main challenges in modern societies and it is part of the concept of circular economy that implies transition from linear systems – “make, use and dispose” to circular ones – “make, use and recycle”, in order to become more sustainable (Elisha, 2020). Byproducts from the food industry can be of different origin and sources and they have been studied by a growing number of researchers from the aspect of their nutritive value, potential use and environmentally-friendly disposal.

Considerable amounts of byproducts from industrial production are treated as waste, and as such pose an environmental, but also an economic issue due to wasting of raw material that still has a considerable use-value. The highest amounts of byproducts and waste material come from the fruit and vegetable processing industry, amounting to 50% of the weight of the initial raw material (Ndubuisi Ezejiofor *et al.*, 2014; Baiano, 2014).

Apple pomace is a byproduct of apple juice production, generated in the stage of pressing apples and extracting the juice, and it makes 20-30% of the weight of the processed apples (Kosseva,

2011). Apple pomace is a solid residue consisting of apple skin, stems, seed pockets, seeds and flesh (Vendruscolo *et al.,* 2008; Madrera *et al.,* 2015). Global production of apple pomace is estimated to be several million tonnes per year. Fresh apple pomace is characterized by high content of moisture (75–85%) and sugars, low pH value (3-4), thus being prone to microbiological contamination, uncontrolled fermentation and spoiling (Wang *et al.,* 2007; Bhushan and Gupta, 2013). Apple pomace is mostly used for pectin recovery, but large amounts are still considered as a waste and are directly disposed to soil in landfills (Reis, 2012). Such inappropriate management of this biodegradable waste, with high biochemical and chemical oxygen demand, causes ecological problems in regards of land, surface and groundwater pollution (Bhushan *et al.,* 2008; Gassara *et al.,* 2011). Therefore, special attention should be put on proper disposal and finding ways to make use of this organic material.
In order to define and describe apple pomace as a perspective raw material in different processes, substantial research has been focused on determination of chemical composition and nutritive value of apple pomace. Apple pomace is rich in dietary fiber and sugars and it also contains low amounts of protein and fat, minerals and vitamins (Gullón *et al.,* 2007; Vendruscolo *et al., 2008*; Sato *et al.,* 2010; Gabriel *et al.,* 2013). Polyphenolic compounds add special quality to apple pomace, being concentrated in apple skin, while only a small amount of them can be found in the juice (Guyot *et al*., 2003; Tsao *et al.*, 2005). In recent decades numerous studies have been conducted with the aim to obtain multiple value-added products from apple pomace. It was found that apple pomace can be used as a raw material for extraction of functional ingredients, such as phenolic compounds and fiber, as well as a substrate in fermentation processes for production of enzymes, organic acids, protein-enriched feeds, natural antioxidants and edible fibers (Vendruscolo *et al., 2008*).
Regarding the nutritive value of apple pomace, one of the strategies for its sustainable utilization is to use it as a feedstuff, especially nowadays when volatile prices and supply of animal feedstuffs in many parts of the word have led to a growing interest in using alternative feeds in animal nutrition (Besharati and Taghizadeh, 2008; Beigh *et al*., 2015). Research during the last decades has been focused on examining the possibility of using certain forms of apple pomace - raw, ensiled and dry, and its content in animal rations, both in roughages and concentrates. Moreover, formulating a proper diet that meets nutritive requirements of a certain animal species/category has been a subject-matter of a large number of research, considering that apple pomace is rich in sugars and fiber and low in protein and fat, containing also anti-nutritive compounds (Joshi and Attri, 2006; Zhong-Tao *et al.,* 2009). Research on the effect of apple pomace feeding was performed on different species and categories of farm animals, i.e. ruminants, namely: dairy cows (Ghoreishi *et al*., 2007; Tiwari *et al*., 2008), sheep, goats and lambs (Alibes *et al*., 1984; Ahn *et al*., 2002; Taasoli and Kafilzadeh, 2008), as well as non-ruminants, namely: pigs (Gutzwiller *et al*., 2007; Sehm *et al*., 2007; Pieszka *et al*., 2017) and poultry (Matoo *et al.*, 2001; Zafar *et al.*, 2005). The aim of this paper is to point out the nutritive value of apple pomace and its perspective use as a feedstuff in the diet of dairy cows.

Nutritive value of apple pomace

Apple pomace is a rich source of complex (insoluble) carbohydrates, i.e. dietary fibers - cellulose, hemicellulose, lignin and pectin, simple carbohydrates, i.e. sugars - fructose and glucose, as well as sucrose. It contains small amounts of protein and fat, minerals and vitamins (Gullón *et al*., 2007; Vendruscolo *et al*. 2008; Sato *et al*., 2010; Gabriel *et al*. 2013).

From the standpoint of energy content in dairy cows' feeding, metabolic energy (ME) of apple pomace amounts to 80% of the metabolic energy of maize silage (NRC, 2001). Furthermore, apple pomace has similar or slightly higher energetic value (ME 7,79 – 10.73 MJ/kg dry matter (DM)) as compared to other feedstuffs commonly used in cattle feeding, such as grass hay (ME 7.78 MJ/kg DM), grass silage (ME 7.78 MJ/kg DM), grass – legume hay (ME 8.60 MJ/kg DM) and grass – legume silage (ME 8.20 MJ/kg DM) and alfalfa meal (ME 8.20 MJ/kg DM) (NRC, 2001).
The moisture content of apple pomace ranges from 70 to 80%. (Gullon *et al*., 2007; Bhushan *et al*., 2008; Gabriel *et al.*, 2013). Wet apple pomace is susceptible to rapid spoilage and the transport, manipulation and storage of such bulky material is difficult (Bhushan *et al*., 2008; Yan, 2012), thus, it is often dehydrated for the purpose of stabilization and using it over an extended period (Constenla *et al*., 2002). The examination of the nutritional value of apple pomace has been the subject of the research by a number of authors. Range of the values of certain nutrition parameters is given in Table 1.

Table 1. Chemical composition and nutrition value of apple pomace

Ingredient	Content, (DM basis)
Crude protein, %	1.3 - 7.7[a, b, c, d, e, f]
Crude fat, %	1.8 - 4.7[e, f, g, h]
Crude fibre, %	18.2 – 25.9[i, j, k, l]
Crude ash, %	1.48 - 2.6[b, g, m, n]
NDF (Neutral Detergent Fiber), %	35.3 – 61.2[g, o, p, q, r]
ADF(Acid Detergent Fiber), %	28.0 – 46.7 [g, o, p, q, r]
Lignin (Acid Detergent Lignin), %	15.4[b]
Pectin, %	3.4 – 16.8[l, s, t]
Sugars, %	31.95[z] / 38.05[u] / 44,42[v]
Glucose, %	13,3 - 25,1 [n, u, v, w]
Fructose, %	6.9 – 21.1 [n, u, v, w]
Sucrose, %	3.6 – 18.6 [n, u, v, w]
K, %	0.24 – 0.73[b, m, q]
Ca, %	0.01 – 0.20 [b, m, q]
P, %	0.07 – 0.17 [b, m, q]
Mg, %	0.04 – 0.09 [b, m, q]
Na, %	0.002 – 0.04 [b, m, q]
Fe, mg/kg	31.80 - 185 [b, m, x]
Mn, mg/kg	3.96 – 17.0 [b, m, x]
Zn, mg/kg	6.90 – 15.0 [b, m, x]
ME, MJ/kg DM	7,79 – 10.73 [b, g, o, y]

[a]Masoodi & Chauhan (1998), [b]NRC(2001); [c]Pirmohammadi et al. (2006), [d]Sudha et al. (2007), [e]Abdollahzadeh et al. (2010), [f]Reis et al. (2012), [g]Mirzaei-Aghsaghali et al. (2011); [h]Madrera et al. (2017); [i]Ensminger et al. (1990), [j]Ayhan et al. (2009), [k]Gazalli et al. (2013) ; [l]Pieszka et al. (2017); [m]Villas-Bôas et al. (2003); [n]Gullón et al. (2007); [o]Maghsoud et al. (2008), [p]Taasoli & Kafilzadeh (2008), [q]Pieszka et al. (2015); [r]Juskiewicz et al. (2016); [s]Marcon et al. (2005); [t]Garna et al. (2007); [u]Chandel et al. (2016); [v]Sato et al. (2010); [w]Queji et al. (2010); [x]Bhushan et al. (2008); [y]Givens and Barber (1987); [z]Maslovarić et al. (2017)

In terms of crude protein and fat content, apple pomace is similar to citrus pulp, containing 6 to 7% crude protein and 2 to 5% fat, on a DM basis (NRC, 2001; Lima *et al*., 2014; Peixoto *et al*., 2015). On the other hand, other fruit processing by-products, such as grape and tomato pomace, contain more crude protein than apple pomace, i.e. it ranges from 9.4 to 15.8% in grape pomace (Abarghuei *et al*., 2010; Voicu *et al*., 2014; Winkler *et al*., 2015; Foiklang *et al*., 2016) and from 19.3 to 22.6% in tomato pomace (DM basis) (Jafari *et al*., 2006; Amirkolaie *et al*., 2015; Saemi *et al*., 2012). Furthermore, fat content in tomato pomace is higher (4,0-10,7%) compared to apple pomace (Jafari et al., 2006, Amirkolaie et al., 2015; Saemi et al. (2012).
High value of crude fiber content in the apple pomace, ranging from 18.2 to 25.9% (DM basis) is a characteristic of bulky feedstuffs that contain more than 18% crude fiber (Đorđević *et al*., 2009). In terms of the fiber content, apple pomace could be compared to the class III sunflower meal, which contains up to 21% crude fiber (Đoređević and Dinić, 2007). High raw fiber content can be also found in grape pomace, which, according to the data of Voicu *et al*. (2014) amounts to 29.2%, as well as in tomato pomace - 36% (Saemi *et al*., 2012).
In terms of crude fiber fractions’ contents - NDF and ADF, apple pomace can be compared with commonly used feedstuffs in animal nutrition, such as maize silage, sugar beet pulp, dry brewer’s spent grain, alfalfa meal, barley malt sprouts, hay, as well as grass and grass-leguminous mixtures’ silage (NRC, 2001). On the other hand, the content of lignin in apple pomace is significantly higher than in the aforementioned feedstuffs. High values of crude fiber fractions are specific to other by-products of fruit and vegetable processing, such as grape pomace, tomato pulp and almond hulls (Abarghuei *et al*., 2010; Moate *et al*., 2014; Winkler *et al*., 2015 and Foiklang *et al*., 2016).
Fermentable sugars are among main components of apple pomace, making up over 30% of the apple pomace dry matter (Table 1). According to the results presented by Sato *et al.* (2010), glucose, fructose and sucrose make up about 80% of the total soluble dry matter of the apple pomace. Research performed by several authors revealed fructose to be the main component of sugars in apple pomace, followed by glucose, while sucrose content was the lowest (Gullón *et al.*, 2007; Gabriel *et al.*, 2013; Maslovarić *et al.*, 2017). The sugar content in dry apple pomace is significantly higher as compared to the most commonly used conventional energy feedstuffs, such as corn (1.91%), rye (1.92%) and oats (1.77%) (Menkovska and Cilev, 2001).

Apple pomace utilisation feeding dairy cows’ nutrition

Given that apple pomace is bulky and highly perishable, its use in animal nutrition is seasonal and limited to the local level, i.e. to the vicinity of apple processing factories (Dhillon *et al*., 2013). Therefore, apple pomace is most often used in animal nutrition either ensiled or dried (Mirzaei - Aghsaghali *et al.*, 2011). Fresh, ensiled or dried apple pomace is mainly considered as an energy feed for ruminants and can partially replace conventional feedstuffs in ruminant diet, such as maize and other cereals, as well as maize silage (Beigh *et al*., 2015).
In the research conducted by Edwards and Parker (1995), fresh apple pomace was used as a supplement to pasture for Fresian-Jersey crossbred dairy cows in late lactation. Three experimental diets were tested – I: 7 kg DM grass silage, II: 3 kg DM grass silage + 4 kg DM apple pomace and III: 3 kg DM grass silage + 3 kg DM apple pomace + 1 kg DM protein supplement. Results of the given research have shown that the partial replacement of grass silage by fresh apple pomace led to higher DM intake (DMI), increased milk yield (by 20-23%) milk fat and protein and milk solids’ content, as compared to the grass silage diet. Improvements in

production performance and milk composition were attributed to higher DMI, suggesting that apple pomace in the diet contributed to improved palatability and utilization of grass silage.
Ensiling is frequently used method of preserving apple pomace. Since apple pomace has a high water content, it is necessary to add dry material as an absorbent (such as cereal straw) in the process of ensiling, in order to prevent the loss of nutrients due to effluent draining from the silage (Alibes *et al.*, 1984; Antov *et al.*, 2004). Research performed by Pirmohammadi *et al.* (2006) has shown that good quality silage was obtained by ensiling apple pomace with 10% wheat straw and 0.5% urea (in relation to apple pomace amount). As stated by Antov *et al.* (2004), very good silage quality was obtained by ensiling 25% apple pomace, 50% sugar beet pulp and 25% brewer’s spent grains. The aforementioned authors also suggested that apple pomace can be successfully ensiled with various amounts of fresh sugar beet pulp, as well as in combinations with up to 35% dry corn stalks and up to 35% chopped oat straw. On the other hand, due to the high amount of free sugars, the fermentation into ethanol occurs in the apple pomace silage and in the rumen, which can cause intoxication of cows, i.e., a condition known as alcoholaemia (Alibes *et al*., 1984; Hall et al., 1998; Arthington *et al*., 2002; Rodrigues *et al*., 2008). According to the research by Rodrigues *et al.* 2008, such condition can be prevented by adding fibrous materials into the silage, i.e. *in vitro* incubations with rumen fluid have shown that mixtures containing from 15% up to 30 % straw were the most appropriate for ruminants’ feeding. In the research by Ghoreishi *et al.* (2007) ensiled apple pomace was used in total mixed rations for lactating multiparous Holstein cows, as a partial replacement of alfaalfa and barley. Treatments were 0, 15 and 30% ensiled apple pomace (DM basis) in the total mixed ration. According to their results, there were no statistically significant differences between treatments in milk yield, milk fat and milk protein contents, but 3.5 and 4% fat corrected milk (FCM) yield were significantly lower for the treatments that included ensiled apple pomace. On the other hand, feed conversion ratio (FCR) was significantly lower, while feed efficiency (FE) was higher, for the treatment with 15% ensiled apple pomace. Therefore, it was suggested that using 15% ensiled apple pomace in the total mixed ration, on a DM basis, may be the most economically viable, but further research on this matter is required. Results presented by Anrique and Dossow (2003) have shown that ensiled apple pomace can supplement from 20 to 30% of the ration (as fed basis) of the Black Friesian early lactating dairy cows, in addition to the base roughage. Such diet improved DMI, which resulted in: better body weight status, increased milk yield - by 9% for standardized and 5.9% for non-standardized milk production. Also, milk fat and protein contents were increased in cows fed 30% apple pomace silage.
The results of several studies have shown that, in addition to ensiled form, dry apple pomace can also be used in the diet of dairy cows. Tiwari *et al*. (2008) reported that feeding concentrated rations with 12% dry apple pomace on a DM basis, substituting 34% of maize, to the crossbred cows Red Sindhi and Jersey, had no adverse effect on the quantity and quality of the milk. In the research performed by Steyn *et al.*, 2017, dry apple pomace was used as an energy source, i.e. maize substitution, for Jersey cows grazing ryegrass pasture. Four treatments were applied, with 0%, 25, 50 and 75% of dried apple pomace in the concentrate (DM basis), replacing 0, 33.3, 66.6 and 100% of maize, respectively. Results of this study have shown that the total DMI and DMI as % of the body weight were lower for cows fed concentrates containing dry apple pomace. Dietary treatment had no effect on rumen metabolism parameters. Milk yield decreased as the level of dry apple pomace increased, but it was not statistically significant. Milk crude protein and fat content were the highest in the treatment with 25% dry apple pomace in the concentrate. On the other hand, 4% fat corrected milk yield decreased with increased dry apple pomace

inclusion in the rations. Replacing maize with dry apple pomace can be successful solution for cows grazing ryegrass pasture, however, the potential economic impact of such diet should not be neglected.

Conclusions

The use of apple pomace, as well as other by-products of food industry, in animal nutrition is one of the strategies for utilizing and effectively managing such biodegradable waste, as well as creating its added value. This is also the way to overcome the problem of feedstuffs’ shortage in certain parts of the world, especially in developing countries.

Apple pomace can be described as low-protein and fat and high-fiber and sugars feed. It is an energy feed and a source of digestible fibers for ruminants and proper alternative for roughage feeds. ME of apple pomace amounts to 80% of the ME of the maize silage and it is similar to the ME of the grass hay, grass silage, grass – legume hay and grass – legume silage, as well as of the alfalfa meal. Due to high moisture content, it is recommended to preserve apple pomace by ensiling or drying. Drying of apple pomace is a viable option for the feed industry where it could be used for producing concentrated feed, but it is an expensive process, therefore ensiling is more feasible on family farms. Fresh or ensiled apple pomace can partially substitute grass and maize silage, as well as alfaalfa and cereals in the dairy cows’ rations. Addition of dry fibrous material to apple pomace in the ensiling process, such as cereal straw, dry sugar beet pulp, dry corn stalks, is recommended since it prevents effluent drainage from the silage and loss of valuable nutrients. Ensiled apple pomace should be introduced into dairy cows’ diets with caution, in accordance with its ethanol and organic acid concentration, in order to maintain rumen health and pH. Ethanol concentration in apple pomace silage can be reduced by the addition from 15 up to 30% straw, as well as silage additives that limit fermentation of sugars into ethanol.

The level of inclusion of ensiled or dry apple pomace in the dairy cows’ diet, depends on the breed of cows, the stage of lactation, as well as on the composition of the ration and on the roughage source. The results of previous research indicate that up to 25% dry apple pomace can be included in the concentrated part of the meal (substituting maize), while apple pomace silage can replace 15% of the total mixed ration (DM basis) and 20 to 30% of the ration (as fed basis), in addition to the base roughage source.

In order to better define apple pomace as a feedstuff, further research should be aimed at determining the optimal proportions of certain forms of apple pomace in dairy cows’ rations, in terms of production properties and health safety. Special attention should be paid to the profitability analysis of using this feed and its economic effects in milk production. Furthermore, the impact of feeding apple pomace on the quality and sensory properties of dairy products should also be explored.

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INFLUENCE OF PROTEOLYSIS AND LIPOLYSIS IN SILAGE ON MILK PRODUCTION AND MILK FAT COMPOSITION IN RUMINANTS

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Abstract

The overview of domestic and foreign research was given in the paper about the influence of proteolytic and lipolytic changes in silage on milk production and milk fat composition. During the preparation (wilting) of plant material for ensiling, lipolysis and oxidation of fatty acids occur, which hurts the content of polyunsaturated fatty acids (PUFA) in silage and milk fat. The exception is corn silage, which is prepared without wilting. However, its use leads to the biohydrogenation of linoleic acid to *trans*-10, *cis*-12 CLA, which is a very potent inhibitor of milk fat synthesis. Lactic acid bacteria decompose carbohydrates during fermentation in ensiled material, resulting in lactic, acetic, and butyric acids. These acids are used for energy production in ruminants, but due to their volatility, they affect the relative changes in the chemical composition of silage. By the influence of enzymes from plant cells of ensiled material or butyric acid bacteria, intensive proteolysis occurs, whose end products are peptides, free amino acids, and ammonia. These products lead to a significant increase in the fraction of degradable proteins in some silages (alfalfa), which hurts the utilization of total meal protein and production. Conversely, red clover is a more suitable material for ensiling due to the partial reduction of lipolysis and proteolysis by polyphenol oxidase. Using modern inoculants, fermentation is intensified, fermentable carbohydrates are used to the maximum, and the aerobic stability of silage is increased. During the fermentation of the ensiled mass and when using some strains of lactic acid bacteria as inoculants (*Lactobacillus Plantarum* AKU 1009a), there is a biohydrogenation of some PUFAs and an increase in the content of conjugated fatty acids in the silage itself.

Keywords: *silage, lipolysis, proteolysis, milk, fatty acids*.

Introduction

During the preservation of nutrients by ensiling, the transformation of some organic nutrients occurs, which leads to an absolute or relative change in their ratio, and a decrease in the nutritional value of silage (Đorđević et al., 2020, 2022). In the first days and weeks after ensiling, lactic acid bacteria (from natural microflora or inoculant) use fermentable carbohydrates and produce lactic acid and acetic acid, methyl alcohol, ethyl alcohol, and carbon dioxide (Đorđević et al., 2018, 2019). These acids and alcohols are used in the body of ruminants as a source of energy, which means that they do not represent a significant loss in the nutritional value of silage. However, their volatility during drying of the sample for laboratory analysis leads to significant relative changes in the content of certain substances in silage. Conversely, non-structural carbohydrates are not subject to change, except in the case of the use of cellulosic additives (in the composition of some modern inoculants). Compared to carbohydrates, changes in protein (proteolysis) are more important for ruminant nutrition when ensiling legumes (alfalfa, fudder peas, Common Vetch) (Tao et al., 2020). These changes lead to a significant change in the ratio of degradable and non-degradable proteins in the diet of ruminants and affect negatively their use and cow production (Đorđević et al., 2004, 2012). Recently, lipid changes (lipolysis) have been significant, although forage contains a small percentage of real fats. However, these changes lead to a reduction in the content of conjugated fatty acids, which are found in milk fat and are important for human health (Liu et al., 2018; Đorđević et al., 2021a,b).

Proteolysis in silage

An important (and cheap) source of protein for cows in Serbia is hay and silage (haylage) of legumes, primarily alfalfa. However, legume silages are characterized by intense proteolytic processes that can significantly reduce the utilization of protein compared to hay, and even fresh food, from the same plants. In the fresh plants, 75-90% of the total amount of nitrogen is in the form of real proteins, while in silage it is only 30-50% (Slottner and Bertilsson, 2006). The second part is peptides, free amino acids, and ammonia, which are less used, with higher losses in the rumen. Besides, the low efficiency of nitrogen use in ruminants from silage and urinary excretion is an environmental problem (Herremans et al., 2019). Due to similar problems, modern norms and systems for ruminant nutrition (for example CNCPS system-Fox et al., 2003) require detailed knowledge of the content of protein fractions in meals, to maximize microbial protein synthesis and minimal ammonia losses (Grubić et al., 2014).

Alfalfa is the most important legume for forage in Serbia, due to its exceptional biological and production properties. However, alfalfa is characterized by the highest degree of solubility of nitrogenous substances in ensiling, compared to other legumes grown for animal feed (Tao et al., 2019). Protein solubility is positively correlated with degradability, which can significantly reduce their utilization or lead to health and reproductive problems (Đorđević et al., 2002).

Some other leguminous (sainfoin, lespedeza, and *Lotus pedunculatus*) are distinguished by a lower degree of protein solubility due to the higher presence of condensed tannins (Broderick, 1995). According to the same author, red clover does not contain condensed tannins, but its proteins have low solubility. The lesser degree of proteolysis in red clover silages was explained by the presence of soluble enzyme polyphenol-oxidase, which in presence of oxygen reacts with O-diphenol creating very reactive O-quinone, which creates polymers with other molecules such as proteins (Getachew et al., 2009; Graber, 2009). When ensiling a mixture of alfalfa and red clover, with increasing proportion of red clover, Le et al. (2018) found a significant decrease in the proportion of non protein nitrogen, free amino acid nitrogen and ammonia nitrogen (as indicators of proteolysis). The significant decrease in total nitrogen content is a consequence of the loss of volatile nitrogenous substances, primarily ammonia (Table 1). Herremans et al. (2019) added tannins (from oak or chestnut) when ensiling alfalfa and determined a significantly lower degree of proteolysis compared to the control. However, the authors caution that excessive concentration in tannins can become an antinutritional factor for animals and reduce ingestion.

Mowing alfalfa in the later phenophases of development reduces the solubility of proteins but also reduces the amount of protein in biomass. By drying the cut material, better conditions for ensiling alfalfa are provided, and at the same time, the activity of plant cell enzymes and microorganisms is reduced. The result is a reduction in fermentation, but also proteolysis, which increases the quality and nutritive value of silage. In addition, better and faster compaction provides better conditions for lactic acid fermentation, faster and greater reduction in pH, and reduction of proteolysis. Some earlier procedures (addition of mineral or organic acids) are no longer used for practical reasons (application problems, negative impact on animal and human health ...), but they have proven to be the most effective for proteolysis control (Đorđević et al., 1999). Instead, bacterial inoculants are used today to intensify fermentation and more efficiently transform fermentable carbohydrates into lactic acid (Wang et al., 2018).

Table 1. Effect of alfalfa and red clover ratio on fermentation parameters, N distribution, and protein fractions of silage on d 30 (Le et al., 2018)

pH and nitrogen distribution in silages	Alfalfa and red clover ration (fresh weight), %				
	100:0	70:30	50:50	30:70	0:100
pH	4.48^{a}	4.37^{ab}	4.29^{bc}	4.46^{a}	4.20^{c}
TN, %of DM	2.94^{a}	2.79^{ab}	2.83^{ab}	2.74^{bc}	2.61^{c}
NPN, % of TN	48.1^{a}	44.1^{b}	41.0^{bc}	38.4^{cd}	35.4^{d}
Peptide-N, % of TN	26.1^{a}	22.8^{ab}	21.0^{bc}	17.0^{c}	20.5^{bc}
FAA_N, % of TN	14.7^{a}	15.2^{a}	14.6^{a}	14.4^{a}	10.5^{b}
NH_3-N, % of TN	7.32^{a}	6.06^{b}	5.40^{b}	7.04^{a}	4.40^{c}

$^{a\text{-}d}$Mean values in the same row with different superscripts differed ($P<0.05$)

TN = total nitrogen; NPN = non protein nitrogen; peptide - N = peptide nitrogen; FAA-N = free amino acid N; NH_3-N = ammonia nitrogen.

Lipolysis in silage

The most important energy sources for ruminants are structural carbohydrates (from bulky food), and for highly productive cows and non-structural (concentrates)(Grubić and Đorđević, 2005). High-fat content in cow diets (over 7%) is not desirable, due to the depressant effect on cellulolytic bacteria, and the reduction of milk fat content (Grubić et al., 2005). The largest quantity of milk fat is formed from acetic acid, which is a product of the activity of rumen microflora and the decomposition of cellulose. However, in recent years, not only has the content of milk fat been relevant, but also the share of PUFA in milk fat due to its great importance for human health (Radonjic et al., 2019). Diet is the most important paragenetic factor that affects the composition of fatty acids in milk fat. The most positive impact (on the content of PUFA) has green foods (Hanuš et al., 2018). However, intensive farm milk production force the use of large quantities of forage, primarily silage, due to the favorable price. Therefore, in recent decades, great attention has been paid to the control of fermentation processes in silage due to their proven impact on the part of PUFA. Drying (for hay) and wilting (for silage) are necessary procedures for preserving many forages. After mowing, drying, or wilting, membrane lipid lipolysis occurs (Van Ranst et al., 2009a,b). The released PUFAs are oxidized, as a result of which their amount is reduced. Lipolysis also takes place during ensiling but is different for different plant species (Van Roast et al., 2009a). For example, in red clover silage, lipolysis is partially reduced by the polyphenol oxidase enzyme, deactivation of lipolytic enzymes, and/or formation of the protein-phenol-lipid complex (Lee et al., 2008). The same enzyme provides less proteolysis in red clover silage, and more efficient use of protein in ruminant diets, compared to alfalfa silage (Dinić et al., 2012). Oxidation and loss of PUFA continue even after silo opening (Liu et al., 2018). Some strains of lactic acid bacteria used for inoculation of ensiled material (Lactobacillus Plantarum AKU 1009a), perform biohydrogenation of some PUFAs and increase the content of conjugated

fatty acids in silage (Kishino et al., 2009). This can be very important shortly for more efficient use of ensiled food (Ding et al., 2013).
Although wilting is a necessary procedure before ensiling legumes and grasses, it negatively affects the PUFA content in silage. Conversely, silages from plants that are directly ensiled (whole corn plant) have better preservation of PUFA. However, the use of corn silage results in the biohydrogenation of linoleic acid to *trans*-10, *cis*-12 CLA, which is a very potent inhibitor of milk fat synthesis (Grubić et al., 2007).

Conclusion

Proteolysis and lipolysis are “regular” biochemical processes during the preservation of forage by ensiling, which lead to a decreased nutritional value. From all examined procedures, wilting and inoculation proved to be the simplest to practice. Although wilting is effective in reducing proteolysis, the same procedure can lead to greater loss of PUFA. Therefore, the use of inoculants is an important opportunity to achieve the greatest possible preservation of nutritional and use value and to increase the content of some conjugated fatty acids. The process of inoculation of ensiled material remains an important scientific topic in the field of forage preparation because it provides completely new possibilities.

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EFFECTS OF PROTEASE, DURATION OF FATTENING PERIOD AND SEX OF BROILERS ON CARCASS CONFORMATION MEASURES

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Abstract

This study analyses the effect of different protein levels in broiler feeds (supplemented with protease), different lengths of fattening period and sex on body conformation in fast-growing Cobb 500 broilers. Complete feeds for broilers in experimental groups E-I and E-II contained 4 and 6% less crude protein than the control (C) and were supplemented with protease (Ronozyme Pro Act) at a concentration of 200mg/kg feed and 300mg/kg feed respectively. At 49 and 63 days of age, 10 male and 10 female broilers were randomly selected from each experimental group and slaughtered. Body conformation measurement included absolute carcass conformation measures (metatarsus length, keel length, breast depth, breast angle, thigh girth) and relative body conformation measures - conformation indices (body weight/metatarsus length, body weight/keel length, body weight/breast depth, body weight/thigh girth). The results showed that dietary treatment did not affect conformation measures, as well as that the time of slaughter and sex of chickens had a significant effect on the examined carcass quality parameters. Namely, the length of the fattening period affected all conformation indices, as well as some absolute measures of carcass conformation - metatarsus length, keel length and thigh girth, while sex had a significant effect on the values of the all absolute values of carcass conformation (except for breast angle) and indices body weight/keel length and body weight/breast depth.

Keywords: *broilers, body conformation, feeds, lengths of fattening period, sex.*

Introduction

For decades, those involved in the production chain of broiler chickens have been concerned with the potential for growth and body conformation of poultry, since these characteristics are related to the efficiency and profitability of the poultry sector. Genetic enhancements have resulted in the current broiler chicken strains, which are characterized by faster weight gain and better feed conversion (Nascimento *et al.* 2018). The feed efficiency, live and carcass weight and percentages of some carcass parts are significantly affected by age and production system (Castelini *et al.* 2002).
Dietary protein plays a significant role in digestive system development and growth performance. Modern poultry primarily focuses on reducing the feed cost to optimize economic benefits since feed is the main factor determining the total production cost, and crude protein are one of the fundamental cost constituents of poultry feed (Kamran *et al.* 2004). As one of the methods to improve the utilization of proteins and amino acids from the feed is proposed protease supplementation. Protease enzyme have several benefits including positive effects on

growth performance, nutrient utilization, lipid peroxidation and modified plasma lipids profile in broiler chickens (Sorbara, 2009; Saleh *et al.* 2018; Moreira *et al.* 2020; Saleh *et al.* 2020).
Therefore, the objective of the present study was to evaluate some carcass characteristics - body conformation of broiler chickens from different dietary treatments (with or without protease enzymes), different lengths of fattening period (49 and 63 days of age) and sexes.

Material and methods

A total of 300 day-old broiler chicks (Cobb 500) of mixed sex were randomly assigned to 3 nutritionally corn-soybean based experimental diets (C, E-I and E-II) comprising 3 levels of protease supplementation (C - control group, without protease enzyme; E-I group - 200 mg Ronozyme ProAct/kg feed and 4% less crude protein than the control group and E-II group - 300mg/kg feed and 6% less crude protein than the control respectively). The nutrient composition of feeds are presented in Table 1.
Broilers were fed *ad-libitum* starter (day 1-21 of age), grower (day 22-35 of age) and finisher mash diets (day 36-63 of age) and water was available freely and were placed within the identical environmental conditions.

Table 1. Nutrient composition of experimental diets for broilers

Calculated composition	Starter stage (1 to 21 d)			Grower stage (22 to 35 d)			Finisher stage (36 to 63 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
ME, kcal/kg	3.081	3.100	3.112	3.157	3.174	3.183	3.181	3.198	3.207
Crude proteins, %	22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09
Total lysine, %	1.33	1.27	1.24	1.15	1.10	1.08	1.05	1.00	0.98
Methionine+cystine, %	0.92	0.90	0.89	0.91	0.89	0.88	0.86	0.84	0.83

On day 49 and 63, ten male and ten female birds from each dietary treatment were weighed and after were fasted for 6 h and slaughtered to determine eviscerated absolute carcass conformation measures (metatarsus length, keel length, breast depth, breast angle, thigh girth). Based on body weight at slaughter data and absolute conformation measurements, relative conformation measurements were calculated (body weight/metatarsus length, body weight/keel length, body weight/breast depth and body weight/thigh girth).
The data thus obtained on various parameters were subjected to statistical analysis according to using analysis of variance technique (for three factors - diet, fattening period and sex of chickens) and Tukey's test (for individual comparisons, $P<0.05$) (Statsoft Inc. Statistica for Windows, *2006*).

Results and discussion

The measured parameters of the absolute values of carcass conformation (metatarsus length, keel length, breast depth, breast angle and thigh girth) of Cobb 500 broiler chickens at the age of 49 and 63 days, are presented in Table 2.

Table 2. Body conformation (absolute values) of broilers across experimental groups

Treatment				ML mm	KL mm	BD mm	BA degrees	TG mm
Protease	Fattening period, days	Sex						
C	49	♂	$\bar{x}$	83.1^{bc}	123.2^{cd}	114.0^{ab}	153.6^{abc}	165.5^{ab}
			Sd	3.14	3.26	3.20	5.17	6.48
		♀	$\bar{x}$	74.3^{d}	117.2^{ef}	107.7^{cd}	145.7^{c}	148.2^{e}
			Sd	2.50	3.79	2.79	6.81	5.47
	63	♂	$\bar{x}$	91.3^{a}	130.2^{a}	118.8^{a}	161.7^{a}	173.1^{a}
			Sd	2.75	1.55	4.08	5.64	6.84
		♀	$\bar{x}$	80.8^{bc}	126.8^{abc}	108.4^{bcd}	154.1^{abc}	158.4^{bcd}
			Sd	3.76	1.81	3.78	6.81	7.44
E-I	49	♂	$\bar{x}$	82.1^{bc}	121.3^{de}	113.4^{abc}	152.6^{abc}	159.6^{bc}
			Sd	2.18	4.08	3.75	4.14	2.76
		♀	$\bar{x}$	75.1^{d}	116.4^{f}	105.2^{d}	148.9^{bc}	150.4^{de}
			Sd	3.25	3.31	3.12	6.62	4.55
	63	♂	$\bar{x}$	93.6^{a}	130.6^{a}	116.4^{a}	159.2^{a}	171.4^{a}
			Sd	2.37	1.07	6.67	7.15	5.29
		♀	$\bar{x}$	80.4^{c}	126.3^{abc}	110.5^{bcd}	147.9^{bc}	154.8^{cde}
			Sd	2.76	3.59	4.50	7.61	7.27
E-II	49	♂	$\bar{x}$	85.1^{b}	124.3^{bcd}	113.3^{abc}	155.2^{ab}	161.5^{bc}
			Sd	3.93	2.16	4.29	5.63	5.40
		♀	$\bar{x}$	75.6^{d}	117.6^{ef}	105.1^{d}	147.0^{bc}	150.9^{de}
			Sd	2.63	4.09	3.18	5.12	7.49
	63	♂	$\bar{x}$	90.3^{a}	129.7^{a}	116.3^{a}	154.5^{abc}	167.1^{ab}
			Sd	4.19	1.89	3.16	6.79	6.71
		♀	$\bar{x}$	81.0^{bc}	128.3^{ab}	109.4^{bcd}	148.5^{bc}	159.4^{bcd}
			Sd	2.36	2.06	1.78	6.62	7.46

ML-metatarsus length, KL-keel length, BD-breast depth, BA-breast angle, TG-thigh girth
$^{a-e}$ Means followed by different superscript letters within columns differ significantly (P<0.05)

The addition of protease enzyme (200 or 300 mg/kg of protease) had no significant influence (P>0.05) on the absolute carcass conformation measures compared to the control group.
The prolongation of duration fattening period for two weeks (both at 7 and 9 weeks of age) significantly increased the metatarsus length, keel length and thigh girth (E-I group in female and E-II group in male chickens) (P<0.05), with a slight increase in breast depth and breast angle (P>0.05). The obtained results are consistent with the results of Dosković *et al.* (2017) who reported that the length of the fattening period affected almost all studied parameters, except breast angle.
Males had higher values for all absolute carcass conformation measures than females (P<0.05), with only breast angle (both 49 and 63 days), keel length (63 day) and thigh girth (49 day, E-1 group and 63 day, E-II group) showing no significance (P>0.05). Similar results on the better conformation of male chicken carcasses compared to females, with no significant difference only for breast angle, were obtained by Pavlovski *et al.* (2007) and Dosković *et al.* (2018), while

Blagojević *et al.* (2009) determined a significant effect of sex on all absolute measures of conformation.

Table 3. Body conformation indices in broilers across experimental groups

Treatment				BW/ML g/mm	BW/KL g/mm	BW/BD g/mm	BW/TG g/mm
Protease	Fattening period, days	Sex					
C	49	♂	$\bar{x}$	41.73^{cde}	28.13^{b}	30.40^{cd}	20.95^{cde}
			Sd	1.89	1.05	1.22	0.83
		♀	$\bar{x}$	39.01^{ef}	24.76^{c}	26.90^{e}	19.56^{def}
			Sd	1.79	1.63	0.99	0.83
	63	♂	$\bar{x}$	48.50^{a}	33.40^{a}	37.28^{a}	25.59^{a}
			Sd	2.61	1.74	1.96	1.45
		♀	$\bar{x}$	44.33^{abc}	28.19^{b}	32.94^{b}	22.54^{bc}
			Sd	4.20	2.30	1.94	1.06
E-I	49	♂	$\bar{x}$	41.36^{c-f}	28.01^{b}	29.97^{d}	21.26^{cd}
			Sd	1.88	1.56	1.71	0.63
		♀	$\bar{x}$	38.40^{ef}	24.74^{c}	27.36^{e}	19.15^{ef}
			Sd	2.45	1.15	1.14	1.00
	63	♂	$\bar{x}$	46.94^{ab}	33.62^{a}	37.73^{a}	25.64^{a}
			Sd	3.54	2.37	1.69	1.93
		♀	$\bar{x}$	44.57^{abc}	28.35^{b}	32.40^{bc}	23.15^{b}
			Sd	3.21	1.70	1.57	1.43
E-II	49	♂	$\bar{x}$	39.94^{def}	27.26^{b}	29.90^{d}	20.98^{cde}
			Sd	3.67	1.64	1.22	1.13
		♀	$\bar{x}$	37.29^{f}	23.97^{c}	26.82^{e}	18.69^{f}
			Sd	1.52	1.02	1.21	0.67
	63	♂	$\bar{x}$	47.21^{ab}	32.78^{a}	36.56^{a}	25.51^{a}
			Sd	3.67	1.47	1.62	2.06
		♀	$\bar{x}$	43.63^{bcd}	27.54^{b}	32.28^{bc}	22.17^{bc}
			Sd	2.52	1.51	1.56	0.99

BW – body weight at slaughter, ML-metatarsus length, KL – keel length, BD – breast depth, TG – thigh girth
a-f Means followed by different superscript letters within columns differ significantly ($P<0.05$)

Results from this study clearly show non-significant differences in body conformation indices between applied dietary treatments ($P>0.05$). Similar results on the effect of different concentrations of protease enzymes on carcass quality parameters and conformation measures (both relative and absolute) of chicken carcasses, with a decrease in crude protein content, were published by Dosković *et al.* (2012, 2016), examining the same hybrid - Cobb 500.
With the extension of the duration of fattening (for 14 days), the value of all relative conformation measurements increased ($P<0.05$). The relative conformation traits were significantly affected by the length of the fattening period in similar growing conditions, according to Dosković *et al.* (2017).
There was a significant effect ($P<0.05$) between male and females chicks in body weight/keel length and body weight/breast depth, while at the same time there was no difference in indices of body weight/metatarsus length and body weight/thigh girth ($P>0.05$). Dosković *et al.* (2016) reported that male chickens had significantly ($P<0.01$) higher relative conformation measures

compared to female chickens, while Blagojević (2011) found that the effect of sex on carcass conformation index value was significant for BW/ML.

Conclusion

Based on the data presented above, it could be concluded that:

- dietary treatments (standard diet without enzyme protease; 4 % less crude protein than the control and enzyme protease (Ronozyme Pro Act) at a concentration of 200mg/kg feed and 6 % less crude protein than the control and with enzyme protease at a concentration of 300mg/kg feed) did not affect carcass conformation measures,
- prolonging the fattening period of Cobb 500 chickens (from 49 to 63 day of age) significantly increased some absolute carcass conformation measures (metatarsus length, keel length and thigh girth), as well as all body conformation indices ($P<0.05$),
- male chickens had better conformation compared to females, and sex had a non-significant effect on the values of the breast angle and indices of body weight/metatarsus length and body weight/thigh girth ($P>0.05$).

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COMPARING THE EGG QUALITY TRAITS OF DIFFERENT LAYER GENOTYPES RAISED IN A FREE-RANGE SYSTEM

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Abstract

The aim of this study is to compare different layer hybrids in free-range system in terms of some egg quality characteristics. Lohmann Brown (LB) at 32 weeks of age as commercial layer hybrid and ATAK-S (AT) genotype, which is domestic layer hybrid material, were used. Egg weight, shell color, eggshell strength and Haugh unit were investigated as egg quality characteristics. Quality analyses were carried out on 20 randomly taken eggs from eggs produced for two consecutive days of each genotype. It was determined that the eggshell color L value of the LB genotype (59.26) was lower than the eggshell L value of the AT genotype (66.69) ($P<0.05$). Eggs obtained from LB genotype were found to be heavier in terms of egg weight ($P<0.05$). Egg shell strength was found to be higher in eggs obtained from LB genotype (4.68 kg) than eggs obtained from AT genotype (3.79 kg) ($P<0.05$). It was determined that the Haugh unit (93.78) of the eggs obtained from the LB genotype was higher than the Haugh unit (84.44) of the eggs obtained from the AT genotype ($P<0.05$). As a result, it was observed that the quality characteristics of eggs obtained from LB genotype in the free-range system were better than the quality characteristics of eggs obtained from AT genotype.

Keywords: *Genotype, egg quality, free-range system.*

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. Many studies have been conducted for this purpose by researchers (Türker *et al.,* 2017). Lohman Brown is a foreign layer hybrid that is grown in Turkey using the free-range system. The Ankara Poultry Institute developed the ATAK-S layer hybrid, which produces brown-shelled eggs (Goger *et al.,* 2016). It is preferred in free-range systems and small family breeding production models in Turkey (Tutkun *et al.,* 2018). Egg quality attributes can be influenced by a number of factors, including genotype (Tůmová *et al.,* 2007; Bozkurt and Tekerli 2009; Rajkumar *et al.,* 2009; Zita *et al.,* 2009; Obike *et al.,* 2014; Hayirli *et al.,* 2015; Sokołowicz *et al.,* 2018; Kraus *et al.,* 2020). According to Bozkurt and Tekerli (2009), white layers produce eggs with higher Haugh units than brown layers. However, Hayirli *et al.,* (2015) reported that genotype had no significant effect on any other quality characteristics besides egg shape index and yolk index. According to Kraus *et al.,* (2020), there were statistically significant differences found in the egg weight, shape index, shell break resistance, Haugh unit, and shell thickness of eggs from different genotypes.

The goal of this study is to compare different layer genotypes in the free-range system in terms of egg quality.

Materials and Methods

This study was carried out at Selcuk University, Faculty of Agriculture, Department of Animal Science. Two different genotypes, commercial (Lohmann Brown; LB) and domestic (ATAK-S, AT) at 32 weeks of age, were used in the study.
The hens were reared in a free-range system. The stocking density in the in-door area is 7 animals/m^2, while the out-door area provides 4 m^2 per hen. Water and feed are given as ad-libitum. Quality analyses were carried out on 20 randomly taken eggs from eggs produced for two consecutive days of each genotype. Egg weight, shell color, breaking strength and Haugh unit were investigated as egg quality characteristics. Egg weight was measured using a balance and was recorded to the nearest 0.01 g. Colour measurement was performed using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The egg shell color was measured at the large pole of the egg (Aygun, 2014).
Eggshell strength (kg) was measured with an Egg Force Reader (Hong Kong, China). 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).

Statistical analysis
In the study, two breeding practices (raising with and without roosters) and two storage temperatures will be carried out according to a randomized plot design in a 2x2 factorial arrangement. Kolmogorov Smirnov and Levene tests will be applied to determine that the data meet the parametric test assumptions. Data that do not show normal distribution will be transformed into a state suitable for parametric test assumptions by rank transformation method. After it is determined that the data have normal distribution and the variances are homogeneous, two-way analysis of variance will be applied. The main effects will be taken as a basis in the analysis, and the interaction effects will not be used in the model due to the nature of the data. Tukey's multiple comparison test will be used to identify differences between treatments. 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 shell color values of genotypes are given in Figure 1. It was determined that the eggshell color L value of the LB genotype (59.26) was lower than the eggshell L value of the AT genotype (66.69) ($P<0.05$). This finding is consistent with the research by Kraus and Zita (2019), which found that genotype had a significant impact on eggshell color. Egg shell color is an important criterion in terms of consumer preference. The LB genotype produced darker brown eggs than the AT genotype. Joseph et al., (1999) stated that genotype has a significant effect on eggshell color.

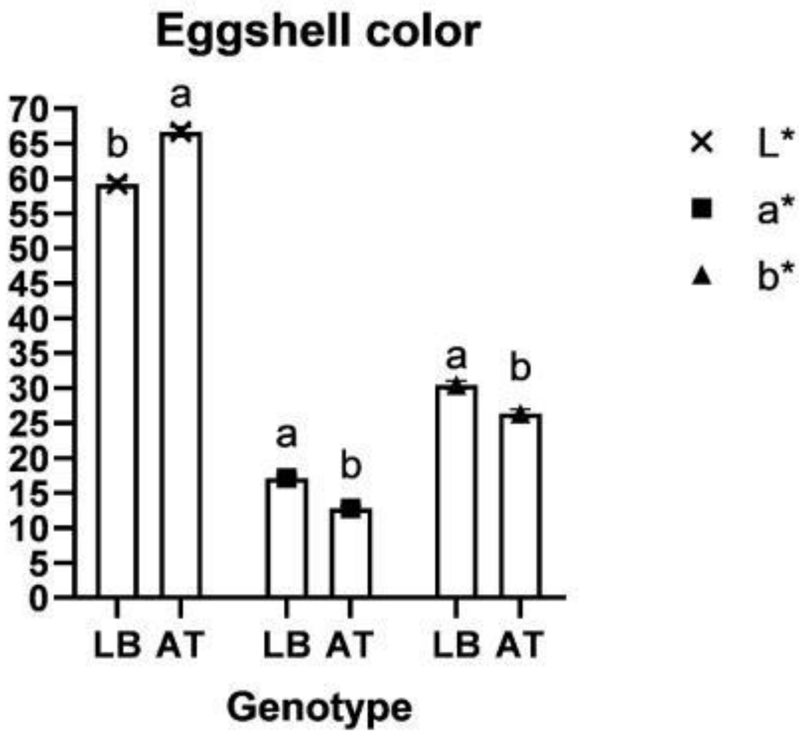

Figure 1. Eggshell colour
LB: Lohmann Brown, AT: Atak-S

The effects of genotype on egg weight, eggshell strength and Haugh unit are given in Table 1. The eggs obtained from the LB genotype were found to be heavier than those obtained from the AT genotype in terms of egg weight (P<0.05). 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).

Table 1. Egg weight, eggshell strength, and Haugh unit values of different genotypes

Group	Egg weight (g)	Eggshell strength (kg)	Haugh Unit
LB	60.91^{a}	4.679^{a}	93.77^{a}
AT	55.38^{b}	3.790^{b}	84.43^{b}
SEM	0,573	0.110	1.221
P value	0.000	0.000	0.000

Egg shell strength was found to be higher in eggs obtained from LB genotype (4.68 kg) than eggs obtained from AT genotype (3.79 kg) (P<0.05). The findings are similar to those of Türker et al., (2017), who found that the ATAK-S genotype's eggshell strength is worse than the foreign genotype's. In contrast, Petričević et al., (2017) found that genotype had no effect on eggshell strength. It was determined that the Haugh unit (93.78) of the eggs obtained from the LB genotype was higher than the Haugh unit (84.44) of the eggs obtained from the AT genotype ($P<0.05$). Similar result, Bozkurt and Tekerli (2009) reported that the Haugh unit value of the eggs derived from the Lohmann White genotype (85.92) was higher than that of the eggs derived from the Isa Brown (79.72). The obtained result contradicts the findings of Türker et al., (2017), who found no significant difference in Haugh unit value between eggs obtained from the A genotype and eggs obtained from the foreign genotype.

Conclusion

As a result, it was observed that the quality characteristics of eggs obtained from LB genotype in the free-range system were better than the quality characteristics of eggs obtained from AT genotype.

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INVESTIGATION OF THE EFFECT OF INTRANASAL LTA AND LPS IMPLEMENTATION ON GENE EXPRESSION IN THE IMMUNE SYSTEM PATHWAY WITH CORRELATION ANALYSIS IN SHEEP

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Abstract

In this study, Lipoteichoic acid (LTA, n=7, 50 µg/kg), Lipopolysaccharide (LPS, n=7, 20 µg/kg) and LTA-LPS mixture (n=7, 50 µg/kg + 20 µg/kg) and PBS (control, n=7, 250 µl) were administered via intranasal route to Akkaraman lambs. The differences in the expression of TLR2, TLR4, MyD88, TRAF6, TNF-α, IL-1ß, IL-6, IL-10, NF-κß and IFNγ genes using GAPDH and ß- actin as reference genes, and the relationship of genes with each other’s both in different and in the same time periods were determined with qRT-PCR in PBMCs isolated at the 24th hour and on the 7^{th} day after administration. The correlation coefficient and statistical significance between the normalized expression data of genes were examined by Spearman rho correlation analysis. Considering the obtained results at the 24^{th} hour; high and statistically significant ($p<0.05$) positive correlations were determined between *TLR2-IL10, NF-κß, NF-κß-IL-10* genes in the control group; between *TLR2-TNF-α, IL-10, TLR4-MyD88, IL-1ß, MyD88-IL-1ß, IFNγ-TRAF, NF-κß, TNF-α-IL-10* genes in the LTA group; between *TLR2-TRAF6, TNF-α, IL-10, IL-10-TLR4, TRAF6, IL-1ß* genes in the LPS group and between *TLR2-IL-10, TLR4-TRAF6* genes in the mixture group. On the 7^{th} day, high level of ($p<0.05$) positive correlations were determined between *TLR2-TLR4, IL-1ß, IL-10, IFNγ, TLR4-IL10, TRAF6-NF-κß, TNF-α, IL1-ß-IL-10* genes in control group, between *TLR2-TLR4, TNFA, IL-10, TLR4-MyD88, NF-κß, TNF-α, NF-κß, MyD88, TNF-α* genes in the LTA group and between *IL-10-IFNγ* genes in the LPS group whereas a statistically significant high level of negative correlation ($p<0.05$) was determined between NF-κß-IL-1ß genes in the mixture group. Considering the correlation between time periods, moderate and high level of negative correlations ($p<0.05$) were observed between genes in all groups except the control group. According to the obtained results, the applied molecules activated different genes in different groups at the 24^{th} hour and the activity decreased on the 7^{th} day.

Keywords: *LTA, LPS, sheep, PBMCs, immunity.*

Introduction

As in all farm animals, infectious diseases in sheep also cause significant economic losses in enterprises. Respiratory system diseases that constitute about 5-6% of the diseases seen in sheep cause significant economic losses in sheep breeding all over the world (Bell, 2008). Therefore, improving the care as well as the protection methods is important in reducing the economic losses caused by respiratory system diseases in sheep breeding (Kennerman, 2017). In understanding the mechanism of natural and acquired immunity against disease agents in farm animals, microarray, RNA-seq, transcriptome, qRT-PCR analyses of genes of cells and tissues playing a role in this mechanism offer new perspectives to researchers (Gao *et al*., 2010; Singh *et* *al*., 2017, Aksel and Akyüz, 2021).These technologies can contribute to the explanation of the genetic basis of the individual responses to the treatment protocols against diseases, and in the further process, to take the necessary precautions in the control and treatment of the disease at an early stage (Davies *et al*., 2009).

Clarifying the genetic mechanism of the immune response of the diseases in the immune system is very important in terms of controlling the disease in the treatment phase (Davies *et al*., 2009). In addition, animal models that mimic human biology are important for the successful transfer of basic science findings to clinical practice. Rodent models, which are widely used in such studies, have been criticized for lacking the mimicking important features of the human immune response to microbial products (Enkhbaatar *et al*., 2015). Enkhbaatar *et al*. (2015) emphasized that sheep can be a good model animal in studies with the immune system due to the similarity between sheep immune system genes and human genes.

Immune responses include two main components as innate and adaptive immunity. Natural immunity defines general threats whereas adaptive immunity occurs after exposure to specific antigen such as pathogen or vaccination. Lymphocytes, monocytes/macrophages in peripheral blood cells play a vital role in the immune system (Gao *et al*., 2010; Siednjenko *et al*., 2009). Among the infectious agents that threaten the immune system, LPS in the cell wall of Gram-negative bacteria (Heumann *et al*., 1998) and LTA in the cell wall of Gram-positive bacteria (Standiford *et al*., 1994) are pathogenicity factors that stimulate the inflammatory reaction.

In this study, unlike the previous study by Aksel and Akyüz (2021), bacterial LTA and LPS were administered separately and in combined form to Akkaraman lambs via intranasal route to investigate the relationship between the expression data of TLR2, TLR4, MyD88, TRAF6, TNF-α, IL-1ß, IL-6, IL-10, NF-κß and IFNγ genes in PBMC cells in all groups, both in different time periods and in the same time period.

Material and Method

The animal material of the study consisted of the 28 Akkaraman lambs which were distributed into four groups as treatment and control groups and 50 μg/kg of LTA (n=7), 20 μg/kg of LPS (n=7) and 50 μg/kg + 20 μg/kg of LTA-LPS mixture (n=7) were administrated to treatment groups and 250 μl of dilution solution, PBS, was administrated to control group (n=7) via intranasal route. RNA isolation was performed by Trizol method from PBMC cells isolated from total blood taken from lambs in tubes containing EDTA at 24 hours and 7 days after the application. RNA quantity and quality (BioSpec-nano, Japan) were determined. Primers of TLR2, TLR4, MyD88, TRAF6, TNF-α, IL-1ß, IL-6, IL-10, NF-κß, and IFN-γ genes as well as GAPDH and β-actin selected as the house-keeping, were designed using Primer 3 (2020) (http://primer3.ut.ee). After RNA isolation, expression levels of TLR2, TLR4, MyD88, TRAF6, TNF-α, IL-1ß, IL-6, IL-10, NF-κß and IFNγ, house-keeping genes were determined by qRT-PCR. For the qRT-PCR, Roche Fast Start Green Master PCR kit (Sigma-Aldrich, Germany) protocol was applied. The mixture completed to 10 μl with reverse primer (0.5 μl) and 0.2 μM forward (0.5 μl) belonging to each gene; cDNA (2 μl) and nuclease-free water (7 μl) were placed into sterile strips. Ten microliters of Fast Start Green Master was added to the mix, and it was loaded into the device (Aksel and Akyüz, 2021). Spearman rho correlation analysis was applied to expression data normalized according to GAPDH and β-actin genes for statistical correlation analysis. All analyses were performed using R Statistical Software with “corrplot” package (v4.1.2; R Core Team 2021).

Results and Discussion

According to the results obtained after the correlation analysis, in the 24th hour, a high and positive correlations ($p<0.05$) were determined between the genes TLR2-IL10, TLR2-NF-κß, NF-κß-IL-1 in control group; between TLR2-TNF-α, TLR2-IL-10,TLR4-MyD88, TLR4-IL-1ß,MyD88-IL-1ß, TRAF6-IFNγ, NF-κß-IFNγ, TNF-α-IL-10 in the LTA group (Figure 1); between TLR2-TRAF6, TLR2-TNF-α, TLR2-IL-10, TLR4-IL-10, TRAF6-IL-10, IL-1ß-IL-10 in the LPS group, and between TLR2-IL-10, TLR4-TRAF6 in the mixture group (Figure 2). On the 7th day, high level ($p<0.05$) of positive correlations were determined between genes TLR2-TLR4, TLR2-IL-1ß, TLR2-IL-10, TLR2-IFNγ, TLR4-IL10, TRAF6-NF-κß, TRAF6-TNF-α, IL1-ß-IL-10 in control group; between TLR2-TLR4, TLR2-TNFA, TLR1-IL-10, TLR4-MyD88, TLR4-NF-κß, TLR4-TNF-α, MyD88-NF-κß, NF-κß-TNF-α genes in the LTA group (Figure 1) and between IL-10-IFNγ genes in the LPS group whereas a statistically significant high level of negative correlation ($p<0.05$) was determined between NF-κß-IL-1ß genes in the mixture group (Figure 2). Considering the correlation between time periods, moderate and high level of negative correlations ($p<0.05$) were observed between genes in all groups except the control group. According to the obtained results, it was determined that the degree of relationship of the applied agents differed according to the groups examined at the 24th hour after the application, and the activity on the 7th day in the experimental groups decreased with the observation of negative correlations compared to the 24th hour.

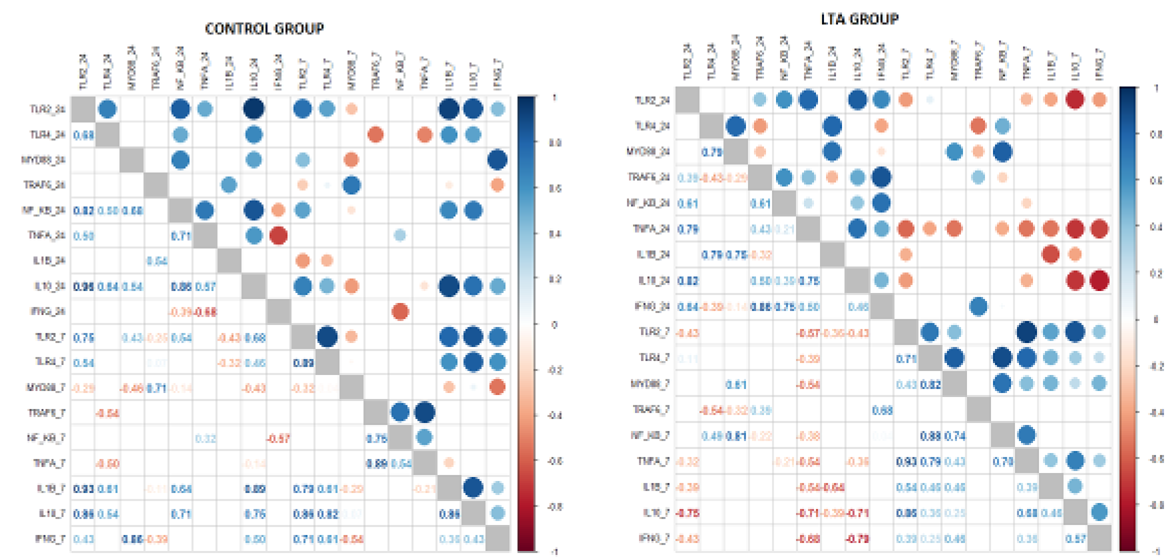

Figure 1. Spearman Rho correlation analysis results of genes belonging to control and LTA groups according to 24th hour and 7th day time periods and groups.

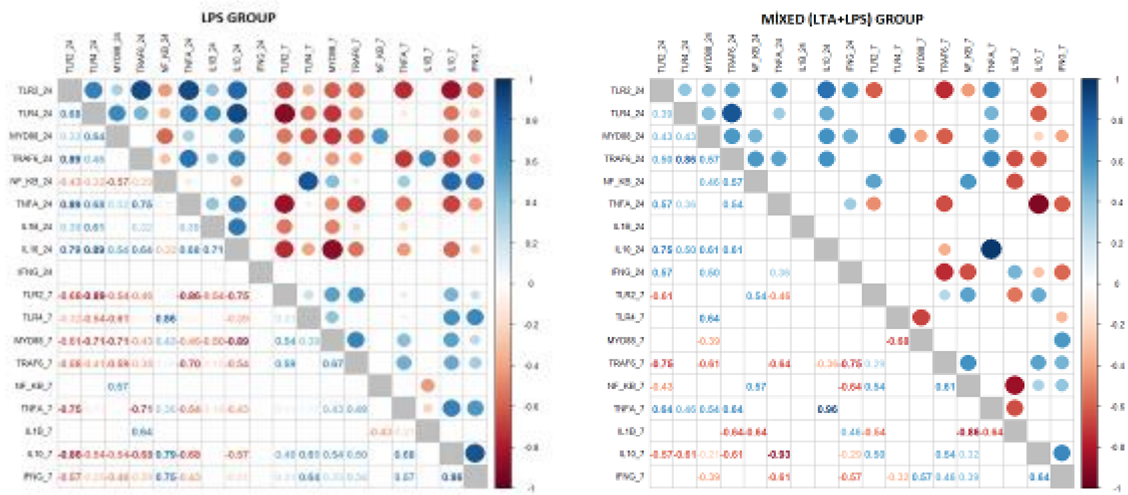

Figure 2. Spearman Rho correlation analysis results of genes belonging to LPS and mixed groups according to 24th hour and 7th day time periods and groups.

In a previous study performed by Aksel and Akyüz (2021), it was reported that the doses applied to activate the sheep immune system significantly increased the expression of TLR2, TLR4, TNF-α, IL-10, IFNγ genes in PBMC cells, especially according to the groups, at the 24th hour. In this study, unlike the study conducted by Aksel and Akyüz (2021), it was aimed to reveal the correlations of all groups over the data normalized according to GAPDH and ß-actin genes by considering the relations of genes within the group, according to time and among themselves. According to the results of the correlation analysis, high levels of negative and positive correlations between different time periods were prominent in the experimental groups (Figure 1, 2). In this in vivo study, the effects of molecules belonging to different bacterial agents on the expression of genes determined in PBMC cells in respiratory system bacterial infections were examined in relational terms, and statistical significance levels between genes were determined.
It was also revealed by the observed negative correlations that the applied doses lost their activity on the 7th day in LPS, LTA and mixture groups, respectively. In some studies, bacterial agents were applied to animals such as mice, pigs, horses, and sheep by different administration routes, and expression profiles were tried to be revealed in different tissues and in different time periods (Ehrentraut *et al*., 2011; Hillman *et al*., 2008). Similar to the results obtained in this study, Hillman *et al*. (2008) reported that the expression of TLR2 and TLR4 increased on the 2nd day and decreased on the 7th day with the LPS applied into the amniotic fluid in sheep. Moreover, Uddin *et al*. (2012), in their study in porcine PBMC cells, investigated the change in time-dependent TLR1-10 genes between the 1st and 48th hours depending on the increase in the LPS dose, and reported the increases in the expression levels of some TLRs depending on the dose. In this study, it was thought that the low dose of LPS compared to LTA might be effective in the earlier observation of moderate and high level of negative correlations between genes observed at the 24th hour and 7th day in this group.

Conclusion

With the obtained results, it was determined that this experimental study designed in Akkaraman lambs was effective. In order to reveal the differences in resistance to diseases between the domestic sheep breeds of our country and the domestic breeds in different countries, it has been recommended to determine both the expression and the pathogen-SNP relationship of different pathogenic factors related to TLR genes involved in the immune system mechanism and to plan in line with the results observed in this study. The expression changes observed in lymphocyte, monocyte/macrophage cells, especially in the immune system of bacterial infectious agents in sheep respiratory system diseases, were revealed in terms of the expression profiles of genes examined with the doses applied in this study. This study is a pioneer for future studies on this subject. The results obtained in this study can contribute to in vivo and in vitro drug and vaccine development studies to be planned in different scientific fields. In addition, according to the obtained results, it has been concluded that it can also contribute to both the studies concerning the respiratory system infections seen in farm animals and in terms of sheep being a bio-model animal for studies to be planned on human.

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POULTRY BEHAVIOUR AND WELFARE

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Abstract

The concept of welfare in poultry has gained importance in recent years and has been shaped by some demands of societies. Behavior is the first indicator for understanding welfare status of birds. If chickens can display their natural behavior within their rearing system, it is widely an accepted approach to say that their welfare status is good. However, as a result of the high stocking density of broilers in the confined systems and laying hens in conventional battery cages, many people are concerned about the welfare of birds. In these intensive and compelling production models, birds cannot exhibit their natural behaviors, their fear levels increase, they are exposed to stress, and naturally their well-being is adversely affected. In order to solve this problem, welfare-friendly alternative rearing systems have been developed for broilers and enriched cages have been used for layers. All these alternative production systems and enriched cages have been designed so that birds can exhibit their natural behaviors and therefore increase their welfare level. Studies on bird behavior have also increased in parallel with these developments. The aim of this study is to introduce what bird behaviors are, how they change, and their genetic and physiological basis. In addition, behavior-welfare relations were also revealed.

Keywords: *Well-being, Poultry ethology, Fear, Focal sampling, Behavior*.

Introduction

The domestication of chickens has been interested since the times of Charles Darwin by many wide range of disciplines (Miao et al., 2013). It is widely accepted that chickens are domesticated from India and Southeast Asia from red jungle fowl (Al-Nasser et al., 2007; Kanginakudru et al., 2008; Marino, 2017). While researchers mostly agree about domestication of chickens there some conflicts about the date of domestication. Some researchers reported that chickens are domesticated about 8000 years ago (Göger and Yenice, 2018; Marino, 2017), some others reported that it is about 2000 – 2500 years ago (Tixier-Boichard et al., 2011; West and Zhou, 1988). However, there is also some molecular evidence that the domestication has begun as early as 58000 years ago (Marino, 2017). Although humans gain much information of its history, it is still open to debate. Primarily chickens domesticated for entertainment or as a pet. Afterwards they are reared for their egg and meat. While they are domesticated by humans their production and behavioural traits are changed. Humans applied some selection to gain more goods and selection caused some changes of chickens' appearance and behaviour. It is reported that although production and morphological traits have important changes by selection behavioural and cognitive traits have small changes (Göger and Yenice, 2018; Marino, 2017). The most important changes on behaviour decreasing to escape and broodiness, increasing aggression and changes in feeding habit (Göger and Yenice, 2018). Also Marino (2017) reported that

domesticated chickens are more aggressive than their ancestors. Briefly movement of chickens, appearance of chickens like feather colour, body weight and size of body are changed by selection.
Animal behaviours can give information about animal welfare; therefore, they are very linked together. While determining animal welfare there are two aspects; one of them is animals' health and the other one is whether animals behave like in their natural habitat (Akbaş, 2013). Behaviours of animals are important to identify of animal welfare and yield status (Akbaş, 2013). The aim of this paper to understand behaviour of chicken from the beginning of evolution to recent times and to determine the relation of their behaviour with welfare and performance.

Physiology of Behaviour

Poultry production is based on profitability and there are many factors to affect this profit. However, while the intensive production system continued, the production, faced a problem with the awareness of consumers which is animal welfare. Welfare has become an ethical problem, but it is realized that it affects production performance as well. Therefore, the most important factor among all production has become welfare of birds and many scientists started to study about this issue. In order to identify of welfare, the most significant parameter is behaviour of birds. While welfare can be detected by observing the poultry behaviour as well as by looking for some physiological parameters.
Behaviours are regulated by hypothalamus in poultry like all vertebrates. Secreted hormones by hypothalamus are regulate some behaviours. Presence or absence of some these hormones affects to display some behaviours and because of this welfare and the production performance decreases. In this section we try to explain physiology of some important behaviours for production and welfare.
In most avian species reproductive behaviours scope the copulation, nest building, incubation, and the care of the chicks after displaying courtship (Ball and Balthazard, 2009). Hypothalamus conducts the endocrine system and behaviour of reproduction system in poultry. The hypothalamus, pituitary gland and gonads are the main elements of reproductive system. Successful production system needs these elements function simultaneously (Ottinger and Bakst, 1995). Avian reproductive system is regulated by hypothalamus pituitary – gonadal axis (Ottinger and Bakst, 1995) and gonadal hormones (Adkins and Alder, 1972; Ottinger, 1983). Gonadotropin-releasing hormone (GnRH) which is produced by hypothalamus stimulates the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) that regulate the ovarian and testicular function. Gonadal steroids, mainly testosterone, estradiol and progesterone are transferred by blood to the central nervous system and give feedback to hypothalamus in order to GnRH production and release (Ottinger and Bakst, 1995). There are some studies which prove for some forms of GnRH has different role on central nervous system. For instance, Beach and Inman (1965) found in a study that castration abolished all sexual behaviours within 8 days, and testosterone pellet implantation restored all sexual behaviours. This supports that sexual behaviour in male depends on the presence of testosterone.
Another important behaviour that affects performance in poultry is aggression. Aggressive behaviour is inherently associated with survival and reproduction (Cheng and Muir, 2007; Duncan, 1998). Caliva et al. (2017) reported that although aggression is a heritable behaviour for all animals, it is an important behaviour that affects both animal welfare and yield traits when considering all livestock. The frequency and intensity of aggressive behaviour and some social

cohesion and behavioural synchronicity may also be good indicators of animal welfare (Duncan, 1998). However, aggression in intensive and controlled production systems, such as in poultry production, causes an increase in social stress and cannibalism due to feather and body injuries (Cheng and Muir, 2007). In order to perceive this behaviour and take the necessary precautions, it is necessary to first understand the mechanism and physiological infrastructure of the behaviour. The density of serotonin and its metabolites and even its receptors is accepted as an indicator of some abnormal behaviours, including aggressive behaviours (Cheng and Muir, 2007). Dopamine is also accepted as an indicator that participates in the control of some behaviours (Cheng and Muir, 2007). There are studies on whether some hormones, which are effective on such behaviours in humans and mammals, have the same function in poultry. Cheng and Muir (2007) hypothesized that the neuroendocrine system has the same function in laying hens. They determined that the serotonin concentration was higher in the low viability group, and this caused high cannibalism, and they reported that the serotonin level was positively correlated with the aggressive behaviour.

Genetics of Behaviour

Darwin (1875) stated that while animals were domesticated, humans modified them based on their preferences for particular characteristics (Siegel, 1979). Although they made unconscious choices during this domestication process, Wirén et al. (2009) specified that it should be considered natural to choose individuals who have the vitality to cope with this intensity, since animals are raised in larger groups than in their natural lives under modern farm conditions. In poultry, the process has accelerated in the last few decades due to increased selection and the development of specialized dam and sire lines in genetic improvement programs (Siegel, 1993). In this process, poultry gave physiological, genetic and behavioural responses to cope with the pressures they felt in the environment (Siegel, 1993). Here, it should be investigated how domestic chickens differ from red jungle fowl, and how much genetic diversity there is among them, and what changes this genetic diversity causes in the behaviour of chickens. According to the studies, the genetic distance between red jungle fowl and white leghorn layer hybrids was found to be similar to the genetic distance between commercial broiler hybrids in DNA studies (Siegel et al., 1992). Although most behaviour appears to be influenced by polygenic systems, there is ample evidence that certain mutants alter behaviour (Siegel, 1993). The degree of effect of these mutants depends on the genetic background of the individual, the effect of the environment or the relationship of both. Kinney (1969), who estimated heritabilities of some behaviour traits, and genetic and phenotypic correlations between the traits in chickens. Researcher reported that the available genetic variations affect some behaviours indirectly, while it affects some behaviours for itself (Siegel, 1993).

When all these data are taken into consideration, it is clear that natural selection can alter genetic background, and that this can influence behavior. Despite the increasing interest and studies in recent years, the effect of genetics on behaviour has not been adequately explained. For this reason, more studies are needed to explain the genetic background of behaviour and to determine the effects of polymorphisms on behaviour.

Behavioural Traits in Poultry

It is possible to determine the behavioural characteristics of poultry and, accordingly, to determine the welfare of the flock. Each behaviour of birds can be converted into numerical data by assessing it as a distinctive characteristic. The welfare status of birds can be understood from the fact that they can exhibit their behaviours in nature and lead a life free from stress and disease.

The most important factors affecting welfare in poultry is fear and stress. Broiler and layer hybrids with high yield levels used in commercial production today have been developed as a result of genetic improvemet studies. In order for them to reflect their superior genetic structures to the phenotype with minimum deficiencies, poultry houses with fully controlled environmental conditions, feeds that can meet all physiological needs of birds and special rearing-management systems are provided. However, in these advanced poultry house and rearing-management systems, birds cannot adequately exhibit their natural behaviours and the fear behaviour in their instincts could not be prevented in this rapidly changing system (Akşit and Özdemir, 2002). The severity of fear varies according to the animal's perception ability, previous experiences, hormonal status, and the size of the factor that causes fear, and low-intensity fear factors increase the animal's adaptability. They react such as escaping, staying still, or resisting when they feel severe fear (Akşit and Özdemir, 2002). While a short-term and low-intensity fear situation normally protects animals against external dangers, when it is long-term and severe, it affects the performance by disturbing the peace of the birds (Akşit and Özdemir, 2002; Jones, 1996). Behaviours such as sand bathing and feather trimming, which are among the natural behaviours of poultry, can be considered as welfare criteria. Since these behaviours are generally short-lived and often observed in small numbers, they have been evaluated in different categories by various researchers. Feather trimming behaviour, Mohammed et al. (2018), Wei et al. (2020) and Li et al. (2019) expressed it as the gentle trimming and combing of chickens' feathers with their beaks. On the other hand, some researchers have considered comfort behaviour together with behaviours such as wing flapping, leg – wing stretching, feather flapping (De Los Mozos et al., 2017; Riber, 2015; Riber et al., 2021; van Emous and Mens, 2021).

Although poultry were domesticated for different purposes at the beginning of the domestication process, today they are used for meat and egg production. Efforts to increase the yield are still continuing for these animals whose yields are increased with the applied selections. Studies on behavior have increased in recent years, and its relationship with productivity has been tried to be revealed. The most important behavior associated with productivity is feed consumption and water drinking. Feed consumption is defined as having the head in the feeder or pecking in the feeder, and drinking water as being in contact with the drinkers (De Los Mozos et al., 2017; Li et al., 2019; Lourenço da Silva et al., 2021; Mohammed et al., 2018; Riber, 2015; Riber et al., 2021; Ross et al., 2019; van Emous and Mens, 2021; Wei et al., 2020). Studies show that feed consumption and water drinking behavior vary according to the content of the feed, the structure of the feed and the environmental conditions of the animal.

The most common harmful behaviors in broilers can be defined as pecking, feather pecking, plucking, aggression and cannibalism as a result of these. While some researchers define the feather pecking or plucking behavior as light or harsh on its own (Riber et al., 2021), some researchers examine it under object pecking (Li et al., 2019). Aggression or aggressive pecking has been defined as jumping, flapping, kicking, pecking at another animal from the front threateningly (Riber, 2015; Riber et al., 2021) or directly pecking its head while walking, sitting or standing (Li et al., 2019). These behaviors are considered as an indicator of animal welfare and a reflection of their emotional state (Lourenço da Silva et al., 2021). In addition, when such behaviors increase in the herd, they can cause injury or even death, thus negatively affecting animal welfare. Pecking, feather pecking, plucking and aggressive behavior can be controlled by practices such as various environmental regulations and feed regulations. Dawson et al. (2021) reported that oral enrichment objects such as hanging ropes and straw bales increased pecking probability and were also associated with gait score. Kristensen et al. (2007), in a study investigating the effects of lighting sources used on commercially broilers, noted that chickens exhibit less feather pecking behavior in warm white light than in biolux light. Similary, Prayitno et al. (1997) reported that chickens reared in red and white light were more active than those reared in green and blue light, in addition, those reared in red light were more aggressive.

Measuring Behavioral Traits

Although it is very difficult to measure and evaluate behavioral traits in poultry, many different methods have been developed and used to detect these traits in studies conducted up to now. These methods include testing animals individually, focal sampling with camera recordings, and evaluation of physiological parameters. These methods are listed below.

- Focal sampling
- Scan sampling
- Tonic immobility
- Open field test
- Emergence test
- Upcoming human test
- Box plus test
- Manual restraint test
- Home cage avoidance test

Conclusions

From the past to the present, great developments have been achieved in poultry production systems, which have reached a high level of production to meet the nutritional needs of the world and are still developing. While the studies carried out so far have mostly focused on feeding and management, today there has been an increased interest in subjects such as behavior. It is understood by the studies that the productivity level can be improved by detecting and understanding the unique natural behaviors of birds in poultry production. Understanding these behaviors also enables to offer alternatives to animal welfare systems, depending on the type of production. In addition, due to the intensity of these production systems in some parts of developed societies, an important sensitivity regarding animal welfare has arisen. Consumers, who care about the comfort behavior of animals, have reacted to the negative conditions under intensive production systems, and this has increased the importance of animal welfare. Therefore, it is important to understand animal behavior in order to provide facilities for the needs of animals within production systems. At the same time, it is an important issue both in terms of improving efficiency in production and meeting the consumer's demand for animal welfare.

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BEHAVIORAL CHARACTERISTICS IN JAPANESE QUAILS APPLIED TO DIFFERENT MONOCHROMATIC LIGHTING

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Abstract

One of the most important environmental factors for poultry is lighting. Recently, LED lamps have been used in poultry houses due to some of their advantages. The aim of this study is to determine the effects of white (400–760 nm), green (560 nm), yellow (580 nm), blue (480 nm) and red (660 nm) monochromatic lighting treatments on behavioral characteristics of Japanese quails throughout the rearing period. A total of 300 Japanese quails were used in the trial. Observations were made at the ages of four and six weeks. Focal sampling method was applied in order to determine the time budgets for the general behavioral characteristics of eating, walking, drinking water, scratching, mating, standing, shaking, cleaning, pecking, wing stretching, lying, and jumping. In terms of aggressive pecking behavior, the mean of time budget of quails treated with yellow monochromatic lighting was found to be higher than those in other groups. It was determined that quails treated with yellow and red monochromatic lighting were more active, and exhibited more reproductive behaviors, but on the contrary, they showed more unwanted aggressive behaviors. In addition, it was observed that quails with green and blue monochromatic lighting were calmer.

Keywords: *Light wavelength, Poultry behavior, Focal sampling, Environmental conditions.*

Introduction

Since most of the commercial production in poultry is carried out with hybrid material, production is carried out in closed and fully environmentally controlled conditions. The importance of environmental factors on yield performance in poultry is high. For the highest level of reflection of the genotype to the phenotype, the improvement of the environment is also required. The most important environmental factors in the house are temperature, humidity, air quality, litter management and lighting.

Light is an integral part of the vision, including both visual clarity and color discrimination, and allows the synchronization of various metabolic and many basic functions required for physiological rhythm, body temperature, nutrition and digestion in poultry. Light stimulates to varying degrees the secretion patterns of various hormones that control growth, maturation, and reproduction. The light is captured by the bird's eye's retinal photoreceptors, which send nerve impulses to the brain via photosensitive cells (extra-retinal photoreceptors). The brain then coordinates the incoming stimulus to stimulate the pituitary gland to secrete the necessary hormones (Lewis and Morris, 2000). The two main hormones involved in this system are serotonin and melatonin, and they affect all other endocrine functions and daily patterns, including locomotor activity, body temperature, migration and seasonal reproduction (Mellor, 2001).

Studies carried out in the last two decades have shown that lighting at blue and green wavelengths close to ultraviolet increases the slaughter weight by 3-5% (Rozenboim et al., 2004). Akyüz and Onbaşılar (2018) reported that broilers were raised under green and blue light, resulting in an increase in body weight, and red light negatively affected body weight gain. They stated that red and white light decreased the age of sexual maturity in laying hens, while blue light increased the mortality rate. Akyüz and Onbaşılar (2018) reported that there was a significant increase in body weight of turkeys raised under red light, whereas light colors did not cause a significant change in live weight gain in geese. When the ducks were reared under blue light, negative results were revealed in terms of blood hormone levels, performance, and carcass quality. In order to optimize profitability, production and animal welfare, and to improve growth and reproduction characteristics, it is necessary to evaluate different light wavelengths and light sources according to the bird species (Akyüz and Onbaşılar 2018).

Solangi et al (2004), who examined the relationship between some behavioral characteristics and light wavelength in broilers, reported that the aggression and fighting behaviors of chickens raised under blue light were lower than those placed under white and red light. In a study conducted by Xie et al (2008) using broilers, the birds were illuminated with red, green, blue and white LED bulbs. Researchers have suggested that lighting with blue light also has positive effects on the stress level. Sultana et al (2013), who investigated the effects of different colored LED lighting on some behavior and welfare levels in ducks, reported that the fear level of ducks with blue and green monochromatic lighting was lower.

The aim of this study is to determine the effects of white (400–760 nm), green (560 nm), yellow (580 nm), blue (480 nm) and red (660 nm) monochromatic lighting treatments on behavioral characteristics of Japanese quails throughout the fattening period.

Material and Methods

The care and flock management of the birds used in this study were carried out in accordance with the relevant laws and regulations of the Republic of Turkey. For the study was carried out with the permission of the Ministry of Agriculture and Forestry, numbered E-22875267-325.04.02-3661514, in accordance with the article 8 (8/k/2) of the relevant regulation, by Akdeniz University Animal Experiments Local Ethics Committee B.30.2.AKD.0.05.07.00 The decision numbered /50 was taken. The study was carried out in the Animal Husbandry Facilities of Akdeniz University Faculty of Agriculture, Department of Animal Science, and Japanese quail (Coturnix coturnix japonica) was used as animal material. The animal material of the study consisted of a total of 300 quail chicks, which were randomly mated and obtained at the same time from a previously unselected parent flock.

In order to make the experimental groups in the study, a total of five lighting experiment groups were created by using chicks. These; It was created from quails in which blue (420nm), white (400-700 nm), green (500nm), yellow (600 nm) and red (660 nm) illumination was provided throughout the rearing period in Japanese quails. Sixty one-day-old chicks, randomly assigned to each experimental group, were placed in the rearing cages in duplicate and their wing numbers were attached. One-day-old chicks were housed in special chick rearing cages in the experimental room, isolated from daylight, with a stocking density of 75 cm^2/quail from hatching to sex determination in the third week. The chicks were housed at 32 °C for the first three days and lowered by 1 °C every three days to 27 °C at the end of the second week. During the experiment, quails were fed ad libitum powder compound feed containing 24% HP and 2900

kcal/kg ME. After the third week, the chicks were transferred to the 5-floor rearing cages with three compartments on each floor and were housed in these cages with a stocking density of 220 cm^2/quail until the end of the experiment. LED lamps are mounted on top of each cage floor and adjusted so that there is no reflection on other cages.
In order to determine the general behavioral characteristics of quails, the behaviors of six female and six male quails from each experimental group were recorded with a digital camera for five minutes, one day a week, in the morning and evening, when the birds were at the age of four and six weeks. Focal sampling method was applied in order to determine the time budgets for the general behavioral characteristics of eating, walking, drinking water, scratching, mating, standing, shaking, cleaning, pecking, wing stretching, lying, and jumping. The camera recordings were then followed by three observers watching from a large screen in the laboratory environment.
In order to obtain time budgets, the total time spent by each individual for 12 behavioral traits during the observation period was determined. Then, parametric test assumptions were tested in the data, and Rank transformation was used for the data that did not fit the Gaussian distribution. Analysis of variance was performed to compare the behavioral characteristics of the lighting groups, and Duncan multiple-range test was applied to reveal the differences between the groups. The significance level was accepted as 0.05 in all statistical tests, and all analyzes were performed using SAS 9.4 software.

Results and Discussion

The time budget usage and statistical analysis results related to performance and locomotor activity behaviors (eating, drinking water, walking, standing, lying) of Japanese quails treated with monochromatic lighting at different wavelengths are presented in Table 1. Similarly, comfort behaviors (cleaning, scratching, shaking, wing stretching) and mating and undesirable behaviors (aggressive pecking, jumping) are also shown in Table 2 and Table 3, respectively.

Table 1. The time budget means (%) and statistical analysis results regarding performance and locomotor activity behaviors

Group	Eating	Drinking	Standing	Lying	Walking
White	22.98	3.27	35.87	17.08[b]	21.38
Green	17.04	2.35	38.53	20.17[a]	18.55
Yellow	20.44	2.90	44.47	6.54[c]	19.39
Blue	24.14	3.58	34.85	16.13[b]	19.14
Red	26.68	4.26	39.91	16.38[b]	18.40
SEM	2.25	0.64	2.54	2.95	1.72
P Value	0.717	0.891	0.775	0.014*	0.982

As can be seen in Table 1, there was no statistical difference between the experimental groups in terms of performance-related behavioral characteristics defined as feed-eating and water-drinking of quails treated with different monochromatic lighting ($P>0.05$). In addition, in terms of lying behavior, which is among the general behavioral characteristics related to the activity of birds, the highest average (20.17%) was observed in quails with green monochrome lighting,

while the lowest average of lying behavior (6.54%) was found in quails with yellow monochrome lighting ($P<0.05$).
There were no statistical differences between the averages of time budgets in terms of species-specific comfort behaviors such as cleaning, wing stretching, shaking and scratching of quails in the experimental groups ($P>0.05$). In addition, in terms of pecking behavior, which is an indicator of aggression, the mean of quails treated with yellow monochromatic lighting was found to be higher than those in other groups (Table 3), while the lowest mean of pecking behavior was found in quails that were illuminated at green and blue wavelengths ($P<0.05$). Similarly, in terms of mating behavior, yellow and red monochrome illuminated quails had the highest mean values ($P<0.05$).
Maddocks et al. (2001) found that ultraviolet wavelengths consistently found higher basal corticosterone levels in birds housed under UV-deprived lighting on broilers, indicating higher levels of stress. Huber-Eicher et al. (2013), who examined the behavioral characteristics of laying hens under green, red and white LEDs, reported that they tended to spend more time feeding under red and white LEDs compared to green LEDs. They reported that the aggressive behavior of chickens was moderate and low under red and green monochrome lighting, but rather high under white monochromatic lighting.

Table 2. The time budget means (%) and statistical analysis results regarding comfort behaviors

Group	Cleaning	Shaking	Scratching	Wing Stretching
White	6.08	1.17	0.93	1.32
Green	5.70	1.49	4.80	1.17
Yellow	6.18	1.24	0.75	1.26
Blue	7.71	1.98	2.49	1.48
Red	4.69	0.80	0.94	0.92
SEM	0.81	0.18	2.38	0.24
P Value	0.844	0.365	0.931	0.968

Table 3. The time budget means (%) and statistical analysis results regarding mating and undesirable behaviors

Group	Mating	Aggressive Pecking	Jumping
White	1.47^{b}	4.81^{b}	0.60
Green	1.30^{b}	1.47^{c}	0.75
Yellow	2.17^{a}	8.50^{a}	0.51
Blue	1.00^{c}	1.97^{c}	1.13
Red	2.14^{a}	6.37^{a}	0.57
SEM	0.26	0.58	0.05
P Value	0.019*	0.005*	0.129

Mohamed et al. (2020) applied blue, green, blue-green combined and white monochromatic lighting in broilers, reported that chickens with blue and green monochrome lighting were more active and less stressful than those in white light, according to the results of the open field test. Sultana et al. (2013a) reported that as a result of different monochromatic lighting applications to broilers, the animals under blue light were calmer and less active, and there was no difference in behavioral characteristics such as eating and drinking water between the experimental groups.

Sultana et al. (2013c), who carried out a similar study in Pekin ducks, reported that animals treated with yellow and white monochromatic lighting were more active and had more social interactions than those in other groups. In the same study, it was reported that the average of comfort behaviors, especially wing stretching, of ducks that were applied blue monochrome lighting was higher than the other groups. When the time budget averages of the behavioral characteristics classified as eating, walking, drinking water, scratching, mating, standing, shaking, cleaning, pecking, wing stretching, lying and jumping of Japanese quails treated with monochromatic lighting at different wavelengths are evaluated, it is seen that the quails that are treated with blue and green monochromatic lighting are evaluated. It was determined that locomotor activities were lower than the other groups. Sultana et al. (2013b) and Mohamed et al. (2014), who reached similar results, reported that green and blue monochromatic lighting creates more calm and sedentary behaviors in poultry, on the contrary, as the light spectrum approaches infrared, birds are more active and an increase in the frequencies of aggression behaviors is observed. The results found in this study regarding general behavioral characteristics were found to be compatible with the findings of Sultana et al. (2013) and Mohamed et al. (2014).

Conclusions

As a result, it was determined that quails treated with yellow and red monochromatic lighting were more active, and exhibited more reproductive behaviors, but on the contrary, they showed more unwanted aggressive behaviors. In addition, it was observed that quails with green and blue monochromatic lighting were calmer.

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FIRST RESULTS ON THE HONEY BEE DISEASES IN BOLU PROVIENCE, TURKEY

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Abstract

Bolu province has a potential to progress in beekeeping due to its rich flora and vegetation. However, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. In this study, first findings of the presence of pathogens and parasites in bee colonies in Bolu province are presented. For the study, 10 apiaries were sampled in the first step of the study. Three diseases, nosemosis, chalkbrood and stonebrood were the first observed diseases in the examined apiaries. However, nosemosis was the most common disease. It was found in the nine (90%) of the examined apiaries. In contrast, chalkbrood and stonebrood were observed only in one apiary. 137 of the 943 examined bee samples were infected by nosema agents. Infection ranged from 1.1 to 51.4%. Average of the infection was 14.52%. The first results stimulate us to think that pathogens and parasites may be important factors of low yield in honey production in Bolu province, Turkey. However, it is needed to increase the number of apiaries sampled to represent the entire Bolu region, investigate other pathogens and parasites and identify each of them at the species level.

Keywords: *Honey bee, Disease, Nosemosis, Chalkbrood, Stonebrood, Bolu, Turkey.*

Introduction

Turkey has a very rich flora because of its geographical location and climate diversity. Despite the richness of the flora in Turkey, the desired increase in honey production cannot be achieved. While the average honey production per hive in the world is 20.1 kg, this rate is 14.4 kg in Turkey. However, the amount of honey production per beehive in the province of Bolu is 8-10 kg, which is below the average (14.4 kg) of Turkey. Despite having sufficient colonies in honey production, bee diseases and geographical and climatic conditions are among the most important reasons for the low yield.

Pathogens and parasites commonly found in bee colonies in Turkey have been identified as bacterial, viral, fungal, protist and mite origin. Among the bacterial diseases, American foulbrood is caused by *Paenibacillus larvae*, European foulbrood is caused by *Melisococcus pluton* and septicemia is caused by *Pseudomonas aeruginosa*. Among the fungal diseases, the causative agents of chalkbrood and stonebrood diseases are *Ascosphaera apis* and *Aspergillus flavus*, respectively (Tutkun 2000; Tutkun and Boşgelmez 2003). *Nosema apis* and *Nosema ceranae* are the leading protozoan diseases (Ütük et al. 2010; Yaman 2018). As a parasitic disease, Varroa spp. parasite is seen. These disease agents are widely known in Turkey. These diseases are among the leading causes of low productivity in beekeeping, particularly in the province and generally in Turkey. However, studies on bee diseases in the Bolu region are scarce. In this study, first findings of the presence of pathogens and parasites in bee colonies in Bolu province are presented.

Material and Methods

Insect Samples and Microscopic Examination

Bee samples were collected from 10 apiaries in Bolu region. Total 943 adult bee samples, 657 dead and 286 living bees were provided in May 2022. After macroscopic examination, dead and living adult bees were dissected in Ringer’s solution and wet smears were prepared. Host fat body, malpighian tubules, gut epithelium, and hemolymph were examined for the presence of pathogens under a light microscope at × 400–1000 magnification (Yaman et al. 2019). When an infection with the pathogen was observed, a part of the material was used for the preparation of Giemsa-stained smears. For this, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain and reexamined under the microscope by using the oil immersion lens. The spores detected by the light microscopy were measured and photographed using a microscope with a digital camera and Soft Imaging System.

Isolation of Fungal Pathogens

Samples of honeybees were collected from apiaries in the vicinity of Bolu, Turkey in May, 2022. The adult bees collected from the hives were placed in plastic boxes with punched lids for ventilation, and were transported to the laboratory for examination. Bee specimens suspected of having fungal disease were subjected to macroscopic examination. Following this, healthy, diseased, and dead adult bees were separated for observation and used to isolate fungi that could cause disease. Isolation and purification of fungal pathogen was carried out individually from dead honeybees suspected according to macroscopic examination. Dead bee bodies covered with fungal mycelium were the most prominent symptom. After macroscopic examination, suspected samples were individually placed into 70% ethanol and gently shaken for 3 min (Poinar 1978). After surface sterilization, considering aseptic conditions samples were washed by a sterilized water. The samples were crushed in 5 ml of sterile phosphate buffer solution. Then, 100 μl suspension was spread on sabouraud dextrose agar (SDA) plates. The plates were incubated at 25°C for one week. Isolates were selected individually by considering colony color and morphology. Pure fungal colony cultures were prepared and stored in petri dishes on SDA.

Results and Discussion

For the study, 10 apiaries were sampled in the first step of the study. Three diseases, nosemosis, chalkbrood and stonebrood were the first observed diseases in the examined apiaries (Fig. 1). However, nosemosis was the most common disease. 137 of the 943 examined bee samples were infected by nosema agents. Infection ranged from 1.1 to 51.4%. Average of the infection was 14.52% (Table 1). The disease was observed with the lowest rate (1.1%) in Yeniçağ/Centre and with the highest rate (51.4%) in Yeniçağ/Kemaller. In total, the nosema infection (15.7%) in dead bees was relatively higher than that rate (11.9%) in living bees. Spores of the nosemosis agents measure ca. 3.30 – 5.17 µm in length and ca. 2.25 – 2.50 µm in width (Figure 1).
Nosemosis disease in honey bees is caused by two microsporidian pathogens, *Nosema apis* and *Nosema ceranae* (Martin-Hernandez et al. 2007; Fries 2010; Higes et al. 2006). These two species have different infection rates and different spore sizes. However, definitive species identification is made using molecular methods (Fries 2010). In the present study, as seen in Table 1, considerable different rates of infection were detected between the examined regions.

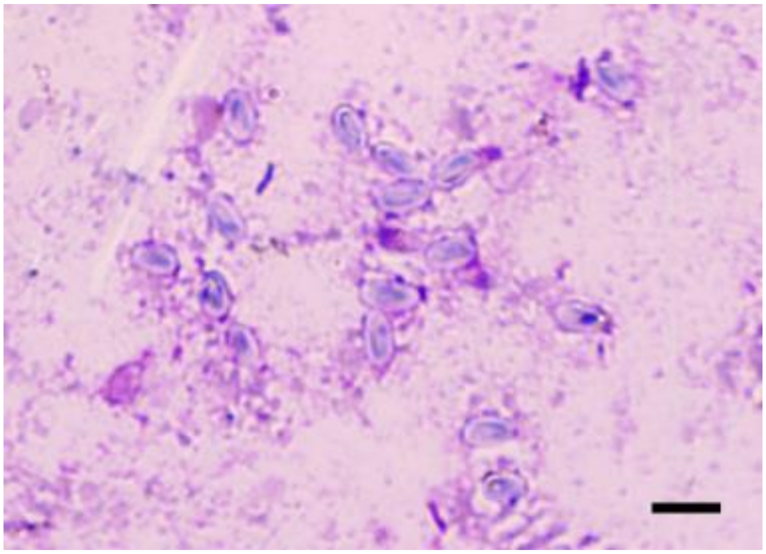

Figure 1. Giemsa-stained *Nosema* spores in honey bees in Bolu, Turkey

Table 1. Occurrence of nosemosis in 10 apiaries in Bolu, Turkey.

Locality	Living bee samples			Dead bee samples			Total		
	Exam. bee	Nosema	%	Exam. bee	Nosema	%	Exam. bee	Nosema	%
Bolu/Kızılağıl	25	4	16	106	28	26.4	131	32	24.4
Bolu/At Yaylası	25	0	0	100	45	45	125	45	36
Bolu/Rüzgarlar	25	3	12	84	5	5.95	109	8	7.34
Bolu/Kındıra	31	0	0	50	0	0	81	0	0
Yeniçağ/Aşağıkuldan	14	0	0	46	3	6.5	60	3	5
Yeniçağ/Aşağıkuldan	9	1	11.1	76	4	5.26	85	5	5.89
Yeniçağ/Aşağıkuldan	40	3	7.5	86	1	1.16	126	4	3.17
Yeniçağ/Centre	69	1	1.4	20	0	0	89	1	1.1
Yeniçağ/ Kemaller	37	19	51.4	50	10	20	87	29	33.4
Bolu/Banaz	11	3	27.8	39	7	17.9	50	10	20
Total	286	34	11.9	657	103	15.7	943	137	14.52

For example, while 36% infection was observed in Bolu/At Yaylası, 1.1% infection was observed in Yeniçağ/Centre, futhermore no infection was observed in Bolu/Kındıra. It is known that *Nosema ceranae* has a much greater effect on bees than *N. apis*, can spread more quickly between tissues, and the disease it causes spreads faster (Paxton et al. 2007; Paxton 2010; Martín-Hernández et al. 2009). In the present study, the nosemosis agents were identified at the genus level as *Nosema* sp. Further studies should be directed to identify them as the species level. On the other hand we observed two fungal diseases in the examined apiaries. Based on the morphological observation of the honeycombs and the colony morphology of the isolates, they were diagnosed as fungal agents causing chalkbrood and stonebrood diseases. It is also needed to identify them as species level.

In contrast to nosemosis found in the nine (90%) of the examined apiaries, chalkbrood and stonebrood diseases were observed only in one apiary. When compared with nosemosis, the fungal diseases show low incidence in honey bees in Bolu.

Conclusions

In this most recent study on diseases in honey bees in Bolu region (Turkey), three diseases, nosemosis, chalkbrood and stonebrood were detected in nine of 10 apiaries examined in Bolu and its region, the diseases occurred at different rates, and nosemosis was the most common disease. The first results stimulate us to think that pathogens and parasites causing diseases in honey bee may be important factors of low yield in honey production in Bolu province, Turkey. However, it is needed to increase the number of apiaries sampled to represent the entire Bolu region, investigate other pathogens and parasites and identify each of them at the species level.

Acknowledgement

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BREEDING POSSIBILITIES AND CURRENT SITUATION OF ANATOLIAN BUFFALO HUSBANDRY IN BİNGÖL PROVINCE OF TÜRKİYE

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Abstract

The aim of this survey is to emphasize the potential of buffalo husbandry and breeding opportunities in Bingöl province of Türkiye. Animal husbandry in Bingöl province is an important source of income for the local people. It is possible to say that water buffalo husbandry has also shown an improvement considerably in the last five years. In fact, the water buffalo husbandry is indispensable for indigenous people. Since there are many wetlands in the province of Bingöl, one of the most important livestock sectors for this region where the people make their living with animal production is water buffalo breeding. For this reason, buffalo husbandry should be expanded in Bingöl province. In this region, the dilemma as such as low yield in buffalo, low number of animals raised, limited marketing opportunity, insufficient reproductive cycle of the animal, insufficient technical knowledge, inadequate cooperatives, inadequate and expensive veterinary service are most problems to create efficient breeding programs. It has been known that breeders perform the water buffalo husbandry in closed housing where they keep cattle and water buffalos together and that they determine the management-feeding requirements of water buffalos according to number of animals. According to the data of 2021, there were a total of 170 head buffalo in Bingöl province. Milk yield, lactation period, birth weight of calves, and daily live weight gain during feeding in Anatolian buffaloes are 800-1000 kg, 200-250 days, 30 kg, and 550-600 g, respectively. The buffalo milk from this breed is consumed by the household as raw milk. In the province of Bingöl, the water buffalo breeding takes place with a very low share in the livestock production sector in Türkiye. However, the preservation and the development of Anatolian buffalo breed as a genetic source is very important in Bingöl province.

Keywords: *Bingöl province, Buffalo meat, Milk, Water buffalo.*

Introduction

Water buffalo husbandry in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications in animal production. With the inquiry works, results are determined as yield of buffalo is low, the number of animals raised are very small, possibility of marketing is restricted, breeding condition of animal is inconvenient, technical knowledge is insufficient, membership at the cooperative is incapable, veterinarian service is expensive. Genetic improvement offers solutions for satisfaction of needs in livestock husbandry.

Water buffalo population in the world was 138 million head whose 97 % are in Asia Continent. Water buffalo were first domesticated in South and South East Asia as a farm animal during the history of human beings (Kreul and Sarıcan, 1993). Water buffalo were raised from very old times past in Türkiye especially as a source of meat, milk and draft power. Water buffaloes were very popular especially for their pull power in the forestry areas and very popular also for their milk fat cream traditionally suits for famous Turkish dessert. Water buffaloes in Türkiye are believed to have originated from Mediterranean water buffaloes, which are a sub-group of river water buffaloes, and are called Anatolian water buffaloes (Soysal, 2006). Water buffalo farming has advantages related to the resistance of water buffaloes to natural conditions and diseases, their ability to benefit from feed and to turn poor feed into meat and milk, and finally, their low cost compared with cows (Canbolat, 2012). The number of animals in Türkiye has been decreased significantly in the last thirty years. The decrease in the number of buffaloes is more serious. In particular, the support given to livestock in recent years has led to an increase in the number of animals in Türkiye. This increase was also reflected in buffalo breeding.

While buffalo breeding had attained importance within the period, buffalo stock of Türkiye demonstrated a declining trend. However, the interest in the activity has been rising due to the supports provided since 2008. As the variety-genre preferences have been rising in the world, Anatolian buffalo gained attention in Türkiye due to external demand. The breeding activities are widespread especially in Blacksea, Central, Eastern and Southern Eastern Anatolia regions. Aegean and Marmara regions of Türkiye followed this geographical orientation (Akpınar et al., 2019). Forty percent (40 %) of water buffalo populations of Türkiye were raised in Central Black Sea region (İzgi et al., 1992), second place with respect of the number of water buffalo was belong to East Anatolian Region. The Aegean and Mediterranean area had lowest number of water buffaloes.

In this study, Bingöl province was chosen as the research area because it will be one of the important provinces for water buffalo breeding in the Eastern Anatolian region of Türkiye. Few studies of water buffalo farming have been found as a result of a literature review. There are no studies on water buffalo farming in Bingöl province. Agriculture is the major source of income in the province, and the pasture and meadow areas suitable for livestock have a high potential for new investments. The most common diseases in buffalo husbandry enterprises of Bingöl province are foot and mouth disease, brucellosis, mastitis. Breeders are generally evaluating their milk themselves. Mostly, farmers are milking by hand.

In the province of Bingöl, the water buffalo breeding takes place with a very low share in the livestock production sector in Türkiye. However, the preservation and the development of Anatolian buffalo breed as a genetic source is very important in Bingöl province. Livestock activities in Bingöl province of Eastern Anatolia are an important source of income for the local people. Bingöl province is one of the most prominent cities of this area in which its public sustain themselves by animal production. For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the water buffalo stock in this province. The aim of this survey is to put forward the current situation and the potential of water buffalo 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, 2022). 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, 2022). Different plant species, distributed in meadow and pasture areas in Bingöl province, are seen as the main food source for nutrition of cattle, buffalo, and small ruminants.

Bingöl city is sixty-second the most crowded city in Türkiye. As of the end of 2021, its population is 283.112 people. The educational status of the buffalo farmers 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, 2022).

The Importance of Water Buffalo Husbandry in Bingöl Province

Despite widespread economic activity in Bingöl province, the animal production sector is not at the desired level. In particular, water buffalo breeding should be one of the most important livestock sectors for the province of Bingöl. Because are;

-the majority of livestock enterprises are small family businesses,

-water buffalo breeding is difficult, animal shelters are not suitable for rearing the productivity of pasture areas is low,

-water buffalo products are not easily marketed, and

-forage crops are insufficient.

It is known that the cultivation made by traditional methods needs some improvements on the basis of the enterprise. With the modern buffalo breeding training to be given to the breeders, the breeding can be beneficial. With the breeding to be carried out under the roof of the organization such as cooperatives and unions, the increase in yield in buffalo can be increased to the desired level, and the evaluation and marketing of products will be made more effective.

As it is known, water buffalo is a late mature when compared to cattle, and the development continues until the age of six. Its lifespan is about 30 years. It reaches the age of sexual maturity later than cattle. Water buffalo heifers reach sexual maturity when 13-14 months old. Therefore, water buffalo heifers can be inseminated when 22-24 months old. However, male water buffaloes can be used as bulls after age of 20-21 months. The breeding age of bulls is approximately 2-15 years (Ermetin, 2017).

The local race bred in Türkiye and named Anatolian Water Buffalo is a farm animal with Mediterranean water buffalo origins, a sub-group of river buffaloes, and it has gained a characteristic structure unique to the conditions of Türkiye. During a period lasting centuries (approximately 1500 years), Anatolian water buffalo has well-adapted to the conditions of Anatolia and Thrace and developed characteristics unique to this area. The color of water

buffaloes in Türkiye is generally black, and they have a backwards curved, arc-like horns. As they have fewer sweat glands than cattle, it is an absolute must-have for them to have a pond or other similar water accumulation in their home range (Ermetin, 2017).
Turkish water buffalo, also called Anatolian Water Buffalo, is practically classified as a river water buffalo of Mediterranean Water Buffaloes group. Mediterranean water buffalo had an origin from Indian water buffaloes according to the data of Dellal (1994). The number of chromosome of Anatolian water buffalo was 25 pair (2n=50) same as river buffaloes. Native Anatolian Water Buffalo breeds were originated from Mediterranean water buffaloes by the results of natural selection. The water buffaloes raised in Trakya region of North West of Türkiye located in South East of Europe have typically black hair and skin colour. They have typically half crescent shape horns directing to back neck. The horns were also big and deep black callow structure (Soysal et al., 2005).
Although water buffalo farming used to be very common in this province, its population has gradually been declined in the last 30 years. Because, roughage and concentrate feed costs have gradually increased. However, an increase in the number of buffaloes has been observed in the last five years due to the incentive practices of the state. But, water buffalo breeders in Bingöl still consider these incentives insufficient. Buffalo husbandry in the study area is carried out with traditional methods. Buffalo production is 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. Buffalo production is indispensable and an important source of income for farmers in Bingöl province. Bingöl 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.
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 internal space 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).
It can be said that the province is rich in terms of underground and surface irrigation sources as well as a suitable land structure for the production of forage crops. However, small ruminant husbandry is a major industrial sector in the Eastern Anatolia of Türkiye (İnan and Aygün, 2018).
To reach the desired level of water buffalo farming, incentives on water buffalo should be maintained, policies should be improved by using input subsidies, producers should be trained on water buffalo farming, and improvements in policies of promotion and benefits of water buffalo dairy products may be useful for establishing a water buffalo culture in Türkiye. Maintaining traditional methods in water buffalo farming prevents targeted efficiency expectations. Therefore, transferring new modern husbandry techniques to producers is an important issue for policy makers (Işık and Gül, 2016).

Number of Buffalo in Bingöl Province

Like the Asian countries, buffalo breeding in Bingöl province has been performed by traditional methods and production per cow is low in comparison with intensive buffalo production systems, but little is known about variation of production traits. Therefore, it is important to carry out the

projects on Anatolian buffaloes for both conserving as a genetic resource and increasing production characteristics.
Characteristics of buffalo husbandry in Bingöl province are small farms with 2 to 3 cows per farm (90-95% of total number of farms). The most common of buffalo breeds in Türkiye is the Anatolian water buffalo breed. It is very important to define the condition, potential and problems of breeding sector especially with respect to the water buffalo, the cattle, sheep, and goat stock in this province. Correspondingly, it could be possible to find short, average and long term solutions for the identified issues. The most important of these problems is roughage. Anatolian buffalo is regarded as an important genetic and cultural source in Bingöl province. However, population size of Anatolian buffaloes was dramatically decreased between 1970 and 2020. It has been thought that we can lose this genetic source, which is important for both the economic and cultural aspects, if we do not have any conservation program for this animal species in future.
Inventory studies constitute the basis of all kinds of studies that are planned to be carried out at national or regional level. Therefore, important suggestions for the Bingöl region will be presented with this study. Number of buffalo and cattle is presented in Table 1 in Türkiye and in Table 2 in Bingöl province.

Table 1. Number of buffalo and cattle in Türkiye (head) (TOB, 2022).

Year	Buffalo	Cattle	Total
2015	133766	13994071	14127837
2016	142073	14080155	14222228
2017	161439	15943586	16105025
2018	178397	17042506	17220903
2019	180826	18070500	18251326
2020	188771	18626219	18614990
2021	185574	17850543	18036117

Table 2. Number of buffalo and cattle in Bingöl province (head) (TOB, 2022).

Year	Buffalo	Cattle	Total
2002	1265	61905	63170
2020	166	144408	144574
2021	124	132183	132307

As can be seen in the table, there has been a serious decrease in the number of buffaloes. There could be many reasons for this decrease. Most importantly, there has been a shift from buffalo to cattle breeding across the country. In addition, the demand for buffalo products has also decreased. The numbers of indigenous water buffalo are very 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 water buffaloes are found to be small in numbers. Therefore, the milk productivity of the indigenous buffalo is very low. Improvement in productive and reproductive performance is the key for increase the milk production. There is a need to increase the milk production in relatively low performing dairy states to achieve future milk demand and make dairy farming sustainable for farmers.

The milk productivity of water buffaloes in the region is low and it is more obvious especially on small farms. Thus, milk yield should be increased by improvements in animal production. In this way, milk yield could reach a desired level (Işık and Gül, 2016).

Buffalo Products in Bingöl Province

Considering the animal production situation in Bingöl Province, a significant part of which is based on animal husbandry, it is not at a sufficient level. Especially the meat production in Bingol province does not show the animal husbandry potential of the province. The main reason for this situation is that the number of slaughtered animals and most of the meat production are not carried out by official institutions or are not reflected in the statistics of Bingöl due to the slaughtering in other provinces (Esen, 2017).
Demand for buffalo products was lower than the other animal products in Bingöl province. In a market-oriented definition, sustainability of production relies on maintenance of marketing and producer satisfaction accordingly. Producer satisfaction is interrelated with consumer satisfaction, raising awareness and development of the market with a marketing point of view. Motivating consumer satisfaction is also related to raising producer satisfaction and improvement of marketing chains and structures in other terms. Lack of market demand and weak market structures also result in negative producer reflections and non-progressive stance in breeding.
It is considered that the size of the establishment has no effect on cattle and buffalo breeding practices (Özyürek et al., 2014). Unfortunately, in most of the enterprises studied in the region, there are no suitable environments for animal husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. As a result, the profits of the breeders will increase with the livestock breeding in the region and will contribute to the country’s economy. Meat and milk products from buffalo in Türkiye are presented in Table 3.

Table 3. Meat and raw milk products from buffaloes in Türkiye (tons) (TOB, 2022).

Year	Meat	Milk
2015	5300	62751
2016	5470	63085
2017	5868	69401
2018	6515	75742
2019	7150	70341
2020	8424	75300
2021	10831	63643

In Türkiye, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of animal farms. In large-scale researches conducted in 2016, it is observed that the bodies of buffaloes and cows in milk processing are unacceptably dirty (up to 70%), thus causing frequent foot, breast and reproductive health and milking hygiene problems (TOB, 2022).
The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the husbandry. Then, solution suggestions should be presented to

remove or minimize these risks. Taking precautions for occupational health and safety are very difficult, costly and time consuming. Also, not all agricultural activities carry same risk, and, as noted above, there are many special populations that must be considered.

Conclusion

All scientific research so far has shown that the water buffalo husbandry in this region is the traditional production system. In addition, we think that databases for Anatolian buffaloes have under developed, showing that they have a wider range of data because of problems in herd management system. Especially in some seasons, buffaloes have been hold at night outside, and therefore in these regions it is difficult to develop and pursue the effective data collect processes. The most common of buffalo breeds in Bingöl province is the Anatolian buffalo breed. The milk from this buffalo breed is either consumed by the household as raw milk or in making cream and yoghurt. As a result, preservation and development of native buffalo breed as a genetic source is very important. If the current potential especially in the Eastern Anatolian Region and in Türkiye is evaluated, it can become important in this region. It can be said that Bingöl province has an important place in terms of its features for buffalo presence and animal production. Buffalo breeding which will contribute to the economy of Bingöl province should be strengthened with plans and projects and should be encouraged again in a way to obtain high yields.

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OVERVIEW OF BREEDERS REGARDING OCCUPATIONAL HEALTH AND SAFETY IN ANIMAL HUSBANDRY IN TÜRKİYE

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Abstract

In this survey study, it is aimed to provide sensitivity of breeders about occupational health and safety and to compile information about occupational safety and accidents that can be encountered by workers at animal production in Türkiye. It is also aimed to provide suggestions for occupational health and safety in animal husbandry. Animal production is associated with a variety of occupational illnesses and injuries. The issue of occupational health and safety in animal production is very important as it is in many other areas. In general, possible dangers of 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. In practical work related to crop and livestock production to ensure safety and to prevent accidents at work, it is important to take necessary precautions. In such an environment and in a certain direction, employees are exposed to occupational accidents. The most common hazards at the animal production in Türkiye are the zoonotic diseases, the ergonomics, the noise, the air conditioning, the chemicals, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases etc. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for the workers at the livestock enterprises, the field, and the factories dealing with the feed, the skin and the meat. However, over the past decade, it has been observed that the relevant ministries have put in legal regulations related to the issue. In Türkiye, preventive measures have started to be taken on occupational health and safety in livestock production.

Keywords: *Animal breeders, Occupational accident, Occupational disease, OHS culture.*

Introduction

Occupational Health and Safety has significant economic implication particularly in terms of medical costs and economic productivity losses. The impact of industrial related accidents and injuries cuts across several sectors of work there by causing substantial human and economic cost to the world at large more so in developing countries where safety at work is always compromise. Available data reveals an alarming and extremely high rate of work-related deaths and injuries in both the developed and developing nations. Research has also shown that each weekday fatal injuries occur every two hours whilst disabling injury occur every eight hours. It is estimated that every year over 1.1 million people worldwide die of occupational injuries and work-related diseases. In developing countries, the risks that foster ill health are estimated to be 20 times higher than in developed countries (Demba et al., 2013; Gyekye, 2006).

The Occupational Health and Safety (OHS) has a vital importance in animal husbandry. It has also significant economic implication particularly in terms of medical costs and economic productivity losses. Occupational safety and accident risk factors of revealing awareness of breeders are not yet fully come to avoid. There are many factors which limit the economic efficiency for production. One of them is production losses due to the workplace accidents and the occupational illness. The issue of occupational health and safety in animal production is very important as it is in many other areas (Aygün et al., 2014; 2019a).

Most Occupational Health and Safety accidents and incidents at workplaces are not reported in Türkiye. The most common hazards at the animal production in Türkiye are the zoonotic diseases, the ergonomics, the noise, the air conditioning, the chemicals, the animal attacks, the bites, the injuries, the accidents in transport, the psychological stress, and the skin-borne diseases etc. Especially, the animal hitting and the zoonotic diseases are very important in animal husbandry. Therefore, the precautions related to the 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., 2019b). The most common complaints of workers are minor cuts and injuries, followed by headaches, and musculoskeletal pains. However, there are major limitations to this data, as many of the organizations never maintain accident/injury records at their facilities. There is urgent need to develop the data collection/reporting system (Demba et al., 2013).

The risk factors that the livestock workers faced vary according to the sector. In sheep-goat husbandry, the most important task in the care-feeding and management of animals falls into workers. Occupational Health and Safety (OHS) has significant economic implication particularly in terms of medical costs and economic productivity losses.

Sheep and goat breeding in Anatolia has been adapted to regional differences and has been characterized by the prominence of different applications (Aygün, 2017). It is true that more occupational health and safety intervention research focusing on preventing illness and injury 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). Animal production is associated with a variety of occupational illnesses and injuries.

The object of this review is to provide sensitivity about occupational health and safety, accidents, diseases, and musculoskeletal disorders in animal production sector. It is also aimed to provide suggestions for occupational health and safety that increase production in animal breeding. This information has been prepared based on the personal observations and the experiences directly in the local area.

The state of occupational health and safety and working conditions were revised periodically either as a whole or by dividing into specific fields in order to detect problems, to develop efficient methods for the solution of these problems, and to evaluate priorities and consequences in this paper.

The Place and Importance of Animal Husbandry in Türkiye

The livestock activities in Türkiye have been characterized by the different regional applications. The activities of animal breeding sector are especially an important source of income for the indigenous people in Türkiye.

Commonly, it was aimed to be offered some information about living culture and stockbreeding activities of nomadic tribes that they come from the south to east because of weather warming up with. Studies made by some researchers in order to achieve their political goals on political and ideological studies relating to a multi-ethnic structure and stockbreeding activities that is the main source of income of the politicized tribes were the main theme of this study. One of the most important issues to be taken into account for sustainable animal breeding sector in Türkiye is no doubt farming culture. Some researchers have emphasized that culture of communities consisted of some narrow range cultures, and that national culture also consisted of many local, regional, or sub-cultural backgrounds (İnan and Aygün, 2019).
In Türkiye, livestock sector has a considerable potential and is an important part of agricultural sector and economy. The livestock products, including meat, milk, eggs, honey, wool, and hides, play a significant role in the Turkish economy. In general, animal production constituted approximately 25 % of agricultural production value. The sector's contribution to farm income is substantial, and activities related to livestock production and marketing are important to the economic development of rural areas in Türkiye. In parts of country where agriculture and farming are limited because of land shape or limited land and high number of population, households make their living with animals, especially in Eastern and Southeastern Anatolia. However, in these regions, mostly traditional techniques are used, and the results are not much satisfactory when compared to developed countries (Aygün, 2021).
Livestock products are an important source of household income for many farmers and households in rural areas. For small farmers, livestock products such as cattle, sheep and goat generate income and ensure food security for these households because an important amount of their incomes comes from the sales of animal and milk.

The Most Common Occupational Accidents in Animal Husbandry

Agriculture and especially animal husbandry are one of the most dangerous industries; it has the highest risk of work-related fatalities and non-fatal injuries in the Türkiye. Currently, it still lacks well-established standards, regulations and guidelines to protect agricultural workers. Part of the reason is the complexity of the working environment in agriculture - the work places and tasks in agriculture have a lot of variety and are hard to control individually. However, it is possible to improve occupational health and safety in agriculture by building up well-controlled and safe facilities, particularly in animal production. Therefore, one of the preliminary purposes in this project is to identify the health and safety issues in animal agricultural operations and then explore the present practices to build a safe facility for improving occupational health and safety in animal production.
The economic activities contribute to the overall national income and to employment. However, there is associated high risk in some occupations in particular the activities and waste generated in health care facilities, activities associated with servicing, manufacturing and building construction industries among others (Von Essen and McCurdy, 1998).
The hazard is anything that has the potential to harm. Hazard can affect the person, the material and the process. Also, hazards can cause accidents, diseases, loss of product, and machine damage etc. The occupational risk refers to the combination of the likelihood and severity of an injury or illness resulting from exposure to a hazard.
Since livestock workers spend a great deal of time outdoors, they are at risk for physical stress from excessively cold and excessively hot environments. The magnitude of heat and cold stress

problems in agriculture is not well documented. Tolerance to such environments varies among individuals and may be difficult to predict. Livestock workers should be provided the means to compensate for extremes of temperature. For example, adequate water supplies while working outdoors in hot climates are essential.

In animal husbandry sector, the most important task in the care-feeding and management of animals falls into workers.

Workers who are away from social habitats and who work in the hills may be exposed to allergies or poisoning caused by the attack of various wild animals, such as bee or insect bites, as well as plants grown in the spring, pollen of fungi or various flowers. Employees are camels exposed to the sun because the work area is mostly open space. Therefore, excessive exposure to sunlight can cause dermatological problems.

Since livestock workers spend a great deal of time outdoors, they are at risk for physical stress from excessively cold and excessively hot environments. The magnitude of heat and cold stress problems in agriculture is not well documented. Tolerance to such environments varies among individuals and may be difficult to predict. Livestock workers should be provided the means to compensate for extremes of temperature. For example, adequate water supplies while working outdoors in hot climates are essential.

Workers in animal husbandry have exposure to other hazards that may increase their risk of health problems: climate-dependent problems, such as heat stroke or cold shock, and occupationally caused infections such as *anthrax, ascariasis, encephalitis, leptospirosis, rabies, salmonellosis, tetanus, and coccidioidomycosis.* Sensory problems are common: eye problems, caused by irritation, infection, or injury from the wind, sun, dust or soil, agricultural chemicals, debris ejected from farm machinery, and allergic reactions to plants, and hearing problems due to noise from farm machinery and cannery work.

The Most Common Occupational Diseases in Animal Husbandry

Disease transmission between animals and between domestic animals and wildlife is an increasing issue for food safety in the location and site design of animal production facilities. Likewise, manure handling can create social conflicts, and almost every action in feeding and caring for animals requires the utilization of equipment. The location of power lines, driveways and movement patterns of machines, and any equipment utilized must be considered an integral aspect of design guidelines to enhance the safety and health of workers and emergency personnel - as typically required for mainstream commercial buildings.

Occupational diseases can go undiagnosed and untreated and worst of all, effective preventive measures are not taken because of lack of awareness to the problem. It is therefore believed that with 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 the taking remedial measures (Demba et al., 2013).

The most hazardous is toxic gas generated from manure, such as ammonia, hydrogen sulfide, methane, and carbon dioxide, because they are usually stored in confined systems without proper ventilation. High levels of toxic gases accumulate fast in enclosed and limited spaces and could be fatal; workers without proper personal protective equipment (PPE) can be killed in only a few minutes. Besides, inadequate ventilation leads to oxygen deficiency and may result in a deadly outcome to workers and animals.

An "occupational disease" is any disease or disorder contracted primarily as a result of an 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 (WHO, 2020).
Occupational diseases and accidents that can be encountered by workers have caused the losses of very serious economic and the qualify persons in animal husbandry. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field (Aygün, 2017).
Zoonotic disease is naturally called vertebrate animals to humans, and humans to animals to diseases or infections. Zoonoses are infections that are spread from animals to humans. 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. The situation is not static, and emerging zoonotic diseases are continually being recognized, both animal diseases which have spread to humans for the first time and existing zoonoses spreading to new geographical areas. 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 waste water 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)
Farmers' lung is one of many forms of *hypersensitivity pneumonitis*. This problem is becoming rare, which is likely due to the reduction of exposure to organic dust from the increasing mechanization of agriculture and the effect of livestock health and safety programs (Von Essen and McCurdy, 1998).
Another danger for farmers 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).

Some Recommendations and Precautions Regarding OHS at the Animal Husbandry

Essentially, the Occupational Health and Safety Unit is mandated to perform many duties including the following:
-Enforcement of existing legislations with regards to Occupational Health and Safety at workplaces;
-Systematic inspection of workplaces including factories, warehouses, building construction sites and related premises;
-Monitoring of Occupational Health and Safety at workplaces;
-Investigation of workplace accidents;
-Training of employers and employees on health and safety issues at workplaces;

-Studies and surveys (Demba et al., 2013).

Due to the lack of sufficient cooperation between institutions and organizations and statistical data, and the fact that this issue has never been dealt with in a systematic way, it is rather difficult to define a system in Türkiye. In spite of these positive developments, it should not be disregarded that there is still a great deal of things to be accomplished in the field of OHS.

Due to the fact that occupational health and safety involves a multi-disciplinary approach, besides the existing definitions of expertise; other OHS experts such as; hygienists, epidemiologists, toxicologists, and ergonomics specialists should also be taken into consideration.

The scattered structure of communication, coordination, policy, planning and organization between the institutions and organizations that are defined as a part of the OHS system and that are involved in OHS activities directly or indirectly should be eliminated and a complementary, contributive and supportive structure should be established.

There are not any scientific research and education institutions specific to the field of OHS. There is a need to carry out new research activities on the casual relationship between OHS and working conditions. In this respect, establishment of departments and areas of specialization specific to occupational health and safety at universities would improve the quality and quantity of the required work. For this purpose, universities should be encouraged to carry out research and investigation in fields related to occupational health and safety.

It is extremely important that the breeders and the organizations engaged in animal husbandry have knowledge of occupational health and safety. The nature of animal breeding requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly 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 production. Workers and animals must be vaccinated against various zoonotic diseases.

With regard to the control of occupational zoonoses, there are some general control measures which 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, 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, 20221).

For certain zoonoses, there is an effective vaccine available and it may be appropriate to administer this 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 recognise 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).

The rules of order and hygiene must be taken into accounted during the milking and the shearing of the animals. Improved water supply should be combined with improved sanitation, special needs of women workers, and a separate toilet in each household to facilitate personal hygiene.
Undertaking studies of occupational health risks in this population with these considerations will not only contribute to the understanding of such risks but can also further preventive efforts and lead to better health in this high-risk population. Effective prevention can reduce suffering and death and contribute to enhanced productivity in the workplace. In this way, both the employers and the employees gain (Aygün and Demir, 2015).
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. Also, not all agricultural activities carry the same risk, and, as noted above, there are many special populations that must be considered. Occupational diseases and accidents that can be encountered by livestock workers at business have caused the losses of very serious economic and the qualify person in animal production (Aygün, 2015).

Conclusion

In this paper, the state of occupational health and safety and working conditions were reviewed periodically either as a whole or by dividing into specific fields in order to detect problems, to develop efficient methods for the solution of these problems, and to evaluate priorities and consequences.
Occupational accidents and diseases that can be encountered by workers have caused the losses of very serious economic and the qualify persons in animal husbandry sector. Therefore, the precautions related to the occupational health and safety must be taken for workers at the husbandry and the field. In Türkiye, preventive measures have started to be taken on occupational health and safety in livestock production.

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TRANSHUMANCE ACTIVITIES AND PROBLEMS IN SMALL RUMINANT HUSBANDRY IN BİNGÖL PROVINCE OF TÜRKİYE

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Abstract

In this survey, the transhumance activities and the problems in small ruminant husbandries in Bingöl province of Türkiye have been discussed. Also, detailed information about highland families in Bingöl province dealing with stockbreeding activities has been given. This information has been prepared based on the personal observations and the experiences directly in local area. In transhumance small ruminant husbandries, sheep flocks are moved to cool highlands with plenty of grassy plains when heat begins to increase and towards the end of spring. The grazing period in animals lasts 3-5 months under the control of shepherds. After weather cools down, highlanders and animals return to villages or to their settlements. Sheep herds usually consist of 300 to 500 heads. The migrants are mostly from Şırnak, Batman, Mardin, and Siirt provinces to the Bingöl highlands. These migrants make as pedestrian their arrival and return to the plateau. Shepherds in their family are responsible for maintenance and feeding of animals on the plateau. Shepherds are taken to the highlands by grazing or by road transport. One of the most important examples of livestock farming is highland small ruminant husbandries in Bingöl province. One of the most important problems of the migrants in the region is undoubtedly related to the education of our children, especially girls, which is emphasized in every platform that is important in the future of the country. The results indicate that solving the problems of nomadic and semi-nomadic families is very important to sustain stockbreeding of sheep and goats, and to benefit Bingöl province economically. Bingöl province has ecological conditions suitable for animal production. If the current potential especially in Bingöl province is evaluated, it can become very important in Eastern Anatolia Region of Türkiye.

Keywords: *Bingöl province, Goat, Nomadic life, Sheep, Transhumance.*

Introduction

Bingöl is a very suitable province for sheep and goat breeding due to its priorities such as superiority in taste and aroma quality of meat due to its natural medicinal aromatic plant pattern. Bingöl province has also an important potential in terms of ecological sheep and goat husbandry. Small ruminant breeding is done more extensively in this province so very low input is used in many branches of animal husbandry. Sheep and goat breeding is mostly based on pasture. 80-90% of the feed requirements of sheep and goats have been met from natural grazing areas such as pastures and plateaus. At the same time, Bingöl province is also a province suitable for small ruminant husbandry in terms of large pasture areas, water resources and climate characteristics. Meadows and pastures constitute 52% of the total land of the province. It can be said that the province is rich in terms of underground and above-ground irrigation resources, as well as a suitable land structure for the production of forage crops.

Eastern Anatolia Region of Türkiye has a different character than other regions in terms of climate conditions. In the winter months, when there are severe storms in this region, extreme cold occurs. According to the investigations in the region, sheep breeding pasture-based are dominant (Aygün et al., 2019; İnan and Aygün, 2019). For this reason, it is very important to define the condition, potential, and problems of breeding sector especially with respect to the small ruminant husbandry in this city.

Although it varies according to the regions, the established system in sheep and goat breeding, highland sheep breeding and nomadic sheep breeding are the most common breeding systems. All three cultivation systems have been practiced in the Eastern Anatolia Region for years. Generally, in plateau and nomadic sheep breeding systems, animals are milked by berivans. Different definitions can be made for women who milk sheep and goats in different regions (Aygün, 2021; Aygün and Alarslan, 2021).

It can be said that the fact that sheep breeding is carried out in herd rather than management level at the enterprise level has an important effect on the spread of plateau and nomadic sheep farming with the effect of structural difference. This situation is more evident in the Eastern and Southeastern Anatolia Region in Türkiye. In this sense, since sheep farming is mostly done by nomadic families or tribes, it is important to compile information about this section and to determine the general characteristics of the nomadic families (Aşkan and Aygün, 2020).

Animal production is one of the most important economic sectors of Türkiye. Sheep production systems in Türkiye depend on factors such as the natural and socio-economic conditions of the regions, the availability of feed resources, the connection to plant production and, the consumption habits of the population. These are systems of the stock breeding, the highland sheep husbandry, and the nomadic livestock breeding (Kaymakçı, 2010).

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).

This review concludes that the transhumance activities and the production habits of breeders in sheep and goat have been discussed. This information has been prepared based on the personal observations and the experiences directly in local area.

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, 2022). 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, 2022). Different plant species, distributed in meadow and pasture areas in Bingöl province, are seen as the main food source for nutrition of small ruminants, cattle, and buffalo.

Bingöl city is sixty-second the most crowded city in Türkiye. As of the end of 2021, its population is 283.112 people. The educational status of the farmers and families in 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, 2022).

The Importance of Small Ruminant Production and Transhumance Activities in Bingöl Province

The province of Bingöl has a suitable environment and a very important potential for the production of all kinds of animal products in terms of its current socioeconomic and geographical characteristics. However, it cannot be said that this potential of the province is used rationally and efficiently. In particular, some macro-economic policies implemented have put the livestock sector in a period of decline over time. For Bingöl province, the livestock sector has also a socio-economic duty to prevent population migration from rural areas to cities.

Sheep and goat production is 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, milk, and wool. There are breeds such as White Karaman, Red Karaman, Awassi, Dağlıç, Kıvırcık, and Karayaka among local sheep breeds of Türkiye (İnan and Aygün, 2019). Small ruminant production is indispensable and an important source of income for farmers in Bingöl province.

Sheep and goat in this region has been adapted to regional differences, and has been characterized by the prominence of different applications. In the highland sheep production, sheep flocks are removed to the highlands with cool and plenty of grassy plains by pressing hot and dry towards the end of spring.

For a period of 3-5 months, sheep remain in control by shepherds in the highland. After the weather cools down, sheep go back to the villages or the farms in the plain. Sheep herds usually consist of 300 to 500 heads. Each sheep is composed of lots of different people with a lot of expenses, depending on the number of animals contributes. Sheep herds were formed by gathering business owners with a different number of animals. Sheep herds are taken to the highlands by grazing or by road transport.

One of the most important examples of livestock farming is highland small ruminant husbandry in Bingöl province. In order to get more abundant the products such as milk, cheese, wool and so on, the people of the region have to go to the highlands with the arrival of spring animals to find better grazing and water areas. With the arrival of spring to the first zone in the region is exited. With the start of the cold days of autumn again return to the settlements. In this province, it is recommended to keep records in order to obtain a sustainable income source from small ruminant production. 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.

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).
The numbers of indigenous sheep and goat are high 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 sheep and goat were found more in numbers. But the milk productivity of the indigenous sheep and goat breeds is very low. Therefore, improvement in productive and reproductive performance of sheep and goat is the key for increase the milk production. There is need to increase the milk production in relatively low performing dairy sates to achieve future milk demand and make dairy farming sustainable for farmers. The population of sheep and goat in its native tract is decreasing steadily and there is no information on its present status in its home tract. The sheep-goat breeders in this region are forced to migrate from the home tract to the cities along without their flocks and herds, due to economic and social problems of the region.

The Importance of Berivans and Shepherds in Small Ruminant Production

Berivan and shepherd workers are inseparable parts of each other. They are the most important workers of highland and nomadic animal production. Their ages may range from 10 to 70. Berivan is called as women milking the ewe and the nannie. Milking in migrant small ruminant breeding systems is done by berivans. Berivans are not the only ones who are responsible for sheep milking. They are laboring at all stages of the processing of the obtained plumbing. Berivans and shepherds have many challenges for in nomadic small ruminant husbandry (Aygün, 2017; Aygün, 2021).
Berivans, who go to the plateau by crossing the rough terrain and steep slopes on horseback, return to their villages from the plateau after loading the milk they milked from the sheep and goats on their horses. They go to the highlands on the backs of horses, mules and donkeys. In the past, they used to walk all the way but now they drive to a certain place. They may encounter various dangers during this journey. Berivans contribute to the family budget by selling the cheese, butter and yoghurt they make from the milk they milk.
Berivans and shepherds are one of the most underserved and understudied populations in the Türkiye. One characteristic of the traditional nomadic lifestyle group is that berivans and shepherds themselves are certain fixed tents that they find themselves under dangerous conditions and for long hours (Aygün, 2021).

Some Recommendations and Overview of the General Problems of Breeders in Small Ruminant Production

One of the most important issues to be taken into account for sustainable small ruminant breeding in the region is no doubt transhumance living culture of tribes that they presented this culture and breeding system to nowadays. Unfortunately, in most of the farms studied in the region, there are no suitable environments and management conditions for small ruminant husbandry. The environmental conditions must be arranged so as to be suitable for animal husbandry. In accordance with the regulation of environmental conditions, breeders must be equipped as technical information. As a result, the profits of breeders will increase with the livestock breeding in the region and will contribute to the country's economy (Aygün, 2021).

Türkiye has an important place in the production of fleece with carpet type in the world. However, the wool industry and production in Türkiye have not developed and fluctuations of the wool prices negatively affected the wool of production. Recently, there has been an increase for sheep production in Türkiye and for use of rough-mixed wool in the world (Öztürk and Odabaşı, 2011).

In this region, there are serious problems in the use of litter that provides dryness and softness in the sleeping and resting places of small ruminant farms. In large-scale researches conducted in last years it is observed that the bodies of animal in milking and shearing processing are unacceptably dirty, thus causing frequent foot, breast and reproductive health, shearing, and milking hygiene problems (TOB, 2022).

It is extremely important that the breeders and the organizations engaged in animal production have knowledge of occupational health and safety. The nature of livestock husbandry requires organization that is its own appropriate in accordance with local conditions for the occupational health and safety. These organizations should be units that are tried to be prevented by determining at the source of the danger. For this aim, the risks at work should firstly be determined. Then, solution suggestions should be presented to remove or minimize these risks.

Sevinç (1972) reported in her study in this region that collected the solutions of the problems of nomadic ovine livestock in 5 items in general and said that it is necessary;

-To protect the pasture and meadow vegetation in the winter lands with the highland and highland slopes and to take continuous measures to ensure their development,

-To identify and secure migration routes in the most convenient way,

-To provide a continuous and secure market order for migratory sheep breeders,

-To improve and increase the efficiency of the nomadic ovine animals with effective measures compatible with the lives of migratory ovine livestock families,

-To bring order to a structure that will modernize ovine livestock within a certain period of time for migratory sheep breeders. Unfortunately, it can be said that these problems are still not resolved today.

The main problems related to animal husbandry can be listed as follows:

- Economic losses due to animal diseases,
- Insufficient appropriations allocated in the budget to combat diseases,
- The enterprises in the province are very small and scattered,
- Inadequate use of technology,
- Insufficient genetic potential,
- Unregistered situations.

One of the most important problems of the breeders in the region is undoubtedly related to the education of children, especially girls, which is emphasized in every platform that is important in the future of the country. Children can always start their education life one step behind. Girls do not even have such a chance, in this way of life.

General recommendations for breeders could be:

-Breeders need to be organized in order to find solutions to their problems more effectively and quickly.

-Quality and hygiene issues should be given importance.

-Breeders need to be trained on occupational health and safety.

-Producers have problems in selling because of the scarcity of the product they obtain. They should come together and organize on this issue.
-It is necessary to learn basic breeding practices in order to obtain more efficiency from animals. Especially making selection in the most effective way is the most important breeding method.

Conclusion

In this survey, the transhumance activities and the problems in small ruminant husbandries in Bingöl province of Türkiye have been discussed. Also, detailed information about highland families in Bingöl province dealing with stockbreeding activities has been given. This information has been prepared based on the personal observations and the experiences directly in local area. It is hoped that it has now been understood by the state that migratory breeders, which have been neglected in many ways for many years, are people engaged in animal production in the Eastern Anatolia Region of Türkiye.
The results indicate that solving the problems of transhumant families is very important to sustain stockbreeding of sheep and goats, and to benefit Bingöl province economically.

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GRANULATED ANIMAL FEED ADDITIVES ON THE BASIS OF SEA BUCKTHORN BIOMASS

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Abstract

Our study aimed to evaluate sea buckthorn (SBT) 'Maria Bruvele' twigs and leaves biomass remaining as waste after berries harvesting, as a raw material for animal feed additives, with the investigation of their composition and nutritional potential. In this study, the initial chemical characterization of SBT waste biomass was carried out using the method of analytical pyrolysis. The results of analytical pyrolysis testified to the presence of condensed tannins (CTs) in the composition of the biomass, which are undesirable components for animal feed due to their possible negative effect on animals' weight gain and taste of the feed. Separation of CTs from biomass was carried out by extraction by a water-ethanol (1:1, v/v) solution. For the evaluation of SBT biomass residue after extraction as a raw material for the production of feed or feed additive, total protein, crude fat, ash, wood fibre, microelements (P, K, Ca, Na), and vitamins (A, C, E) content in the dry matter were determined. The amount of heavy metals (Hg, Pb, Cd) was determined as well, and by the Commission Regulation (EU) No 1275/2013, their amount in the analyzed biomass samples did not exceed the maximum permitted amount for animal feed. The SBT biomass granulation was carried out using the laboratory scale flat die pellet mill KAHL 14-175. The SBT-based feed pellets' properties, such as durability, moisture, bulk density, disintegration in water, and average pellets length were investigated. The investigation proved that SBT biomass is a unique underutilized source for animal feed additives.

Keywords: *Sea buckthorn twigs and leaves, biomass, granulation, animal feed additives, pellets.*

Introduction

The development of the market for feed additives is stimulated by the economic need to optimize the cost of feeding the industrial herd and by the current unstable situation with the supply chain. Problems associated with the insufficient amount of raw materials, the growing cost, and quality indicators, lead to a mismatch of diets with the physiological needs of animals and an imbalance in nutrients. This all leads to a decrease in zootechnical indicators. The situation opens the “gateway” to the market for a wide range of active compounds, forms, compositions, and combinations of feed additives, which should, to one degree or another, increase the effectiveness of diets. The wood and leaves of sea buckthorn (SBT) contain an extensive complex of general nutritive and biologically active compounds that are not inferior to grasses, so they can serve as a valuable top dressing for animals in winter, as well as for field fodder production.
SBT (*Hippophae rhamnoides L.*) is a nitrogen-fixing and pest-resistant deciduous shrub tree, which grows widely in Europe, the high-altitude cold regions of Asia, and North and South

America. The industry uses fruits, but there are almost no applications for the green part of SBT, which comprises around 12-15% of the harvested mass. Upon harvesting at the production scale, a large amount of SBT biomass waste is produced. Finding applications for the SBT biomass waste is necessary for the sustainable use of resources, which is the task of the European Green Deal, and for the creation of additional income for SBT growers and workers in rural areas (Stobdan, 2017; Bruvelis, 2014). It was reported in the literature that SBT twigs contain respectively: Na (1.3-27.7 mg/kg biomass), Ca (600-2000 mg/kg biomass), K (40-120 mg/kg biomass), and P (6 g/kg biomass), carotene (42-61 mg/kg biomass), C vitamin (50-200 mg/kg biomass) and E vitamin as tocopherol (60-390 mg/kg), as well as 10-19 g/kg of protein, 270-320 g/kg of wood fibres, up to 100 mg of chlorophyll, and a number of essential amino acids (Janceva, 2022).

The SBT biomass contains a high amount of condensed tannins. Our previous investigation on the chemical characterization of SBT biomass showed that the content of CTs ranged from 6 to 8 % (Janceva 2022). Condensed tannins (CTs) contained in SBT biomass under investigation are valuable components for the pharmaceutical and cosmetics industries, but they can be dangerous for animals. According to many authors, the addition of CTs to the diets of animals provides diminished weight gains and lowered efficiency of feed utilization, as well as increased fecal nitrogen. These effects have been explained by the inhibition of the dietary protein digestion by tannins (Butler, 1989). Therefore, the isolation of tannins should become a necessary technological part of the production of lignocellulosic-based feed additives for livestock.

The aim of this study was to evaluate twigs and leaves biomass of SBT 'Maria Bruvele', remaining as waste after berries harvesting, as a raw material for animal feed additives, with the investigation of their composition, separation of CTs, granulation of the remaining biomass, and evaluation of the obtained feed additives nutritional potential.

Material and Methods

The twigs and leaves of SBT cultivar '*Maria Bruvele'* were collected from the SBT plantation in Latvia under the same growing conditions in the late summer of 2020. The twigs were dried at room temperature, ground with a knife mill (Cutting Mill SM100, Retsch, Haan, Germany), and sieved to select the particles between 1- and 4 mm.

The chemical characterization of SBT biomass was performed by analytical pyrolysis (Py-GC/MC/FID) analysis using a Frontier Lab (Fukushima, Japan) Micro Double-shot Pyrolyzer Py-3030D (pyrolysis temperature 500˚C, heating rate 600˚C s^{-1}) directly coupled with the Shimadzu GC/MS/FID-QP ULTRA 2010 apparatus (Japan) equipped with a capillary column RTX-1701 (Restec, Metairie, Louisiana, USA) and a 60 m × 0.25 mm × 0.25 mm film (injector temperature of 250˚C, ion source with EI of 70 eV, MS scan range m/z of 15-350, carrier gas helium at the flow rate of 1 mL min^{-1} and a split ratio of 1:30). The identification of the individual compounds was performed based on GC/MS using Library MS NIST 11 and NIST 11s, whereas the relative area of the peaks of individual compounds was calculated using Shimadzu software based on GC/FID data. The summed molar areas of the relevant peaks were normalized to 100%, and the data of four repetitive pyrolysis experiments were averaged.

CTs isolation from biomass was provided by convective extraction with ethanol-water (1:1, v:v) solution, at the temperature 60ºC. After SBT biomass extraction, SBT biomass residue was analyzed as an animal feed additive. The total protein content in SBT biomass residue was determined by the Kjeldahl method and the protein content was estimated using an appropriate

Nitrogen Factor (NF-6.38). Crude fat content was determined by extracting the fat from the SBT biomass residue using a solvent (hexane), then determining the weight of the fat recovered. Ash content was measured as a residue after ignition at 550 °C in a Carbolite ELF 11/6B furnace. The content of microelements including heavy metals will be measured using ICP-MS method.
For evaluation and analysis of SBT biomass residue as a feed additive, it was mixed with a chemically characterized commercial animal feed. Both biomasses were mixed in an AVA HL 30 mixer (drum volume 0.020 m^3). Commercial feed (CF) composition (Rigas kombinetas lopbaribas rupnica Ltd., Riga, Latvia) was: moisture-12%; total protein 16.25, wood fibres 4.70 %, crude fat 2.60%, P 0.62%, Ca 3.70%, Na 0.17%).
SBT biomass granulation was carried out using the laboratory scale flat die pellet mill KAHL 14-175 (small-scale analog of the industrial scale KAHL granulators) with a channel diameter of 6 mm, and a ratio of channel length to a diameter equal to 4:1. The feeding of raw material was started after the die was preheated to 50 °C that which testified that equilibrium temperature was achieved. During extrusion of biomass, the temperature in the die was raised to 70-80°C and then almost did not change. The enhanced temperature in the die led to a decrease in moisture content in biomass after granulation. The size of one batch used for granulation was 2 kg. Bulk density and durability of pellets were determined in accordance with European standards EN 15103, EN 15210-1 correspondingly. All experiments were done in triplicate.

Results and Discussion

The results of analytical pyrolysis show that the products of thermal degradation of SBT biomass (twigs, leaves) consist of a wide range of aliphatic and aromatic compounds (Table 1). Carbohydrates are the dominant organic matter as per the analytical pyrolysis of SBT biomass. The carbohydrate content was 21.4 and 27.6% for twigs and leaves, correspondingly. The volatile substances of both SBT biomasses have a significantly high content of phenol derivatives, including G- and S-type phenols, related to lignin and other polyphenolic compounds. The content of phenol derivatives is approximately similar (8%) for both biomasses. N-containing compounds, the source of which could be amino acids or serotonin are found more in the leaves than in the twigs.

Table 1. SBT biomass characterization by analytical pyrolysis method

Products of thermal degradation of SBT biomass	**%, from the chromatogram**	
	Twigs	Leaves
Summary: Carbon dioxide, Water	51.95	58.09
Carbohydrates	27.16	21.43
Lignin/Phenols derivates	8.44	8.87
N-containing	4.84	8.03
Other compounds	7.62	3.58

Aromatic pyrolysis products of SBT biomass were studied in more detail. Maria Bruvele's twigs have a high content of 1,3-diols in pyrolysis products, which allows us to consider Maria Bruvele twigs as a potential source of condensed tannins (CTs) (as an antioxidant or antibacterial agent for pharmacology or cosmetics, or for production of the adhesives). But since CTs are not desirable in animal fodder, before SBT biomass assessment for obtaining of feed additives, the CTs were separated from biomass by extraction with 50% ethanol-water solution. After CTs separation, the main components indicators of feed additives were determined: total protein, crude fat, and wood fibre. It was found that the leaves and twigs (Table 2) are characterized by a high content of protein (18.4 and 18.9%) and wood fibres (13.2 and 27.1), low ash content (2.9 and 3.7,) and moderate fat content (5.8 and 1.3%). Fat reduction, especially for leaves, can be achieved by additional pre-treatment of the SBT biomass, such as extraction with a non-polar solvent, e.g. hexane, or by a freon extraction that today is one of the most innovative, effective, and topical methods for extracting of lipophilic components from lignocellulosic biomass.

Table 2. Main macronutrients content in SBT biomass and in the samples of granulated animal feed on its basis

Samples	**Total protein, %**	**Wood fibres, %**	**Crude fat, %**	**Total ash, %**
100% leaves	18.4	13.2	5.8	3.7
100% twigs	18.9	27.1	1.3	2.9
20% twigs+80 % CF*	16.8	10.4	2.3	8.6
20% leaves+80% CF*	17.0	6.4	3.2	9.3
50% twigs after extraction+50 % CF*	17.3	18.9	2.1	6.4
50% leaves after extraction+50 % CF*	17.6	8.9	4.2	8.4
10% leaves+10% twigs+80% CF*	16.9	7.8	2.7	9.0
10% leaves+10% twigs+80% CF* with molasses sugar (3% on biomass weight)	16.9	7.8	2.7	9.0
Commercial feed (CF*)	16.3	4.7	2.6	-

Particular attention must be paid to the inorganic part of the feed additive, play an important role in the structural and physiological processes of the animal's body. The obtained data for 100g of SBT biomass showed that it contains: Na (1.72-22.5 mg/100 g biomass), Ca (281-989 mg/100g biomass), K (1109-1376 mg/100g biomass) and P (220 - 225 mg/100g biomass). In addition, SBT biomass contains micronutrients as an extensive complex of water-soluble and fat-soluble vitamins (Table 3). The content of C vitamins in the twigs is much higher than in the leaves. The obtained data for 100g of sea buckthorn showed that leaves and twigs contain, correspondingly: C vitamin (15.6 and 178 mg/100g biomass) and E vitamin, as tocopherol (30.9 and 17.3 mg/100g biomass).

Table 3. Content of macro-and micronutrients (water-soluble and fat-soluble vitamins) in SBT biomass

Samples	**P, mg/100g**	**K, mg/100 g**	**Na, mg/100g**	**Ca, mg/100g**	**C vitamin mg/100g**	**E vitamin mg/100g**
Leaves	225±22	1376±113	1.72±0.40	989±237	15.6±4.4	30.9±4.3
Twigs	220±22	1109±107	22.5±5.2	281±67	178±50	17.3±2.4

An important characteristic is the content of heavy metals in the composition of feed raw materials, that constitutes one of the threats to the health of living organisms. According to the Commission Regulation (EU) No 1275/2013, impurities of heavy metals have not to exceed the permissible norms (Table 4).

Table 4. Heavy metals content in SBT biomass under study

SBT samples	**Cd, mg/kg**	**Hg, mg/kg**	**Pb, mg/kg**
Leaves	0.011±0.003	0.0069±0.0012	0.086±0.0022
Twigs	0.027±0.006	0.0031±0.0006	0.084±0.0018
Maximum permissible values, EU Commission Regulation No. 1275/2013	1	2	10

For obtaining feed pellets, Maria Bruvele’s twigs and leaves with/without commercial feed (Figures 1 and 2) were granulated using a KAHL laboratory granulator (3 kW) equipped with a flat die (channel diameter = 6.00 mm), which is usually used to produce fuel pellets samples.

Figure 1. Feed pellets: A - 50% twigs after extraction+50 % CF; B - 10% leaves+10% twigs+80% CF; C - 50% leaves after extraction+50 % CF

Figure 2. Feed pellets from 100% *Maria Bruvele’s* leaves (on the left); feed pellets from 100% *‘Maria Bruvele’s* twigs (on the right)

The obtained pellets had the following characteristics: length 8-12 mm, durability 62-97%, bulk density 687-749 g/cm^3 (Table 4). In addition, the granulation with molasses (MS) as an additive (3% of total biomass) was performed, where MS not only made the biomass sticky, increased the durability and diminished the amount of fines in the pellets) but also gave a sweet taste to the feed.

The feed pellets’ disintegration in water was observed visually. The feed pellets from 100% twigs or leaves swelled within 30-60 min., but in composition with commercial feeds, feed pellets swelled within 2 min.

Table 4. The properties of feed pellets obtained from SBT biomass with/without addition of CF and MS

Samples (T – twigs, L – leaves, LAE / TAE – leaves/twigs after extraction)	**Pellets durability, %**	**Pellets moisture, %**	**Bulk density, g/cm^3**	**Average length, mm**
T 100%	96.8	5.8	712.6	12
L 100%	97.2	5.6	715.4	12
20% T % CF*	76.4	5.8	687.5	8
20% L +80% CF*	62.0	5.6	749.9	8
50% TAE +50 % CF*	83.9	5.4	697.2	10
50% LAE +50 % CF*	68.4	5.6	610.4	10
10% L +10% T +80% CF*	83.0	5.8	689.1	8
10% L +10% T +80% CF*+ MS	83.2	5.7	701.3	8

Conclusions

The investigation proved that underutilized SBT biomass, after separation of the condensed tannins, could be a valuable unique source for animal feed additives. The high content of protein, wood fibre, macronutrients, and vitamins of SBT biomass can improve feed quality. SBT biomass can serve as a valuable top dressing for animals in winter, as well as an alternative feed for field fodder production. The granulation of the SBT biomass, which is useful for logistics purposes, and could potentially improve also digestibility and decrease the number of harmful pathogens, could provide the animal husbandry industry with a diversified source for animal nutrition, using different combinations of biomass with or without commercial feed additives or molasses. This opens possibilities for the creation of various feed compositions relevant to the nutritional needs of the animals.

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INFLUENCE OF BIOR PREPARATION ON BOAR SPERMOGRAM

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Abstract

The aim of the research was to study the influence of biologically active preparations, extracted from Spirulina platensis (BioR) on the quantitative and qualitative indices of the usual spermogram in boars. The biological studied material was the Landrace boars and the semen material taken from them, raised at M. S. Moldsuinhibrid, Orhei, Republic of Moldova. The experiments were carried out between July and August, the months with hot temperatures. The boars were administered BioR preparation intramuscularly 0.3 ml / 100 kg live mass for 5 and 10 days, the preparation being produced at the Institute of Microbiology and Biotechnology of the Republic of Moldova. The sperm indices were studied before administration and 50 days after cessation of administration. The results showed that the mobility of sperm after 5 days of administration was 90%, after 10 days - 91%, compared to the control group where the mobility was 82% lower; the volume of ejaculate was 213 ml after 5 days and 229 ml after 10 days of administration of the preparation or by 25 ml and 41 ml, compared to the control group, and the fecundity increased by 3.29% and 4.57% compared to the control group where this index was only 75.83% (80.4% when the BioR preparation was administered for 10 days, and it was 79.12% after 5 days of administration).

Keywords: *Spirulina platensis (BioR), boars, sperm indices.*

Introduction

Animal husbandry is a dynamic branch of science and practice mainly imposed by the economic factor. The means by which the reproductive process is coordinated are diverse and often having mutual independent influences. Directing the breeding activity contributes to increasing the obtained production and the economic efficiency, respectively, by making the most of the potential of genetically superior animals. The breeding strategies used to raise pigs are in a continuous renewal along with the "old" ones, elaborating new strategies that are more and more efficient. They mainly refer to artificial insemination, spermatogenesis management, embryo transfer, etc. (V. Nauc, 1991; Darie G.; 1983; 2006, Boronciuc G., 2003; Bogdan A, 1999; Bogdan L.M. 2000). These biotechnologies make it possible to obtain the greatest genetic advances in farm animals. The success in the application of artificial insemination is determined by the knowledge of the particularities of spermatogenesis and their mechanism of regulation. Therefore, the opportunity of studying the efficient improvement of the sperm production, at the same time with the increase of the sperm material resistance with the conditions of its conservation, especially in the critical periods of the year, is of a perspective.
The research conducted at the Institute of Microbiology and Biotechnology of the Republic of Moldova (Rudic V et al., 2006; Darie G and cool., 2006) have demonstrated the beneficial effect of biopreparations of algal origin obtained from the strain Spirulina Platensis on the breeding

animals’ spermograms from zootechnical point of view. They are of interest for the investigation of the biologically active preparation obtained from Spirulina Plantesis on the function and the reproductive system and the resistance of the semen material under conservation.

Material and Methods

The research was carried out on Landrace pigs. First of all, 3 batches of calves of 5 heads each were formed, two experimental and one control. The experimental groups were administered the preparation BioR, with a concentration of 0.5% for a duration of 5 and 10 days, the administration dose 0.3ml/100 kg live weight, head/day.
The BioR preparation was obtained in the Microbial Products laboratory of the Institute of Microbiology and Biotechnology in the Republic of Moldova.
The preparation has a varied content of proteins, carbohydrates, fats, amino acids, lipids, vitamins, macro and micro elements, etc.
The BioR preparation has the ability to be included to a different degree in the complex mechanism at the level of the hypothalamus - pituitary gland - testicles, thus, sensitizing and influencing them beneficially.
At the same time, the following indices were determined: the homeostasis of blood lipids and proteins - which was determined using the semi-automatic analyzer STAT-fax 3300; the spermogram indices being evaluated by the computerized method "CEROS". The inoculation of semen in sows was carried out intrauterinely using the Golden-Pic instrument in a volume of 1 ml/kg live weight but not more than 150 ml sperm with a total number of sperm cells with rectilinear forward movements equal to 3 billion/dose. The fecundity rate of females after the first insemination was previously established according to the number of non-returning females 16-21 days after inoculation and definitively according to the results of parturition.

Results and Discussion

The experimental data on blood lipids homeostasis under the influence of the administration of the BioR preparation to the reproductive boars are presented in Table 1.

Table 1.The effect of the BioR administration on blood lipids homeostasis in breeding boars

Experimental groups	Total lipids	β-lipoproteins, Un / l	Triglycerides, mmol/l	Cholesterol, mmol/l
I (10 days)	2,67±0,256* 3,80±1,329**	0,103±0,010 0,076±0,012	0,315±0,025 0,378±0,064	1,998±0,107 2,188±0,233
II (5 days)	2,69±0,183* 4,95±1,744**	0,107±0,07 0,053±0,004	0,413±0,032 0,223±0,030	2,140±0,296 2,33±0,174
III (control)	2,83±0,419* 4,73±1,666**	0,092±0,007 0,070±0,001	0,227±0,022 0,240±0,030	1,360±0,126 2,010±0,330

* Data obtained after 10 days (II) and 5 days (III) of administration of the preparation;

** Data after 35 days after the cessation of administration;

The experimental data on the homeostasis of blood lipids show that, along the way, both the total lipids and the studied fractions underwent changes. The total lipids after administration of the bioextract showed a tendency to decrease the blood concentration by 4.95% in the animals from group II in which the preparation was administered for 5 days compared to the control group. At the same time, when doubling the administration period of the preparation (duration of a boar spermatogenesis cycle) in group I, the value of this index shows a decrease equal to 5.66% compared to the control group. After 35 days from the cessation of the administration to all groups of animals, there is an increase in the concentration of total lipids in the blood with the intensification of the differences between the control group and the experimental ones. The blood concentration of total lipids in animals from group I is lower than in the control group by 19.34%. The concentration of total lipids in the animals from group II is 30.26% higher than the results recorded in group I and under the same conditions it prevails the results of the control group by 4.65%.
The experimental data on the influence of algal preparation (BioR) administration on blood protein homeostasis are presented in Table 2.

Table 2. The effect of BioR preparation on blood protein concentration

Experimental groups	Duration of admin.	Proteins	Albumin
I (control)	0	81,37±1,03 *	41,87±0,203*
	35	82,17±1,087**	36,53±2,769**
II (10 days)	10	83,37±3,424*	38,12±1,104*
	35	77,6±5,263**	47,67±1,231**
III (5 days)	5	83,90±5,970*	34,33±1,317*
	35	77,60±5,263**	38,03±1,690**

* Data obtained after 10 days (II) and 5 days (III) of administration of the preparation;

** Data after 35 days after cessation of administration;

The data presented in Table 2 show contradictory changes in blood protein concentration. After the administration of the preparation, there is a tendency to increase the concentration of blood proteins in the boars from the experimental groups compared to the control group, constituting 3.90% in group III and 2.45% in group II.
After 35 days from the cessation of the drug administration, the prevalence of blood protein concentration is maintained only in the boars from group III, the value of blood protein concentration being identical to the control group. We note that the protein metabolism proved to be more indifferent to the administration of the bioextract and prevailed the control group only in the situation when during the administration period it was doubled.
The administration of the BioR preparation to the breeding boars favored some indications of sperm production. The unique properties of the cells are some of the factors that move it to its destination - the anterior third of the oviduct to achieve its biological destination. In the practice of reproduction it serves as an index of fecundity prediction, we evaluated the effects produced by BioR on the mobility of semen cells after collection (fig.1).

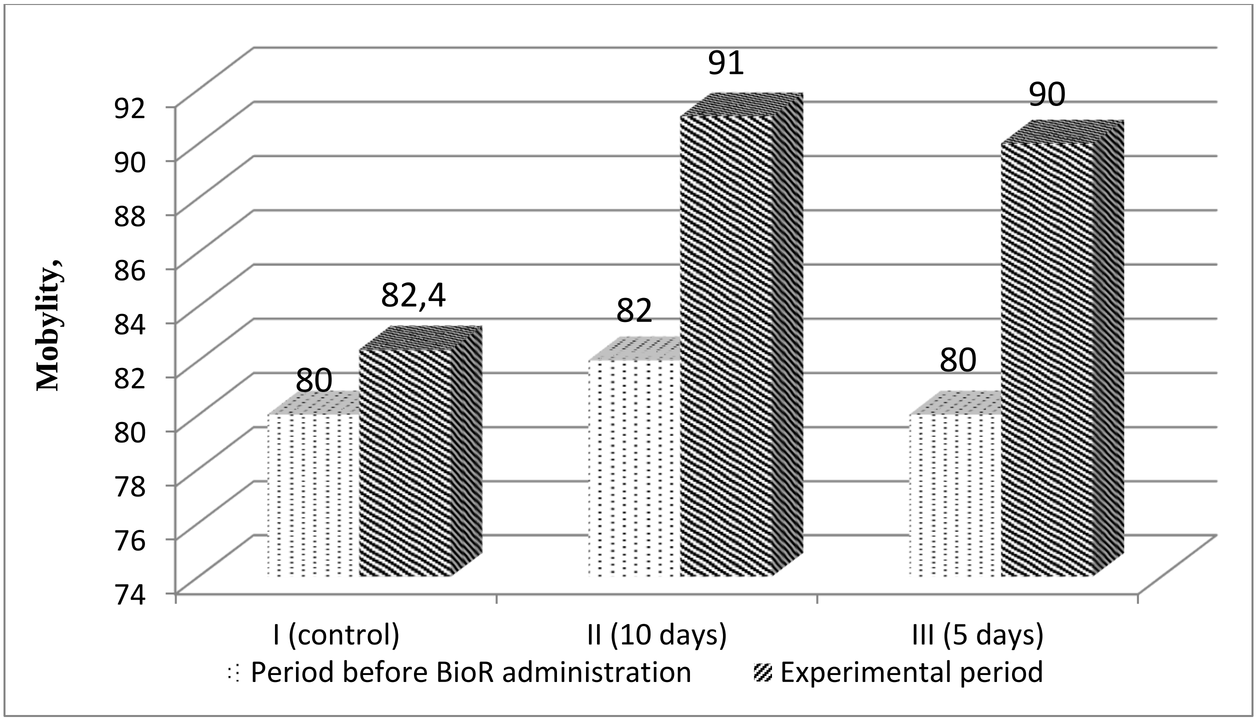

Figure 1. The dynamics of semen cell mobility under the influence of BioR administration, %

The data in Fig. 1 indicate the presence of differences between the mobility of native sperm cells in boars from the experimental groups, compared to those from the control group. As the mobility was identical until the administration of the preparation, at the end of the experimental period the spermatic mobility in group II progressed over the control one by 10.44%. The mobility in the semen collected from the animals from group III increased by 9.22% compared to the control group.

At the same time, we evaluated the influence of the bioextract on the ejaculate volume (fig.2) in the breeding boars.

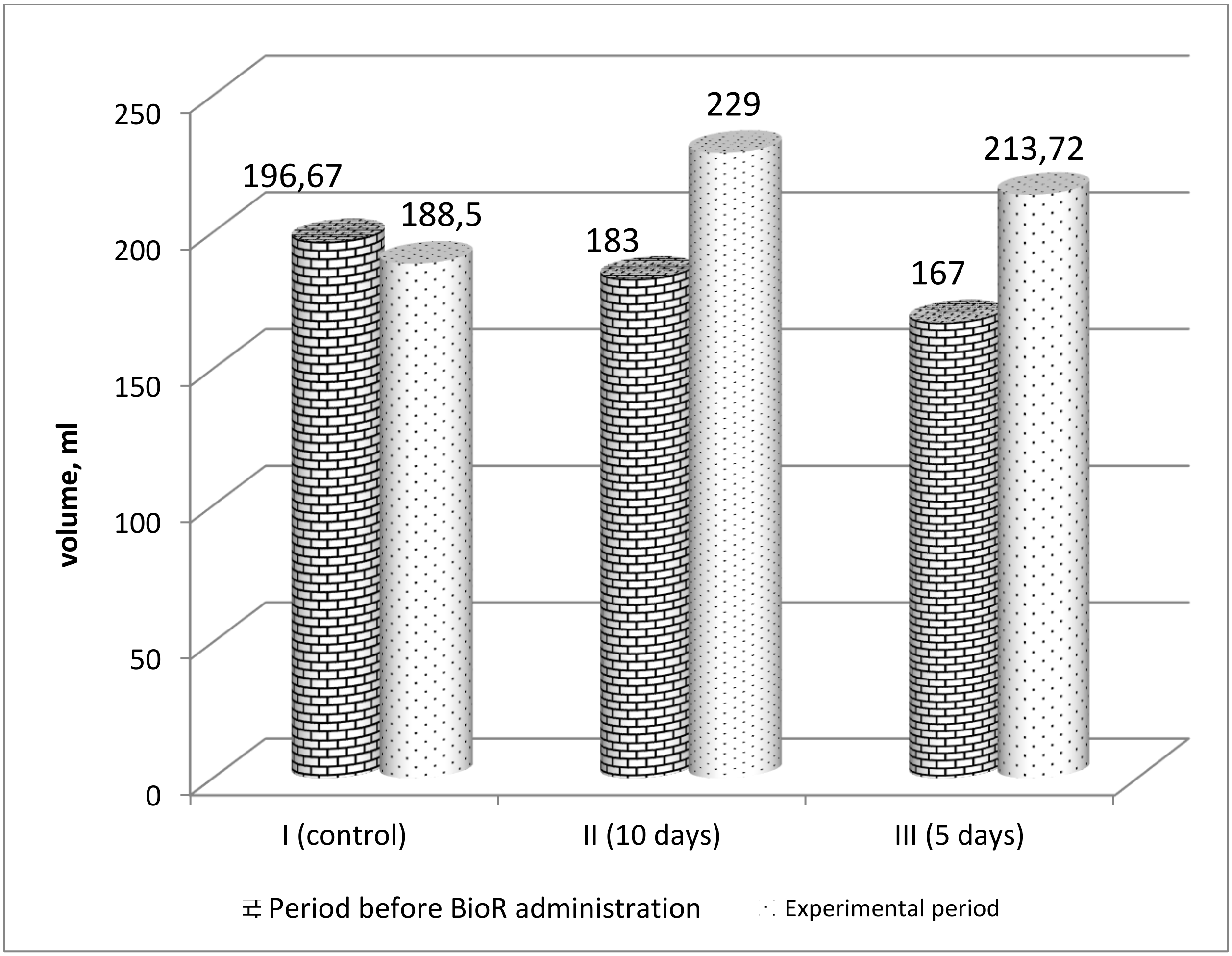

Figure 2. The influence of BioR administration on the ejaculate volume in breeding boars, ml

The experimental results reflect changes in favor of the experimental groups. At the end of the experimental period, the sperm mobility in the boars from group II increased by 21.48% compared to the control group. The same trend is manifested in the boars from group III, regardless of the fact that the administration period was 5 days, the volume increase was 13.38.
The next step was to establish the effect induced by the experienced preparation on the fertilizing ability of the semen obtained from the experimental boars (fig. 3.).

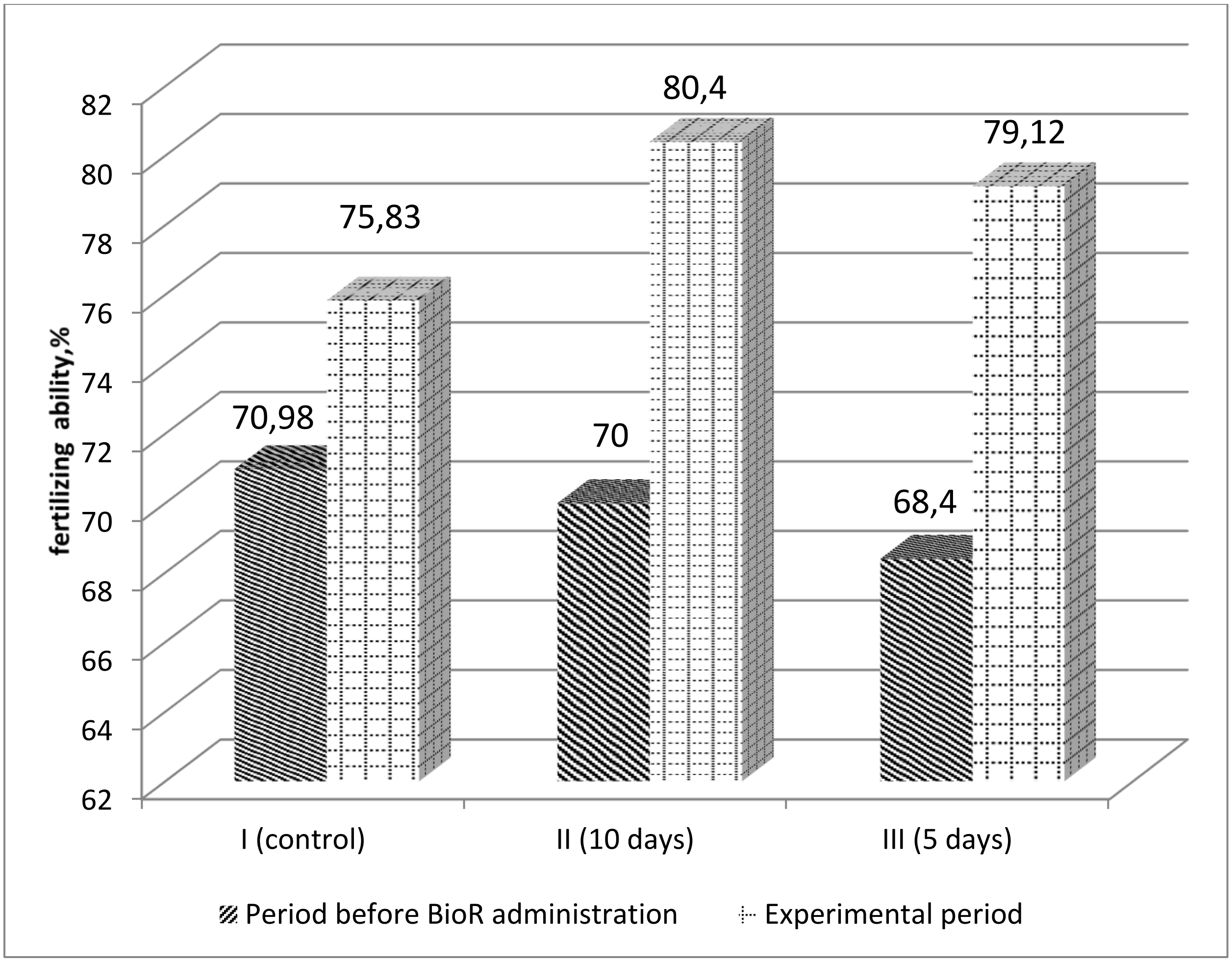

Figure 3. The effect of BioR administration on the fertilizing ability of the semen,%

The results presented in Fig. 3 show that the administration of algal bioextract contributed to the increase of the sows’ ability to fertilize. The most relevant results were obtained in the animals from group II, in which an increase in fertility was by 4.57%. In group III, the fertilizing capacity of the sperm prevails over the control group by 3.29%.

Significant progress has been made in the prolificacy of sows, inseminated with the semen obtained from the boars during the experimental period (Table 3).

Table 3. Prolificacy of sows according to the semen used for insemination.

Experimental groups of boars	Proliferation, heads		±, in relation to the control during the experimental period %
	Experimental period	Experimental period	
I (control)	10,83±1,30	10,84±1,22	100
II (10 days)	9,06±0,61	11,83±0,55	+9,13
III (5 days)	9,19±0,49	11,23±0,71	+3,60

The analysis of the experimental data shows that the prolificacy of the sows inseminated with the sperm of the boars from group II increased by 9.13% compared to the control group. For the sows inseminated with the semen collected from the boars in group III, the increase was 3.6%.

Conclusions

The BioR preparation of algal origin produced anabolic effects depending on the duration of the administration period of the preparation, it favors the metabolism of total lipids (when administered in 5-day regimen), triglycerides and blood β-lipoproteins and cholesterol (when administered in both regimens) and the concentration of these fractions in the blood;
It favors the indications of the usual spermogram in boars, mainly the ejaculate volume and the moderate mobility of the sperm cells in the ejaculate;
By improving the indices of the usual spermogram in boars, it moderately favors the percentage of fecundity and the prolificacy of the sows.

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PRESENCE OF SEXUAL DIMORPHISM BUT NO ECOTYPES AMONG FREE-RANGING MUSCOVY DUCKS IN GUATEMALA

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Abstract

In this study, we investigated the sexual dimorphism between males and females of Muscovy ducks raised freely in three different local communities in Guatemala. The analysis was based on 14 important morphological traits -arm length, shank length, total height, head length and head width, body length and width, dorsoesternal length, croup height, body, breast and abdominal perimeters, metatarsus perimeter and body weight- in a sample constituted by 137 males and 225 females. Males presented higher massiveness than females, but being less stocky and legged. The discriminant analysis revealed that males could not be grouped into groups, but females from Camotán appeared separated from the rest. Detailed analysis demonstrated that most differential variables **(**abdominal and chest perimeters) were not related to ethnological traits, so Camotán group can be considered as a mere topotype (associated with artificial selection and breeding) rather than an ecotype (associated to local ecological conditions). Sexual dimorphism is well manifested. The smaller body exhibited by the females as compared to the males can be a reflection of their adaptive strategy to the stressful environmental and nutritional conditions, as smaller size would reduce the maintenance feed requirements and increase feed efficiency in this free range system where feed resources are limited in terms of quantity and quality. The results of this study might be effective in characterization and conservation of the Muscovy duck in Guatemala and to register this breed internationally. But there is a need to study carcass and egg quality as well as variability at molecular levels to clarify the similarity with ducks from other Iberoamerican countries.

Keywords: *biodiversity; criollo; drake; indigenous; morphological variation.*

Introduction

A breed is a homogenous group of livestock with definable, specific, and identifiable physical traits (FAO, 2006) its individuals being distinguished on the basis of morphological and biometrical traits. Improvement of domestic animals to meet human needs is dependent on variation within and between breeds because such variation gives room the opportunity for selection.

Duck production is largely a traditional enterprise and has not yet been industrialized as that of chicken (Yakubu, Kaankuka, & Ugbo, 2011) (Veeramani, Prabakaran, Selvan, Sivaselvam, & Sivakumar, 2014). Even though, duck is still a neglected species among researches on domestic animals. If the volume of work pertaining to the phenotypic and genetic constitution of the indigenous breeds of Guatemala is still very small, information on indigenous domestic ducks of Guatemala is null.

The wild Muscovy duck (*Cairina moschata* Linnaeus 1758) is native to the Americas (Donkin, 1989), its distribution comprising both coasts of Mexico, Central America and most tropical regions in South America (Hernández, Muñoz, Valencia, Posso, & Muñoz, 2007) (Schaaf et al., 2018). The wild species, called too Greater Wood Duck or Forest Duck, has the hindlegs projected more to the front with respect to the base of the body than the ducks of the genus *Anas*, has strong and sharp claws, well-developed hind fingers and broad wings (Donkin, 1989), and an average male size of 66 to 86.5 cm, a body weight of 2 to 4 kg, the male being considerably larger than the female (Donkin, 1989) (Baéza, Williams, Guémené, & Duclos, 2001) (Almeida et al., 2014).

The derived domestic species is called *criollo*, *pato real*, *pato perulero*, *pato almizclado*, *pato mudo* and Berbery duck. It is a rustic animal that does not require complicated installations for its breeding, is resistant to diseases, has a high prolificity, and presents a good precocity in fattening as well as a great capacity to take advantage of food rations (Igwe, Okoli, & Okeudo, 2003) (Almeida et al., 2014) (Abdeltawab, Salha, Allam, & Fandy, 2017). They are primarily raised for meat and eggs, although, they provide other materials of economic value such as feathers (Etuk, Abasiekong, Ojewola, & Akomas, 2006). In small villages around Guatemala, Muscovy duck represents an interesting meat and egg source. Its feeding base is scavenging, consisting of anything edible found in the immediate environment, as well as small amounts of grain supplements provided by the locals. So feed supplies from home and the environment is varied according to farming activities in the household, season of the year and the life cycle of insects and other invertebrates. Under this present system Muscovy duck probably performs rather poorly. The use of multitude of different body measurements available has lead several researchers to use multivariate techniques to simultaneously examine the relationship among body measurements and production traits in chicken (Jáuregui, Flores, Vásquez, & Oliva, 2015) (Fitsum, 2015), turkey (Ogah, 2011), pigeon (Uribe, Senar, & Camerino, 1985) (Parés-Casanova, 2013) and duck (Cuesta, 2008) (Yakubu, 2011). The first step for effective conservation of Muscovy duck in Guatemala is to perform a characterization based on the phenotypic morphological traits (Oguntunji & Ayorinde, 2014). Hence, the work was proposed to study morphometric traits of this species. Phenotypic character study is the basis for the differentiation of groups and/or breeds and provides support for conservation programs; it is a technique used to characterize genetic resources in many countries. Many tools are available that can help determine the discriminatory power that variables have in describing breed patterns. These also aid in evaluation of the relative importance of variables, saving time and financial resources invested by researchers. In this field, multivariate analysis techniques have been very useful in support of characterization studies of breeds. Multivariate analysis (cluster analysis, main component analysis, canonical analysis, discriminant analysis and correspondence analysis) refers to all statistical methods that simultaneously analyse multiple measurements in a single individual and that are interrelated. It has been widely used in studies of breed characterization and genetic diversity as it provides descriptive analysis of the differences between populations, considering all variables together, providing a data overview. Multivariate analysis allows for greater discriminating power, eliminating those difficult to measure variables and those that contribute little towards explaining variation. The objectives of this study are (1) to examine the morphometry of the free-range Muscovy duck from Guatemala and the sexual dimorphism; (2) to determine possible ecotypes (understood as a locally adapted population assumed to be a result of the action of natural selection). The present results might aid in better tools for the conservation and improvement of this species in the country.

Material and Methods

Sampled animals

We studied randomly selected 376 adult Muscovy ducks extensively reared in three different local communities, which have different cultural and geographical features, in Guatemala: Camotán (Chiquimula), San Manuel Chaparrón (Jalapa) and Olintepeque (Quetzaltenango). Due to logistical constraints, not all measurements were possible on all birds, and those with one or more lacking measurements were excluded from the study. Final sample was of 362 adult Muscovy ducks (137 males and 225 females). Measurements were restricted to apparently healthy birds that conformed to the species' classification descriptors. Handling animals was practiced in accordance with ethics.

We studied the following parameters, according to standard literature (Cuesta, 2008) (Yakubu, 2011):

- Arm length (humerus+cubitus+phalanges) (ArL)
- Shank length (tibia+fibula+phalanges) (ShL)
- Total height (TH)
- Head length (HdL) and head width (HdW)
- Body length (BL)
- Body width (BWd)
- Body perimeter (BP)
- Dorso-sternal length (DEL)
- Croup height (CrH)
- Breast perimeter (BrP)
- Abdominal perimeter (AbP)
- Metatarsus perimeter (MtP)
- Body weight (BW).

Four morphological indices (massiveness -ratio of live BW to BL × 100-, stockiness -ratio of BrP to BL × 100- long-leggedness - ratio of total shank length to BL × 100- and condition index -ratio of live BW to arm length × 100-) (Yakubu, 2011) were obtained. Conformation type and meatiness of the ducks could better be assessed using massiveness, stockiness, long-leggedness and condition index. These principal selection indices state the ratio of measurements that characterizes the proportionality of bird's body. Massiveness and stockiness which, are used to assess musculature development, are traits for solidity of the body and clearly defined traits for meat-type ducks (Yakubu, 2011). Determining body condition is a valuable tool as it can be used to indicate how animals manage environmental variation and stressors (Angel et al., 2015). So if BW is corrected for body size using condition ratio, it gives a better indication of a bird's ability to meet its present and future energy requirements than using BW alone (Yakubu, 2011).

Statistical analysis

To assess the differences between sexes and localities we employed a two-way Non Parametric Multivariate ANalysis Of VAriance (NPMANOVA) with sex and locality as factors and traits, excluding indices, as the dependent variables, using correlation distance. A MANOVA assessed sexual dimorphism. Ulterior one-way NPMANOVAs and p-Bonferroni corrected values were applied to the differentiation between local communities for each gender separately. A correlation table was obtained with Spearman’s r_s. Hierarchical clustering among morphometric variables was assessed based on Ward’s method. Finally, a Principal Component Analysis (PCA) was performed from var-covar matrix.

All analysis were done with PAST v. 2.17c package (Hammer, Harper, & Ryan, 2001). For multiple statistical testing we used sequential Bonferroni-adjustment and 9,999 permutations, with a 95% level of significance.

Results and discussion

A preliminary two-way NPMANOVA reflected statistical differences between local communities and genders (Table 1), so we proceed ulterior analysis with sexes separately. The means, standard deviations and coefficients of variation of the lineal body parameters ducks are presented in table 2. The significance with the minimization of Wilks' lambda corroborated the difference between genders (Wilk’s λ=.254, F14,347=72.8, p<.001) and provided validity for the sexual dimorphism. For females there appeared no statistical differences between animals between San Manuel Chaparrón and Olintepeque (F=3.919, p=.109), but there appeared for those from Camotán. For males, no community differences appeared.

Most of the correlations between the assessed variables were significant (p<.001) and ranged from 2.8% to 73.4% (Table 3). The presence of positive correlations between the assessed variables justifies the use of multivariate analysis. Group analysis (Figure 1) demonstrated relationships between the morphometric variables performed in two distinct groups. One group was formed by variables which define animal lengths and perimeters (BL, ArL, ShL, AbP and BrP) which correlated significantly. The other group comprised the remaining variables.

Two factors explained 100% of total variance of studied morphometric variables (PC1+PC2=9.2+9.7%). The commonalities found in this study ranged from .617 to -.015 and explained how much a particular characteristic contributes to explain the number of factors being considered (Figure 2). HdW showed low commonality, that is, contributed little to the total observed variance. AbP showed the highest commonality. BrP was the second most important variable in the first principal component. The first factor can be termed ‘perimeter factor’, and the loading characteristics were highly correlated variables. But these characteristics appear no to be important for describing a breed, but rather the management.

Meatiness trait was better described in males using massiveness (5.14 vs. 4.09%; p<.001 for males and females respectively) while in females, it was better explained via stockiness (84.55 vs. 8.15%; p<.001 for females and males respectively). Long-leggedness was higher in females (84.43%) compared to males (82.60%, p=.012). The condition index was found to be higher in males (6.08%) compared to female counterparts (4.92%) (p<.001). Higher coefficients of variation were recorded for males than females in all the morphological indices except for condition ratio.

Table 1. Results of 2-way NPANOVA with sex and community as factors and traits as the dependent variables, using correlation distance and 9,999 permutations, in a sample of 362 adult Muscovy ducks (137 males and 225 females) belonging to three different local communities in Guatemala. There are reflected statistical differences between local communities and genders.

Source	Sum of squares	Degrees of freedom	Mean square	F	p
Community	.00747	2	.0037377	12.612	.0001
Sex	.00590	1	.0059076	19.934	.0001
Interaction	-.02524	2	-.0126240	-42.599	.4894
Residual	.10550	356	.0002963		
Total	.09363	361			

Table 2. Main simple statistics for females (n=225) and males (n=137) of Muscovy ducks. Measurements in cm, except for BW (in kg). Acronyms in the text.

	♀					♂				
	Min	Max	Mean	Sd	CV	Min	Max	Mean	Sd	CV
ArL	3.5	42.0	31.5	2.89	9.2	25.5	44.0	37.7	3.21	8.5
ShL	22.0	38.0	3.9	2.19	7.1	21.9	44.8	36.8	3.38	9.2
HdL	1.0	14.5	12.7	.66	5.2	1.0	17.0	14.3	.95	6.6
BL	31.0	49.0	36.7	2.65	7.2	34.0	54.0	44.7	3.69	8.2
BWd	3.4	8.3	6.2	.72	11.7	4.2	9.3	6.9	.92	13.4
DEL	5.0	11.0	8.2	.99	12.0	5.5	11.7	9.2	1.19	12.9
BP	11.0	19.0	14.3	1.18	8.2	12.0	21.0	16.8	1.56	9.3
CrH	9.0	21.0	14.7	1.49	1.1	11.0	22.0	17.0	1.87	11.0
BrP	25.5	37.0	3.9	2.31	7.5	26.0	44.5	35.7	3.58	1.0
AbP	18.0	38.0	25.8	3.02	11.7	18.0	47.0	28.2	4.06	14.4
MtP	3.2	5.0	4.0	.34	8.5	3.5	7.0	4.8	.56	11.7
HdW	2.4	4.0	3.4	.20	5.9	3.0	4.6	3.9	.30	7.6
TH	13.0	24.0	17.4	1.67	9.6	16.0	26.0	19.9	2.09	1.5
BW	1.0	2.4	1.5	.24	15.9	1.1	3.7	2.3	.54	23.6

Sd: Standard deviation
CV: Coefficient of Variation (%)

Table 3. Spearman’s correlations between the evaluated morphometric variables. r_s values below the diagonal row; *p*-values above. Acronyms into the text.

	ArL	ShL	HdL	BL	BWd	DEL	BP	CrH	BrP	AbP	MtP	HdW	TH	BW
ArL		.000	.000	.000	.075	.000	.000	.015	.000	.001	.000	.000	.014	.000
ShL	.421		.000	.000	.000	.000	.000	.007	.000	.000	.000	.000	.002	.000
HdL	.264	.381		.000	.104	.000	.000	.000	.000	.003	.042	.000	.008	.000
BL	.364	.506	.334		.018	.000	.000	.000	.000	.000	.000	.000	.000	.000
BWd	.119	.347	.109	.157		.066	.069	.937	.000	.001	.001	.142	.122	.000
DEL	.232	.440	.301	.413	.123		.005	.000	.000	.002	.274	.001	.001	.000
BP	.289	.383	.256	.283	.121	.185		.016	.000	.000	.000	.001	.025	.000
CrH	.162	.178	.234	.243	-.005	.319	.160		.038	.673	.017	.013	.000	.001
BrP	.259	.483	.299	.360	.391	.301	.295	.139		.000	.000	.001	.000	.000
AbP	.229	.406	.196	.296	.226	.208	.271	-.028	.586		.000	.027	.108	.000
MtP	.289	.325	.136	.245	.230	.073	.249	.159	.431	.258		.000	.000	.000
HdW	.348	.343	.364	.253	.098	.214	.217	.166	.217	.148	.248		.091	.000
TH	.163	.209	.175	.314	.103	.212	.150	.734	.249	.107	.269	.113		.000
BW	.404	.500	.273	.310	.345	.336	.280	.213	.725	.535	.396	.384	.246	

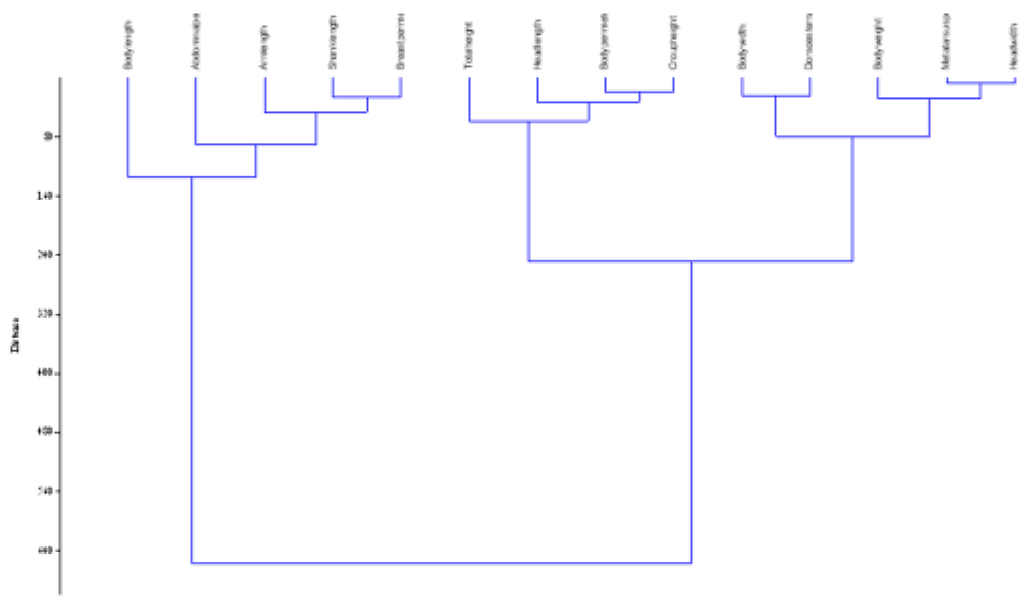

Figure 1. Variables analysis by Ward’s algorithm for the 14 studied traits. It demonstrates relationships between the morphometric variables performed in two distinct groups. Acronyms into the text.

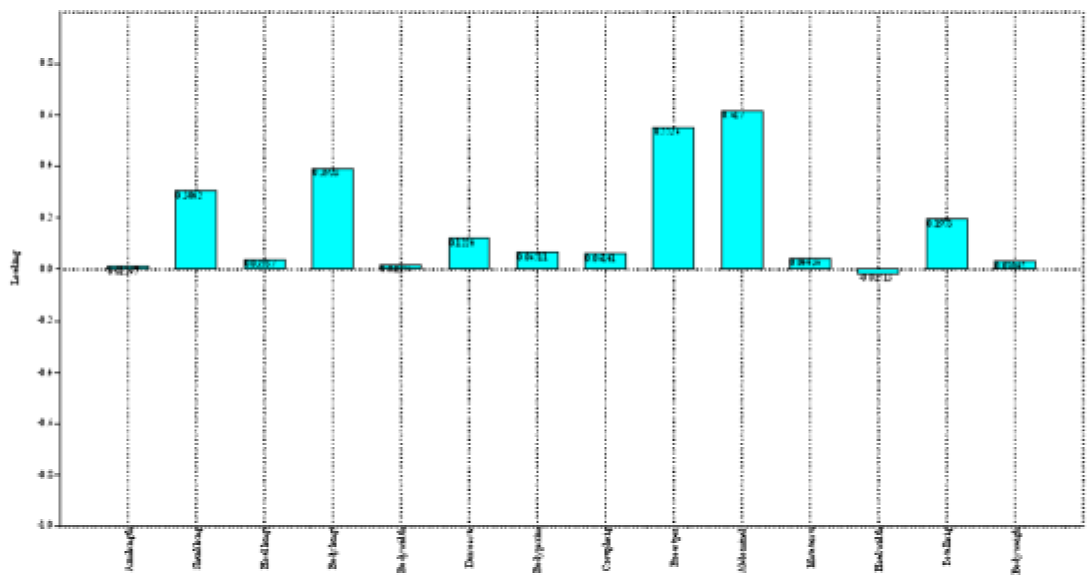

Figure 2. Loadings for Principal Component Analysis in a sample constituted by 137 males and 225 females Muscovy ducks. The found communalities study ranged from .617 to -.015 and explained how much a particular characteristic contributes to explain the number of factors being considered. As all loading values were positive, sexual differences can be considered due to size.

Duck is one of the most important domestic avian species in the world (Abdeltawab et al., 2017). Authors have observed that free-range Muscovy ducks in Guatemala are well adapted to the local climatic conditions, feed, and management stresses, with a good resistance to tropical diseases mainly as a result of frequent heat stress and drought. In general, it appeared a low phenotypic variation of traits which may indicate, a low genetic variation. The high uniformity of traits in both genders would be an indication of the low environmental sensitivity, which could impair a sufficient selection response.

In Muscovy ducks sexual dimorphism is well manifested. The smaller body exhibited by the females as compared to the males can be a reflection of their adaptive strategy to the stressful environmental and nutritional conditions, as smaller size would reduce the maintenance feed requirements and increase feed efficiency in this free range system where feed resources are limited in terms of quantity and quality. The higher leg-body ratio of the females is an indication of longer legs while males have relatively longer body and while the females display a narrower body, which is suitable for egg production; the males exhibit a blockier appearance, which is more a characteristic of meatiness. The higher condition index detected in males is of physiological importance because it measures the metabolic activity (Yakubu, 2011).

The indigenous Muscovy ducks in Guatemala are raised in different ecology matrices and, thus, the differentiation could be noted in different regions. Morphological traits among females signal also a possible different group in Camotán, which for us would be a topotype, e.g. a group associated merely with artificial selection and breeding, not with natural selection.

Conclusions

Morphological traits of Muscovy ducks from Guatemala represent genes of adaptation to their own environment and must be viewed as gene reservoir, reflecting unique adaptation to their agro-ecological or tropical environments. Our study revealed that there are marked sexual differences, with no clear ecotype. Now there is a need to study carcass and egg quality, as well the molecular levels, in order to clarify the similarity with ducks from other origins.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ENVIRONMENTAL SUSTAINABILITY FOR POULTRY PRODUCTION

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Abstract

Environmentally sustainable production models are one of the most important issues for all production industries. Precautions must be taken today so that human beings can access the resources they need in the future. Various legal regulations are being studied for sustainable production models. The world population is increasing rapidly, while agricultural land and food options for human beings are decreasing. The poultry industry, which is the most important producer of animal protein, will have to make environmental regulations in the near future. Environmental pollution, global warming and carbon emission are the most important environmental problems. The poultry industry, like other animal production areas, causes significant damage to the environment. The biggest environmental problems of the poultry industry can be listed as pollution and wastes in feed raw material production, polluted water and greenhouse gas emissions in production, slaughterhouse residues, poultry residues, hatchery residues. The aim of this study is to evaluate the current situation and offer new suggestions for sustainable poultry production. In particular, there is a need to carry out many studies on obtaining feed raw materials from waste products, and global decision makers and governments need to support these studies.

Keywords: *Sustainability, Industry, Global warming, Green recycle, Carbon footprint.*

Introduction

The world population is increasing rapidly, and it is estimated that it will exceed 9 billion in 2050. On the contrary, agricultural areas and the amount of food per capita are decreasing. In addition, although the population growth rate is higher in Asia and Africa, access to food is more difficult in these continents than in others. Poultry meat and eggs are the cheapest animal protein sources, and it is estimated that 50% of the world meat production will be met by poultry species in 2050. Poultry production is the first thing that comes to mind as a solution to the problem of increasing food demand due to increasing population growth. The biggest reasons for this situation are the cheap and abundant products obtained as a result of studies in the field of genetic improvement and feed technology.

As sustainability is important in every field, it also draws attention in poultry production in order to meet the protein deficit that will occur due to population growth in the coming years. Sustainability is a broad, complex, and multifaceted concept that encompasses the environment, economy, and social criteria. Therefore, it does not have an exact definition, but it is defined as follows in the report (Our Common Future) of the World Commission on Environment and Development: "the ability of the present generation to meet their own needs without harming or destroying the chances of future generations to meet their needs". In order to ensure sustainability, it is necessary to transfer environmental and economic resources to future generations without diminishing or deteriorating. For this reason, it is possible to define sustainability as “added value to production” (Vaarst et al., 2015; Kalkan, 2019). Sustainability for the poultry industry; production systems that do not harm the natural environment, protect and improve the social and economic conditions of producers, workers and local communities; it also means the efficient production of safe, high-quality meat and egg products, protecting the health and welfare of the animals used for production (Sustainable Agriculture Initiative, 2015; Kalkan, 2019). For the poultry industry, sustainability means the efficient production of safe, high-quality meat and egg products that do not harm the natural environment, protect and improve the social and economic conditions of producers, workers and local communities, while protecting the health and well-being of the animals used for production. (Sustainable Agriculture Initiative, 2015; Kalkan, 2019). The aim of this study is to evaluate the current situation and offer new suggestions for sustainable poultry production. For this purpose, the current situation has been summarized under headings and suggestions have been made.

Environmental Pollution

The poultry industry has a low impact on greenhouse gas emissions, as birds do not produce methane gas like ruminants because they have a single stomach, and in addition, poultry species have good feed efficiency (Gerber et al., 2013; Cesari et al., 2017; Işık and Kırkpınar, 2019). However, from the perspective of society, focusing only on greenhouse gas emissions may not be enough to determine the impact of the poultry industry on the environment. For this reason, soil and water pollution caused by nitrogen and phosphorus from the feeds used should also be considered (Sutton et al., 2008; Boggia et al, 2010; Tallentire et al, 2017; Işık and Kırkpınar, 2019). With the legal regulations, studies to reduce these negative effects are increasing, and at the same time, scientists make various suggestions based on their research. Ferket et al. (2002) presented five suggestions to reduce environmental pollution caused by animal production. These are reducing animal production, transferring animal wastes with nutritional value to suitable regions and using them in feed, using the minerals in feed again, using waste manure effectively, and improving the efficiency of nutrients in feed (Işık and Kırkpınar, 2019). The first of these recommendations is unacceptable in terms of public health and nutrition, but studies are underway on the others.

Global Warming

In addition, the other negative effect of the intensive production model used in the poultry industry on the environment is its contribution to global warming. Although the effect of methane gas is small, carbon dioxide (CO_2), nitrous oxide (N_2O), hydrofluoride carbons (HFCs), perfluorocarbons (PFCs) and sulfurhexa fluoride (SF6) are emitted to the atmosphere to a significant extent thanks to the intensive production model in poultry industry (Kılıç et al., 2018). With global warming, the concept of carbon footprint has emerged in order to determine the effect of industrial enterprises or various human activities on global warming. Carbon footprint is the measure of the damage caused by human activities to the environment in units of carbon dioxide (Çınar 2007; Kılıç and Amet 2017; Kılıç et al., 2018). In the carbon footprint, which is divided into primary and secondary footprints, the primary footprint is a measure of CO_2 emissions from the combustion of fossil fuels, including domestic energy consumption and transportation. Secondary footprint, on the other hand, is a measure of CO_2 emission that starts from the life cycle of the products used and emerges indirectly as a result of the manufacture and deterioration of these products (Kılıç et al., 2018). The concept of carbon footprint has emerged in order to determine the effect of industrial enterprises and various human activities on global warming. In the study conducted by Ibidhi et al. (2017), it was determined that there is 3 kg CO_2 equivalent for 1 kg of chicken meat production, and it was determined that the carbon footprint of chicken meat is lower than the carbon footprint required for mutton.

Waste Management

In the poultry industry, as in all other animal production branches, the first process in which the amount of emission occurs in large quantities is feed production. Elements such as water, fuel, pesticides used in the production of feed raw materials increase the amount of emission due to the spread of CO_2, CH_4, CO, NOx and volatile organic compounds (VOC) to the atmosphere (Yaylı, 2019). Recently, environmentally friendly production alternatives have been developed for protein supplements and soy. Incentive applications should be made for the production of feed raw materials by using various larvae and worms fed with domestic and non-domestic waste organic materials. Thus, both emissions in feed raw material production will be reduced and waste materials will be converted into reusable valuable organic feed raw materials. Perhaps the most important sustainable production element for the poultry industry is the possibilities of green recycling of feed raw materials.

The most important emission source in poultry production residues is feces. An adult chicken produces 140-160 g of fresh manure per day, and an average poultry-house produces tons of manure. NH_3, N_2O and CH_4 emissions, which are released after the manure is removed from the henhouse after it is formed in the henhouse, its storage and its application to agricultural lands, have a significant impact (Leinonen et al. 2014; Yaylı, 2019). Another alternative is the use of chicken manure as a feed additive in the nutrition of ruminant animals by drying and turning into flour, apart from plant nutrition. Dried and ground fertilizer is purified from germs by sterilization and pasteurization processes. Although it is not a good source of energy in this respect, it is a rich source of protein, calcium, and phosphorus. In addition, while producing biogas from chicken manure contributes to energy savings, it can be offered as a solution to the prevention of environmental pollution as it will not create a suitable environment for the removal of gases such as CH_4 and H_2S and for harmful microorganisms. It is not legally possible to use the valuable protein and fat resources obtained by the production of larvae and worms in poultry feces in the poultry industry. However, if it is revealed that there will be no health problems with the necessary regulations, it is estimated that this practice for a recyclable environment will be approved in the future.

Residues such as infertile, fertile but not hatched eggs, eggshell, and dead chicks are considered as hatchery waste. Incineration is generally used to remove incubator residues from the hatchery. Very high heat is required for combustion. In addition, another way to get rid of hatchery waste is to bury it in the ground. In both cases, significant damage is done to the environment. However, eggshell, eggshell membranes, dead embryos or infertile eggs contain very rich minerals and nutrients. It is necessary to carry out studies for the use of incubation wastes within the scope of green recycling.

Head, feet, feathers, blood, inedible internal organs, and abdominal fat are also considered as slaughterhouse waste. In some cultures, chicken feet are consumed as human food. Blood, internal organs, and fat residues are generally converted into feed raw materials. Feathers constitute the highest share of environmental pollution among the residues produced in slaughterhouses. A small amount of waste consisting of feathers is used in the textile industry by going through different processes. Feather, which contains 80% crude protein in its dry matter, can be used in small amounts for amino acid production. Studies should be carried out to increase the digestibility of feathers with various processes and make them suitable for consumption.

Dead chickens and their disposal practices are a very important problem for the poultry industry. This problem is also significant worldwide, requiring its destruction or appropriate evaluation by biologically safe methods. Burning or burying are the first methods that come to mind getting rid of dead chickens, but they have negative effects on the environment. In fact, dead chickens are a valuable and inexpensive source of protein and fat when properly evaluated. Transforming dead chickens into a biologically safe and valuable by-product can have positive effects on the economy and the environment.

Conclusions

It is necessary to recycle the wastes generated in the poultry industry, which is growing gradually due to human needs, into by-products in order to both reduce environmental pollution and reuse the products obtained from these wastes. These new products will not only pollute the environment less but will also make an extra contribution to the economy. This situation is also important in terms of the increase in production due to the increase in the world population in the near future, but the effective use of the decreasing natural resources and the sustainability of industrial production. In short, it is necessary to focus on issues such as greenhouse gases, carbon emissions, carbon footprints, which have negative effects on the environment, and take necessary precautions. Creating efficient production models that cause the least damage to the environment is the most important issue for the sustainability of the poultry industry.

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ORGANIC WAY OF GOAT BREEDING AND GOAT PRODUCTION

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Abstract

Today, domestic goats are widespread throughout the world, with the exception of extremely cold areas. They are most represented in countries with extensive agricultural production, but in the last decade their population has also grown in richer countries, where there is a trend of increasing consumption of goat meat and milk. Goat meat is a food rich in proteins, vitamins and minerals, and contains very little fat, especially cholesterol. However, the biggest advantage, apart from the extremely high quality nutritional values, is that this type of meat does not have opposite religious and cultural aspects of consumption.
Organic goat production is essentially reduced to returning to the original way of breeding through natural - grazing diet. Today's grazing is essentially different from the previous extensive grazing and is reflected in the fact that this diet is now approached from the modern aspect of sustainable agricultural production, which includes all elements of preservation and improvement of grazing areas, their cultivation, floristic composition and above all, avoiding chemical contaminants that would endanger the quality of the obtained green mass and thus the residual effect in the meat and milk of goats. Having in mind the pronounced trend of increasing the production and consumption of goat meat in the world and in Serbia, the aim of this paper is to show the impact of grazing as an organic way of obtaining the quality of goat meat.

Key words: *goats, meat, organic production, pastures.*

Introduction

Goat has a less pronounced ability for meat, but it is more fertile than sheep, so the production of quality goat meat is more important. Goat meat has less fat and roasts it has an extraordinary taste. Meat from older, discarded heads can be dried, it has a specific smell and taste, so it is priced by consumers who are used to it. Goat meat has approximately the same nutritional and digestible value as sheep meat (more precisely, more protein and less fat compared to sheep meat). Based on its chemical composition, goat and goat meat, in terms of nutritional and biological value, does not lag behind other types of meat of other types of slaughter cattle [Todaro et al.2004, Ivanović et al.,2011,2014,Ivanović and Pavlović,2015]. As a food of animal origin, goat meat is rich in proteins, vitamins and minerals, and contains very little fat, especially cholesterol. This type of meat does not have opposite religious and cultural aspects of consumption. In Serbia, as well as in the whole world, there is a trend of increasing the production and consumption of goat meat [Grace,1992].
The quality of meat is difficult to define because the meat has a heterogeneous composition, and it is specific to each species. A strict definition of meat quality would be that it is its physical and

chemical properties, and / or consumer perception. Although, as indicated, the quality of the ingredients is constantly being redefined, because the quality of meat is influenced by production and technological factors. Production factors are biological factors and factors of production systems [Webb et al.2005]. Biological factors include race, sex, productivity and adaptation to stress, and factors of production systems - environment, management, nutrition, body weight at slaughter and health status. The quantity and quality of goat products are also influenced by climatic conditions, relief and soil properties, primary abiotic and biotic factors and organic conditions of milk and meat production [Zujovic et al.,2008, Pavlovic et al.,2009]. Organic goat production is essentially reduced to returning to the original way of breeding through natural - grazing diet. The essential difference of today's grazing attitude in relation to the previous extensive grazing is seen in the fact that this diet is now approached from the modern aspect of sustainable agricultural production, which includes all elements of preserving and improving grazing areas, their cultivation, floristic composition and above all avoidance of chemical contaminants that would endanger the quality of the obtained green mass and thus the residual effect in goat meat and milk [Pavlović et al.,2014]. It also introduces the application of the latest knowledge on the method of pasture control, grazing, regular control of animal health on them in order to avoid contamination of pastures. The combination of all these factors creates the conditions for sustainable organic production in goat farming [Roe et al.1959].
Having in mind the pronounced trend of increasing the production and consumption of goat meat in the world and in Serbia, the aim of this paper is to show the impact of grazing as an organic way of obtaining the quality of goat meat.

Pastures

By definition, pasture is a land area used for feeding and raising livestock by grazing. According to the altitude it can be mountain or lowland pasture. They are important sources of fodder for the production of green mass for grazing and haymaking, and increasingly for the production of quality silage and haylage, especially in hilly and mountainous areas. However, large areas of meadows and pastures in our country are poorly utilized, they yield small yields of poorer fodder, primarily because grasslands are poorly nurtured and poorly utilized [Ivanović and Pavlović,2015]. According to the method of origin, we distinguish:

- natural pasture - overgrown with natural cover (various grasses and weeds),
- artificial pasture - is created by sowing clover and various types of grass.

By raising sown artificial meadows and pastures, sowing cultural, productive and quality grasses and legumes, ie grass and grass-legume mixtures, the production of fodder can be significantly and faster improved. In terms of vegetation, when our pastures can be used in the period from the first half of May to mid-October, and in favorable weather conditions until mid-November. In the first half of the summer, pasture regeneration lasts from 18-24 days between grazing rounds, while in the second half of the summer, due to lower rainfall, regeneration is slower and the interval shifts to 30 days, and from September to 40 days [Roe et al.1959, Ivanović and Pavlović,2015].
In terms of zootechnical norms, keeping goats on pasture has an incomparably more favorable effect on the organism of individuals. Movement in the fresh air during grazing has a very favorable effect on the proper development of skeletons, muscles and tendons, as well as the development of internal organs - especially the lungs and heart. Goat raised on pasture is more lively, healthier and more resilient, with well-developed muscles and strong joints. The influence of light stimulates the formation of red blood cells and hemoglobin in the blood of animals,

which has a beneficial effect on the nervous system and resistance. The influence of UV rays accelerates the production of vitamin D, which is important for the regulation of metabolism and favorable utilization of calcium and phosphorus [Truong and Baker,1998].

Green young pasture has a favorable chemical composition, with a favorable ratio of digestible proteins and a sufficient amount of minerals and vitamins, which explains the relatively large gains achieved by especially young categories of cattle on pasture. Partial or complete losses in some very important chemical constituents occur during drying or ensiling [Grace,1992]. Depending on the stage of development, grass composition of pastures and applied fertilization, green fodder for grazing can contain 18-24% dry matter, with 18-25% crude protein, 40-50% KDV (acid detergent fiber) and 6.4-7.0 MJ NEL (net lactation energy) in kilograms of dry matter [Zujovic et al.,2008]. In the last few years, there has been a growing interest in the nutritional role of pastures in meat and milk production. Grazing individuals had or yielded significantly higher levels of linoleic acid (CLA) and omega (ω) fatty acids. Linoleic acid (CLA) is known to be a powerful anti-oxidant. Grasses contain linoleic acid, and more than half of the total fatty acids are ω-3 linoleic acid. Milk and meat produced on pastures contain linoleic acid and have a much higher nutritional value in this regard. Greater introduction of grazing in the existing way of milk and meat production on our farms requires a higher level of knowledge and engagement than in the already well-established production on our farms (Ivanović and Pavlović, 2015).

But grazing also has its side. Permanent pastures pose the greatest danger to sheep and goats from a health point of view, especially if they are used unplanned and for many years. Uncultivated pastures with poor floristic composition cause nutritional imbalances, but also cultivated pastures that are improperly used are also places of constant infections, especially parasitic agents [Vlassoff, 1976; Quesada et al., 1990; Ash and Truong,2003, Pavlović et al., 2012].

Cultivation of permanent pastures and bringing in a larger number of animals entails their constant contamination with feces and other secretions, and thus the accumulation of infections in the soil and grass. The cumulative effect of accumulated agents is reflected through reinfections and superinfections and conditions their permanent circulation [Truong and Baker, 1998, Truong et al., 2000]. Pasture cultivation directly depends on the geological and pedological composition of the soil, hydrological conditions (standing and running water), microclimatic conditions and floristic composition on it. The main goal of cultivation is to obtain pastures that contain grass mass that must be of optimal quality, density and nutritional value. Successful cultivation must be based on real data, which means controls and analyzes of floristic composition and pedological composition of pastures [Ash and Truong,2003, Pavlović et al.,2014,2018]. The earlier opinion that plowing permanent pastures destroys infectious agents was refuted by later research which showed that in this way (plowing) certain infectious agents, especially parasites, only successfully protect themselves from unfavorable external conditions - direct insolation, drying, showers, frost, etc. Without the use of certain disinfectants, the primary effect of this measure is short-lived [Truong and Baker,1998, Whitehead, 2000, Vellinga et al.,2004].

Many authors have recommended the use of artificial fertilizers in order to destroy infectious agents and parasites on pastures. It is, of course, a measure of short-term effect and long-term harmful consequences. Fertilization of the soil without knowledge of the pedological composition can cause a serious imbalance of minerals that are incorporated into plants and are necessary for the normal physiological needs of goats [Grace,1992, Ash and Truong,2003]. Any subsequent fertilization, after leveling the quantity and quality of the soil, is done through the

floristic composition of the plant mass and the application of manure, which must be adequately prepared. In any case, such interventions suppress organic production.
Interventions on pastures can be a good precondition in controlling and preventing diseases. Pasture drainage is extremely effective in combating certain parasites that require a sufficient amount of moisture (flukes, etc.) as well as bacterial diseases that sometimes have a district character [Quesada et al.,1990, Pavlović et al.,2010,2018]. In addition, drainage greatly improves vegetation [Pavlović and Rogožarski,2017, Vlassoff,1982]. Mowing the grass has the effect of mechanically removing infections, most of which die during drying. Unfortunately, in heavily infected pastures, such hay is a source of winter infections in sheep and goats [Pavlović et al.,2012,2018].

Types of grazing

Goat grazing is essential both for the maintenance of pastures and for the organization of goat health care. The first principle is that younger animals always go ahead of older ones in order to get a better quality of green mass and thus a better nutritional value that will affect their further development and health status. Next, perhaps most important, is the way pastures are used and the type of grazing. It can be mixed and persecution, and the limitation of the number of individuals on pasture can also be applied [Grace,1992, Ash and Truong,2003, Ivanović and Pavlović,2015].
Mixed grazing means grazing different herbivores on one pasture, which certainly has a positive effect on the reduction of parasitic infections. In the case of bacterial or viral diseases, which are common to endangered species, grazing is a constant source of infections and superinfections [Roe et al.,1959, Truong and Baker,1998]. Another inconvenient side is that in this way the pasture spoils quickly due to the depth of the bite during grazing, so that there is no food for other species of hebivoras on the pasture where sheep graze almost to the root. In addition, goats browse all green plants, including saplings of trees and shrubs, and all other green nutrients they come across.
Distillation grazing is a method of using pastures to move animals from one part of the pasture to another at certain time intervals and return to them only after a certain period of rest. Chases are usually carried out after 5-7 days, and in 2-3 months the animals return to their original location. The cleanest part of the pasture should be used for young animals, which is achieved by grazing them in front of older individuals. In some places, this is solved by making movable fences that can be used to block places where grazing is not desirable (next to the bar, etc.) [Pavlović et al.,2010,2018].
Limiting the number of livestock on pasture is a method of trying to raise animals in almost natural conditions and is very reliable for permanent pastures. Reducing the number of individuals automatically reduces the number of infections - especially parasites, since the prevalence of parasites in small ruminants and in the best conditions is 90-100%. On the other hand, this enables better and better nutrition and, which is unusually important, the introduction of a small number of infections that promote the development of natural immunity to certain agents [Pavlović and Rogožarski,2017, Vlassoff,1976].
Watering can hygiene is also essential in preventing infection. Power from ponds, stagnant waters and ditches carries a constant danger of infection with fluke and other infections. Therefore, there must be watering places or wells on the pastures from which the animals will be fed [Ivanović and Pavlović,2015, Pavlović et al., 2014].

Preventive deworming proved to be the most effective solution for reducing pasture contamination - autumn, which is done 3-4 weeks after withdrawal from pastures and spring before expulsion to pasture. All animals are treated with antiparasitics [Roe et al.,1959, Pavlović and Rogožarski, 2017].

Conclusion

The organic way of obtaining goat meat has become a world trend of the last decade. The increase in goat production today is of global proportions, and there are several reasons for that. Goat meat is a food of animal origin rich in proteins, vitamins and minerals, and contains very little fat, especially cholesterol. More importantly, this type of meat does not have opposite religious and cultural aspects of consumption, so it is accessible and acceptable from all aspects. In Serbia, as well as in the whole world, there is a trend of increasing the production and consumption of goat meat. After decades of embargoing and destroying herds of goats that began in Serbia at the end of World War II, they have regained their well-deserved place in livestock production in recent decades.

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OCCURRENCE OF CHEMICAL ELEMENTS IN EWE TISSUES FROM UNDISTURBED AND SLIGHTLY DISTURBED AREAS IN SLOVAKIA

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Abstract

This study aimed to measure concentrations of selected essential and toxic elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) in animal tissues of sheep originating from the area with a slightly environmental burden (Horehronie region) and undisturbed area (Orava region) in Slovakia. Liver, kidney, mammary gland, and muscle from 22 ewes for two consecutive years (2020 and 2021) were analysed by an inductively-coupled plasma optical emission spectrometry. Various concentrations of monitored elements were obtained after subjecting the results to statistical analysis. Contents of five toxic elements (arsenic, cobalt, nickel, lead, antimony), and chrome and selenium, were below the detection limits. Content of cadmium exceeded the maximum permissible level in kidneys from the slightly disturbed area. Although the content of copper did not exceed the permissible limit, the concentration of Cu was higher in the liver in both areas in 2021 compared to the results from other studies. The concentration of lithium in the kidney, liver, and muscle from the Orava region was significantly ($P < 0.05$) higher than that of the Horehronie region. Concentrations of Mg, Ag, Cd, Mo, and Zn were significantly ($P < 0.05$) higher in samples from region Horehronie than that in the Orava region. Statistically significant differences ($P < 0.05$) were noted between concentrations of K, Li, and Mo in kidneys; Fe, K, and Li in the mammary gland; Cu, Zn in the liver and Mn in muscles of sheep across the reference years. In summary, monitoring the occurrence of elements found in commonly consumed dietary raw materials contributes to ensuring the quality and safety of food. Most of the tissue samples analysed are relatively safe for regular human consumption. However, the concentrations of Cd in the kidneys from the slightly disturbed area and potentially Cu in the liver from both areas pose a health risk for the consumers.

Keywords: *toxic elements, essential elements, animal tissues, sheep, Slovakia,*

Introduction

The presence of various chemical elements in animals is of interest from both animal and human health perspectives. Levels of these essentials and toxic elements can indicate the nutritional status of animals and the value of the animal products. Meat and meat products are important components of the human diet as a source of high-quality protein and highly bio-available essential trace elements (Biel et al., 2019). Monitoring the levels of contaminants such as residues and heavy metals in living organisms to protect human health and ensure the quality and safety of food is a very important part of research and national programmes (EFSA, 2009). The capacity to concentrate and cumulate different metals for each organ was calculated. The liver and kidneys are organs which remove toxic metals from the body and end up accumulating them, and because of that, they are target tissues for monitoring metal contamination in animals (Swaileh et al., 2009; Akoto et al., 2014, Abou- Arab, 2001). Cadmium and lead also can accumulate in sheep muscles, though concentrations are naturally lower in comparison with kidneys and livers (Massanyi et al., 2001). Due to grazing of a herd of sheep on contaminated soil, higher concentrations of metals are found in sheep (Sabir et al., 2003) The goal of this study was to investigate and compare the occurrence of essential and toxic elements in sheep´s tissues in two different regions of Slovakia with the different environmental load.

Materials and methods

Collection of samples

Sheep tissues, specifically the liver, kidney, mammary gland and muscle were used as samples in this study. Samples from 22 ewes together were taken for two consecutive years (2020 and 2021) from two areas with different environmental loads located in Slovakia. Farm from Horehronie region represents an area with a slightly environmental burden and farm from Northern Slovakia, Orava region, which is considered as a control group and originates from the potentially undisturbed area. Division of the country according to the degree of pollution is taken from the document Environmental regionalisation of the Slovak republic made by the Ministry of Slovak republic in (Bohuš and Klinda, 2020). All animals used in this study (5 ewes in 2020 and 6 ewes in 2021 in the Orava region, and the same number of animals in Horehronie) were humanely killed in a registered slaughterhouse. All samples were taken immediately after slaughter and stored in plastic bags in freezers at -18℃ until analysis was carried out, same as in the work of Pšenkova and Toman (2020). Used animals were 4-5 years old and they were improved Wallachian x Lacaune breed from region Orava and Tsigai breed from region Horehronie.

Analysis of samples

Concentrations of 22 selected elements (Ag, Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sr, Zn) were measured in animal tissues of sheep. A pre-analytical procedure such as homogenization was made at first. The weight of the experimental samples ranged from 1.0 to 2.0 g and was reflected in measurement. The samples were mineralized in the high-performance microwave digestion system Ethos UP (Milestone Srl, Sorisole, BG, Italy) in a solution of 5 ml $HNO_3 \geq 69.0\%$ (TraceSELECT®, Honeywell Fluka, Morris Plains, USA), 1 ml $H_2O_2 \geq 30\%$, for trace analysis (Sigma Aldrich, Saint–Louis, Missouri, USA) and 2 ml of ultrapure water (18.2 MΩ cm –1; 25°C, Synergy UV, Merck Millipore, France). The method of determination consists of heating and cooling phases. Analysis of the elements was determined using an inductively coupled plasma-optical emission spectrometer (ICP OES 720, Agilent Technologies Australia (M) Pty Ltd.) with axial plasma configuration and with auto-sampler SPS-3 (Agilent Technologies, Switzerland). Detections limits (μg/kg) of measured trace elements were follows: Ag 0.3; Al 0.2; As 1.5; Ba 0.03; Ca 0.01; Cd 0.05; Co 0.2; Cr 0.15; Cu 0.3; Fe 0.1; K 0.3; Li 0.06; Mg 0.01; Mn 0.03; Mo 0.5; Na 0.15; Ni 0.3; Pb 0.8; Sb 2.0; Se 2.0; Sr 0.01 and Zn 0.2. and wavelength of determination (nm) follows Ag 328.068; Al 167.019; As 188.980; Ba 455.403; Ca 315.887; Cd 226.502; Co 228.615; Cr 267.716; Cu 324.754; Fe 234.350; K 766.491; Li 670.783; Mg 383.829; Mn 257.610; Mo 204.598; Na 589.592; Ni 231.604; Pb 220.353; Sb 206.834; Se 196.026; Sr 407.771; and Zn 206.200.

Statistical analysis

All results of this study were processed using Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA). All obtained results are listed as mean values with standard deviation. A probability level of $p < 0.05$ and $p < 0.01$ was considered statistically significant.

Results and discussion

Measured concentrations of selected essential and toxic elements in animal tissues are summarized in Table 1–4.

Concentrations of Li and Mn in the liver were significantly ($P < 0.01$) higher in samples from region Orava (potentially undisturbed area) than in samples from region Horehronie (area with a slightly environmental burden). In liver samples from Horehronie concentrations of Cd, Ag and Mo were significantly ($P < 0.05$) higher than in samples from Orava. Content of K, Mo and Mg in samples of kidneys from Horehronie is significantly ($P < 0.01$) higher in comparison with kidney samples from Orava. However, the concentration of Li ($P < 0.01$) and K ($P < 0.05$) is higher in kidney samples from Orava. Further content of Li is higher ($P < 0.01$) in muscle samples from Orava, on the contrary, lower content in a sample of a mammary gland from the same region was found. Content of Zn was higher ($P < 0.05$) in muscle samples from Horehronie than in samples from Orava. Comparing contents year-on-year, higher ($P < 0.05$) contents of Li in kidney and mammary gland from Orava, K in kidney and mammary gland from Horehronie, Mo in a kidney from Horehronie and Fe in mammary gland from Orava were recorded in 2020. Statistically significantly higher contents were found for K in a kidney from Orava, Fe and Mn in muscle from Horehronie, Li in mammary gland from Horehronie and Cu and Zn in the liver from Horehronie as well.

Content of Ca was considerable higher in samples from all animal tissues from both monitored groups and both years in comparison with study from region Orava and Western part of Slovakia, where it was at level 92.20 ±20.32 mg/kg and 57.02 ±5.17 mg/kg for liver, 129.40 ±27.88 mg/kg and 132.40 ±5.31 mg/kg for kidney, 196.40 ±274.03 mg/kg and 63.42 ±8.68 mg/kg for muscle, 2,224.80 ±1,598.11 mg/kg and 1,474.00 ±1,223.04 mg/kg for mammary gland (Pšenková and Toman, 2020). Content of Mg was higher in samples of liver than in the study by Pšenková and Toman (2020) and comparable in samples of kidney and in muscles from both areas. However, while the content of magnesium in the mammary gland in control group was higher, in experimental group it was lower than in control and experimental group in mentioned study. Concentrations of Zn in this study were much lower than in sheep from Iran (105.19 and 102,05 mg/kg) (Bazargani-Gilani et al., 2016). Although Zn and Cu are essential elements for the human body, their excessive consumption through diet could be toxic and cause adverse health effects, liver and kidney damage or anaemia caused by Cu (ATSDR, 2004) or anaemia and other haematological effects caused by Zn (ATSDR, 2005). Excess of Zn may cause also Cu deficiency (ATSDR, 2005). Chronic Cu toxicosis is found in ruminants but not in monogastric species and only rarely in humans (McDowell, 2003). Ruminants, particularly the sheep, have a higher potential for copper accumulation in their liver than other species and are more susceptible to copper toxicity (Miranda et al., 2006). The highest mean concentration of copper (101.95 mg/kg) was observed in the liver of undisturbed area in 2021, which is comparable with a concentration of Cu in sheep liver from Obuasi, Ghana (106.63±111.24 mg/kg) (Akoto et al., 2014) and lower than highest copper level (126.14 mg/kg) found in the sheep liver from Iran (Bazargani-Gilani et al., 2016). According to Authority ANZF (2001) permissible limit represents 200 ppm. The maximum copper consumption intake for meat and meat products has been proposed as 0.90–30 mg d^{-1} per person (Alturiqi and Albedair, 2012). However, the concentration of Cu in sheep liver from both regions was significantly higher than 0.040 mg/kg in the organs of Australian sheep (MacLachlan et al, 2016). The highest content of Mn was found in kidneys from areas with a slightly environmental burden and in the liver from the undisturbed area. Manganese is ranked as one of the least toxic trace elements in mammals, mainly because homeostatic mechanisms keep tissue manganese levels within a limited range (Miranda et al., 2006). The mean content of Fe was higher in all our samples in comparison with samples from Saudi Arabia (El- Ghareeb, 2019), similarly in Slovakia (Pšenková and Toman, 2020).

The highest mean content of iron was found in kidneys from the Horehronie region in 2021. A concentration of Cd in the liver in group of animals from Horehronie in 2020 exceeded the MRL (maximum residual limit; 0.5 mg/kg for liver) according to Commission Regulation (EC) No 1881/2006. However, cadmium concentrations under MRL were found in kidney samples from Horehronie, an area with a slightly environmental burden. In an earlier study, concentration of cadmium in liver in an experimental group under MRL was found in Slovakia (Pšenková and Toman, 2020). The high levels of cadmium content in the liver and kidneys are supposedly on account of their specific function- the liver as a storage and metabolic organ and the kidney as an excretory organ (Stoyke et al., 1995; Massanyi et al, 2014). In addition, cadmium appeared only in one sample of muscles from a potentially undisturbed area with a concentration of 1.13 mg/kg which is under MRL but is lower than in a study by Airyaee et al (2015), where the mean value represents 2.8±3.7 mg/kg.

The mean Cd concentrations in this study in liver and kidney (listed in tables 1 and 2) were significantly higher when compared with 0.007 mg/kg and 0.18 mg/kg from Obuasi, Ghana (Akoto et al., 2014) and comparable with 0.33 mg/kg from Lahore (Mariam et al., 2004). Alarming values were shown in the animals raised in the Campania region, Italy in kidney (1.53; 1.22 and 1.1 mg/kg) and in liver (0. 72; 0.64 and 0.61 mg/kg) and in muscle (0.16 mg/kg) (Barrasso et al., 2018). The following elements As, Co, Ni, Pb, Sb, and Se were not detected in any animal tissue used in this study at all, Cd was not detected in mammary glands, and Cr was not detected in any samples of liver, kidney, mammary gland and in muscle was detected only in one sample. Mo was not detected in livers and kidneys. Nevertheless, in other countries traces of heavy metals were found:

nickel in Iran and Turkey (Ariyaee et al., 2015; Tuncer, 2019) lead under permissible limits in Iran (Bazargani-Gilani et al., 2016), Cr in Nigeria (Bristone et al., 2018), As in Saudi Arabia (El-Ghareeb et al., 2019), As and Co in Austraia (MacLachlan et al., 2016).

Table 1 Levels of essential and toxic elements in the liver of sheep from undisturbed area and area with a slightly environmental burden (mg/kg)

	undisturbed area				area with a slightly environmental burden			
	2020 (N=5)		2021 (N=6)		2020(N=5)		2021(N=6)	
Element	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Ag	0.06	0.10	0.02	0.04	0.06	0.09	0.13*	0.07
Al	1.58	2.49	0.82	2.02	0.00	0.00	0.36	0.89
Ba	1.07	1.82	0.27	0.03	0.43	0.29	0.22	0.14
Ca	676.22	996.31	211.56	20.99	218.54	62.48	171.43	83.17

Cd	0.24	0.17	0.23	0.08	0.75	0.74	0.35*	0.08
Cu	67.24	56.85	101.95	81.57	30.99	28.91	93.03	51.09
Fe	70.13	32.31	90.95	46.54	44.39	21.32	57.57	33.77
K	2016.51	612.91	1920.04	53.38	1647.10	288.41	1875.72	64.59
Li	0.01**	0.00	0.01	0.01	0.01	0.00	0.02	0.01
Mg	178.08	38.18	159.62	9.59	142.16	22.12	160.47	13.73
Mn	2.23	1.29	2.71**	0.51	1.49	0.99	1.72	0.31
Mo	1.03	0.71	1.03	0.20	1.13	0.29	1.33*	0.19
Na	751.97	223.16	596.17	50.94	890.23	473.62	579.63	40.00
Sr	2.86	1.15	2.33	0.21	2.15	0.27	1.65	1.26
Zn	35.45	14.39	31.89	2.74	22.80	10.97	35.57	6.18

SD – standard deviation; *P <0.05; **P <0.01

Table 2. Levels of essential and toxic elements in kidneys of sheep from undisturbed area and area with a slightly environmental burden (mg/kg)

	undisturbed area				**area with a slightly environmental burden**			
	2020(N=5)		**2021 (N=6)**		**2020(N=5)**		**2021 (N=6)**	
Element	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Ag	x	x	x	x	0.04	0.08	x	x
Al	x	x	0.67	1.64	1.64	3.68	116.35	282.22
Ba	0.47	0.09	0.47	0.16	0.96	1.01	2.25	3.58
Ca	315.00	161.51	293.16	54.40	298.35	157.28	318.93	118.32
Cd	x	x	x	x	1.10	0.77	1.46	0.74
Cu	3.48	0.23	3.61	0.95	30.61	37.19	3.26	0.75
Fe	29.92	4.65	45.00	40.35	47.34	27.24	174.16	366.61
K	1459.02	135.94	1788.94**	170.01	1781.71**	126.01	1597.62	94.60
Li	0.04**	0.02	0.01	0.01	0.01	0.00	0.21	0.44
Mg	130.24	7.19	155.09	20.37	150.31**	8.75	151.10	59.43
Mn	0.61	0.03	0.71	0.31	1.25	0.84	3.57	7.24
Mo	x	x	x	x	0.82**	0.16	x	x
Na	1388.58	207.94	1402.36	501.96	1186.34	556.07	1522.71	112.95
Sr	2.56	1.00	2.47	0.36	2.45	0.54	2.45	2.01
Zn	15.68	1.51	17.76	2.73	x	x	15.70	2.17

SD – standard deviation; *P <0.05; **P <0.01; x – element was not detected

Table 3. Levels of essential and toxic elements in the muscle of sheep from undisturbed area and area with a slightly environmental burden (mg/kg)

	undisturbed area				area with a slightly environmental burden			
	2020(N=5)		2021 (N=6)		2020(N=5)		2021 (N=6)	
Element	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Al	x	x	x	x	x	x	1.52	1.68
Ba	0.24	0.03	0.28	0.04	0.23	0.05	0.20	0.15
Ca	221.58	49.52	217.62	15.74	185.27	29.37	222.14	83.67
Cd	1.13	(1)	0.19	0.46	x	x	x	x
Cr	x	x	x	x	0.22	(1)	0.04	0.09
Cu	1.95	1.13	2.06	0.86	1.59	0.29	1.95	0.35
Fe	11.48	0.83	18.00	7.50	15.37	4.81	21.49	4.14
K	2145.35	137.43	2079.12	299.08	2068.21	109.11	2066.31	149.95
Li	0.01**	0.00	0.01	0.01	0.00	0.00	0.01	0.00
Mg	185.24	13.64	187.54	29.51	191.08	12.10	201.18	15.31
Mn	0.10	0.03	0.22	0.28	0.08	0.02	0.13	0.03
Na	494.71	34.05	x	x	465.85	51.01	412.91	46.93
Sr	2.32	0.45	2.36	0.15	2.07	0.37	1.87	1.39
Zn	15.70	1.95	17.77	1.15	17.59	1.76	19.53*	1.32

SD – standard deviation; *P <0.05; **P <0.01; (1) only in one sample was selected element detected; x – element was not detected

Table 4. Levels of essential and toxic elements in the mammary gland of sheep from undisturbed area and area with a slightly environmental burden (mg/kg)

	undisturbed area				area with a slightly environmental burden			
	2020 (N=5)		2021 (N=6)		2020 (N=5)		2021 (N=6)	
Element	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Al	1.71	1.58	2.54	2.22	1.31	1.80	10.72	13.96
Ba	8.08	5.69	3.11	2.57	5.92	7.35	7.46	11.36
Ca	3831.52	1957.10	2613.44	2636.74	2566.11	1885.90	2544.16	2505.78
Cu	1.69	0.18	1.59	0.18	1.80	0.19	1.51	0.30
Fe	31.88	6.53	21.78	7.41	28.35	2.82	34.84	20.74
K	1493.28	196.51	1455.43	219.43**	1371.58	159.29	1154.70	68.36
Li	0.02	0.01	0.01	0.00	0.01	0.01	0.03*	0.02
Mg	285.56	129.94	202.19	108.25	225.83	138.53	221.07	164.38
Mn	0.29	0.05	0.33	0.08	0.30	0.02	0.56	0.31
Na	1247.22	277.50	1256.23	267.58	1197.98	269.81	1344.45	171.95
Sr	7.27	3.49	4.35	2.25	6.44	5.15	5.66	5.94
Zn	16.10	7.10	10.90	5.70	11.76	7.55	13.87	8.59

SD – standard deviation; *P <0.05; **P <0.01; x – element was not detected

Conclusion

Statistical analysis of the results showed significant differences in concentrations of Cd, Mo, and Zn in animal tissues of the two animal groups. We found as expected that kidneys and liver cummulate more metals than others animal tissues in this study. Consumption of meat and internal organs from Horehronie (area with a slightly environmental burden) are connected to a higher risk of toxicity because of Cd content under maximum residual limit according to Commission Regulation (EC) No 1881/2006 in liver and kidney. In one sample of muscle from Orava region, potentially undisturbed area, Cd content under MRL was found too. Concentrations of Cu in livers from both areas were not exceeded permissible limit, but in 2021 they were relatively higher in comparison with studies from other countries. Regular and prolonged consumption of Cd and Cu in diet, for example via sheep organs with their high concentrations may lead to accumulation of these metals in the human body and cause toxicity. There is a recommendation to reduce consumption of contaminated tissues from animals from these regions, even exclude their consumption for people belonging to risk groups. Continuous monitoring and further research are needed in terms of maintaining food security and safety.

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COMPARISON OF FEAR RESPONSES AND GROWTH CHARACTERISTICS OF QUAILS REARED IN DIFFERENT CAGES AND STOCKING DENSITIES

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Abstract

The aim of this study was to compare tonic immobility durations and growth characteristics of Japanese quails reared in individual and battery type cages at seven different stocking densities. A total of 436 quails were used in the study. Stocking densities per quail in battery type cages were set as 160 cm^2, 180 cm^2, 200 cm^2, 220 cm^2 and 240 cm^2. In individual cages, stocking densities were 280 cm2/quail and 360 cm2/quail. In the study, there was no statistical difference between the experimental groups in terms of the six-week live weight averages of the birds. Similarly, there were no statistical differences in the parameters of the Gompertz growth curve between the groups. However, a statistical difference was determined in terms of tonic immobility averages of quails in the experimental groups. While the lowest tonic immobility average (56.49 sec) in battery type cages was measured in quails housed in the 240 cm^2/quail stocking density group, the averages of those in the other groups were found to be higher ($P<0.05$). Similarly, quails reared in larger individual cages had lower tonic immobility average (83.55 sec.) than the other group. As a result, it is possible to say that stocking density and cage type affect the fear responses of Japanese quails.

Keywords: *Japanese quail, Tonic immobility, Stocking density, Battery cage, Individual cage.*

Introduction

Optimum environmental conditions must be provided for poultry species raised for meat production to display their genetic potential. Management, feeding, breeding system, climatic environment are some of the most important environmental factors. Deviations from these optimum environmental conditions may cause a decrease in the yield performance of birds. One of the most important factors causing performance loss in meat type birds is the stocking density. While determining the stocking density in birds; slaughter weight, slaughter age, genotype, cage type, poultry house capacity, feed content, equipment volumes, gender, climate, breeding system, and economic conditions are taken into account (Gous, 2014). Considering these conditions, losses due to excessive stocking density can be prevented and maximum income can be obtained from the unit area. However, while it is aimed to increase the income in production, some behaviors of the birds are restricted, the immune system of the animals weakens, and some disorders occur due to welfare problems and related stress factors. For these reasons, stocking density standards have been determined for broiler chickens in EU countries according to rearing types. Many studies have been done on stocking density in Japanese quails, but as in broilers, specific standards have not been developed. Japanese quail farming is a production area that does not require much investment and provides an economic return in a short time. Japanese quail is used as a model animal in research studies with its small size and superior reproductive characteristics as well as being an economical production tool. Quail is used both in breeding, feeding and genetics in order to obtain complementary information for other poultry, as well as in experiments in many fields from space science to sociology.

Fear, which emerges as the psychophysiological response of the organism in the face of any danger, is a phenomenon that creates reactions similar to the reactions given in case of stress (Akşit and Özdemir, 2002). For this reason, most researchers confuse fear and stress, whereas fear is only one of the factors that cause stress in birds. In the case of fear in poultry, there are two physiological and behavioral processes that follow each other, the first of which is the process of escape, immobility or counter-attack in the face of danger, which is called the alarm period. Depending on the severity of the stimulus causing fear and the psychological and physiological conditions of the animal, the length of the alarm period and the behaviors that occur in this process are shaped. When the bird comes out of the alarm period, it either got rid of the fear element or developed a defense against this element. In both cases, with the beginning of the adaptation period, stress arises depending on the presence and severity of the threat. Stress causes significant yield losses in poultry. For this reason, it is necessary to remove the elements that cause stress from environment.

Tonic immobility, which is one of the most important measures of fear in poultry, is a reaction that restricts the movement of the animal for a short time. It is thought that tonic immobility is caused by the bird's temporary loss of ability to stand up due to fear, slowing of sympathetic nerve conduction, and inability to respond to external stimuli (Jones, 1989). Birds with a long tonic immobility period are evaluated as more passive, more timid and more cowardly than those who stand up in a short time. Gvaryahu et al. (1987) it was revealed that environmental enrichment had a reducing effect on the level of fear. There are some studies on the level of fear in Japanese quails, the relationship between fear and productivity traits, and the genetic background of this trait (Jones 1986; Minvielle et al., 2002; Mignon-Gresteau and Minvielle, 2003; Calandreau et al., 2011). Investigating the fear levels in lines with two-way long-term selection for body weight in Japanese quails, Jones et al. (2005) revealed that quails in the line with increased body weight had lower tonic immobility duration. Researchers have suggested that there is a negative relationship between the level of fear and growth performance. Contrary to this, Mignon-Gresteau and Minvielle (2003) examined the relationship between tonic immobility duration and some yield characteristics using factorial correspondence analysis, which is one of the multivariate analysis methods, and found that body weight, feed efficiency and egg production characteristics were not significantly associated with tonic immobility duration. In another study, two-way selection was made according to tonic immobility duration, and the relationship between some yield characteristics and fear level was investigated in control group Japanese quails (Minvielle et al. 2002). Researchers have reported that individuals with low fear levels perform better in terms of some growth and reproductive characteristics. As can be seen, no clear results could be found in studies examining the relationship between tonic immobility duration and yield characteristics. In this study, it was aimed to investigate the effects of housing type and stocking density on the level of fear in a Japanese quail flock. For this purpose, a total of 218 Japanese quails housed in individual and group cages were raised in 7 different stocking densities and their tonic immobility durations were measured.

Material and Methods

The experiment was carried out in an environmentally controlled quail coop in the Animal Production Research Unit of Akdeniz University Faculty of Agriculture. The quails used in the study were obtained from a randomly mated flock without any genetic improvement studies. The quails were assigned wing numbers at hatch and were housed in rearing cages with 96 x 43 x 21 cm compartments on each floor for the first three weeks. During the rearing period, quails were given mixed feed with 24% crude protein and 2900 kcal/kg metabolic energy content. Quails were placed in individual chambers measuring 14 x 20 x 21 cm from the third week. During the rearing period, 23 hours of daily lighting was applied to the quails. Randomly selected 108 quails were placed in groups in five chambers measuring 96 x 42 x 20 cm. Quails were placed in these cages using stocking densities of 120, 140, 160, 180, 200 cm^2/quail. Randomly selected 110 quails were placed in quail cages with individual compartments at a stocking density of 300 and 360 cm^2/quail.

In order to examine the growth in quails, the following form of the three-parameter Gompertz nonlinear regression model, which has been demonstrated in similar studies, was used (Akbaş and Oğuz, 1998):

$Y = \beta_0.\exp(-\beta_1.\exp(-\beta_2.t))$

The meanings of the terms used in the Gompertz model are as follows:

t : time

β_0 : mature (asymptotic) weight

β_1 : growth rate (integration constant)

β_2 : growth rate

Using the model parameters, the inflection point weight (IPW) and the inflection point age (IPT) were calculated as:

IPW: β_0/e

IPT: $\ln(\beta_1)/\beta_2$ Model parameters estimated in SAS 9.3 NLIN procedure with Levenberg-Marquardt iteration method (SAS 2003).

When the quails were six weeks old, immobility durations (TI; tonic immobility) were measured in order to determine the fear level of all quails. During the application of this test, the operator taking the test laid the quail to be tested on a special device on its back with its head hanging down and placed one hand on the quail's chest without applying any pressure and waited for the bird to become immobilized (calm) for 10 seconds. At the end of 10 seconds, the hand was slowly withdrawn, and the chronometer was started. In the measurement of inactivity time, the highest value was accepted as 5 minutes (Campo and Davilla, 2002).

In order to measure the effects of housing type and stocking density on tonic immobility duration, sixth week body weight, and growth curve parameters, variance analysis was performed according to nested-design, and the following statistical model was used:

$Y_{ijk} = \mu + b_i + ys_{j(i)} + e_{k(ij)}$

Yijk; Trait, μ; mean, b_i; effect of i^{th} housing type, $ys_{(i)j}$, the effect of the j^{th} stocking density in the i^{th} housing type, $e_{(ij)k}$; error term.

All statistical analyzes were performed using SAS 9.3 statistical software.

Results and Discussion

The tonic immobility durations, body weights, and parameters of Gompertz growth model measured in Japanese quails housed in group cages and individual cages according to different stocking densities are presented in Table 1. The tonic immobility durations determined in the study are consistent with the results of many studies performed in Japanese quail (Benoff and Siegel, 1976; Mignon-Gresteau and Minvielle, 2003). According to the short and long tonic immobility duration in Japanese quails, at the end of long-term (43 generations) mass selection, TI durations were found to be 113 seconds in the randomly mated control group, 399 seconds in the line selected according to the long TI duration, and 22.3 seconds in the line selected according to the short TI duration (Calandreau et al. .2011). The TI durations found in this study are consistent with the TI duration reported by Calandreau et al (2011) for quails mated by chance in control group.

Tonic immobility durations were found to be 68.47 seconds for quails housed in group cages and 83.55seconds for quails housed in individual cages. The difference observed in terms of tonic immobility times between housing types was found to be statistically significant ($P<0.05$). Salzen (1963) and Jones (1996) reported that tonic immobility duration measured in group housed animals were higher than in individually housed birds. In addition, in a study conducted by Kujiyat et al. (1983) with 1, 5, 15 and 17 laying hens in the cages, it was reported that the TI durations of the hens housed individually were higher ($P<0.01$). Salzen (1963) suggested that the level of fear of poultry isolated from their social environment increases, which will lead to stress and lead to loss of productivity. However, in many studies investigating the relationship between TI time and yield traits in poultry, it was reported that TI time did not have a significant effect on yield traits (Minvielle et al., 2002; Mignon-Gresteau and Minvielle, 2003; Skinner-Noble et al., 2003; Buijs et al., 2009).

Table 1. The mean values and statistical analysis results for tonic immobility duration, body weight, and parameters of Gompertz growth model according to stocking density and rearing type

Rearing Type	Stocking Density (cm^2)	TI (sec)	Body Weight (g)	β_0	β_1	β_2	IPW	IPT
Colony	160	72.18^b	204.42	256.49	3.45	0.068	94.36	18.21
	180	74.49^b	201.78	248.69	3.51	0.073	91.49	17.20
	200	75.21^b	199.86	252.81	3.55	0.066	93.00	19.20
	220	63.99^d	208.42	253.27	3.48	0.071	93.17	17.56
	240	56.49^e	207.54	245.89	3.62	0.069	90.46	18.64
Mean		68.47^B	204.40^B	251.43	3.52	0.069	85.48	18.24
SE		3.21	4.56	3.55	0.18	0.001	1.56	0.29
Individual	280	82.43^a	221.48	259.29	3.53	0.073	95.39	17.28
	360	84.67^a	218.56	255.57	3.54	0.073	94.02	17.32
Mean		83.55^A	220.02^A	257.43	3.54	0.073	85.26	17.32
SE		4.23	3.78	3.76	0.13	0.001	1.24	0.27
Variation Sources					P Value			
Rearing Type		0.001*	0.458	0.741	0.332	0.886	0.741	0.546
Stocking Density		0.001*	0.034*	0.484	0.128	0.749	0.484	0.548

*P<0.05. Lower case letters in the same column denote the difference between stocking density groups, and upper-case letters denote the difference between rearing types.

Japanese quails housed in individual cages have higher body weight averages at 6 weeks of age than those reared in colony cages ($P<0.05$). Cage type and stocking density applications did not affect the Gompertz growth curve parameters.

Conclusions

In conditions where fear factors are effective, irreparable economic losses occur both in terms of poultry and breeders. For these reasons, the importance of fear for poultry should be well investigated. For this purpose, some developed fear tests were tested on birds. Although fear tests alone do not provide sufficient information about bird welfare, it is necessary to know to what extent these tests can detect fear. It would be useful to investigate the contribution of this factor to general stress, without evaluating fear as a single factor on productivity characteristics.

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IS THE JAPANESE QUAIL A PRODUCTION MATERIAL OR A MODEL ANIMAL?

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Abstract

Japanese quail has been used for many years both for commercial production and scientific studies. In the past, the commercial production volume was quite low due to its low yield characteristics. However, with the widespread use of lines developed for meat and egg production in recent years, the production volume has also increased. Especially in developing countries, quail consumption is expected to increase much more. The Japanese quail, whose commercial importance is increasing, is also increasing in the rate of taking part in scientific studies. In particular, obtaining information that can be transferred to other poultry species more easily increases the scientific value of Japanese quails. As a result, Japanese quail, which was defined as a "model animal" in the past, is now turning into a "production animal". However, this does not mean that this transformation will negatively affect its importance in scientific studies. On the contrary, the Japanese quail is both a breeding material and a model animal.

Keywords: *Japanese quail, Genetic improvement, Commercial production, Meat, Egg yield.*

Introduction

In taxonomy, quail is included in the *Phasianidae* family among domestic birds, together with species such as turkey and partridge. Although there are different breeds such as California and Bob White, there are the most Japanese Quail (*Coturnix coturnix Japonica*) in the world (Chang, 2005). Many sources state that Japanese quails have been raised in Asia for their eggs and meat since the 17th century. However, as it can be understood from the verse "And we overshadowed you with clouds, we sent you halvah and quail, eat from the good blessings given" in Surah Baqarah in the Qur'an, quail has been produced in many parts of the world for food since ancient times.

When the scientific studies carried out in the twentieth century are examined, it is seen that the mature body weight values in flocks without genetic improvement were presented as 80-100 g by some researchers and as 180-220 g by some researchers. It is understood that this situation represents the transition period from wildlife to cage adaptation. It can be accepted that this process took place in the 20th century (Genchev, 2014). Egg weight of quails is between 8-12 g and increases with age similar to other poultry. The live weight at the end of the six-week fattening period is 180-220 g and the carcass yield is 68-72%. Unlike other poultry species, females are heavier than males. Life expectancy is only 2 to 2.5 years. There is gender discrimination based on plumage color; the female is characterized by long, pointed feathers with black spots on the throat and upper chest, while the males have rusty brown throat and chest feathers. Sexually active males also have cloaca glands, a bulbous structure located at the upper edge of their orifice that ejects a white foamy material. Reproductive performance is very high, even females without genetic improvement study have higher egg production than pure breed

Leghorn hens' annual egg production. There are lines with an annual egg yield of more than 300 in flocks that have undergone long-term genetic selection for egg production. Among the poultry species, quail has the highest ratio of testes weight to body weight. Since the sexual maturity age is 6 weeks, the production period is short, and 4 generations of genetic selection can be applied per year. Fertility and hatchability are quite high, but these characteristics vary considerably, especially depending on the feed content. Since quails are from the *Phasianidae* family, the crude protein ratio should be high in their feed rations. While the fertility rate of quails fed with commercial chicken breeder feed is around 70%, the fertility rate can exceed 90% in Japanese quails fed with breeder feed with a protein content of over 24%. Feed consumption is approximately 25 g per day in adult birds, and the feed conversion ratio in non-selected flocks is between 2.5-3.0. Quail eggs and meat are more nutritious than chicken eggs and meat. Quail eggs have proportionally more vitamins A and B and higher amounts of phosphorus and iron than chicken eggs. Due to the high concentration of valuable nutrients, quail eggs are not only a food product, but also a valuable therapeutic agent whose use for health is widely recommended by medical professionals (Arthur, 2017). Quail eggs are also superior to chicken in terms of the proportional content of essential amino acids such as tyrosine, threonine, lysine, glycine and histidine (Genchev, 2012). Quail eggs have antibacterial, immunomodulatory, antitumor properties, normalize the activity of the gastrointestinal tract and cardiovascular system. In Japan, quail eggs are well known for their ability to remove radionuclide from the body (Costăchescu, 2018). One of the biggest advantages of quail breeding is the small space requirement, an optimum area of 160 cm^2 is required in cage conditions. For optimum fertility, the family structure is 1 male and 3 females. Quail is perhaps the most resistant to adverse environmental conditions and diseases among the poultry species. Because of all these positive features, Japanese quails are used both in commercial production and are evaluated as model animals for other poultry species in scientific studies. The aim of this study is to review the information about the commercial production of Japanese quail and its use in scientific studies.

Japanese Quail as Production Material

About 1.5 billion quails are raised every year for egg and meat production in the world. With approximately 270 million quail production, approximately 20% of world production takes place in China. More than 100 million quails are produced in European Union countries, especially in the last 20 years, there has been an annual increase of 5-10% in meat consumption and 15% in egg consumption worldwide. The main reason for this is the prevalence of high-yielding lines developed through genetic improvement in South America and Southern Europe. In this way, both production has become more profitable and production volume has increased. China leads the world in egg production with 100 billion pieces per year, followed by Japan, United States, Brazil, Ukraine, Russia, and Turkey. The quail meat market is derived from the quail egg market and is generally lagging behind in its development. The main quail meat producing countries are China, Brazil, Spain, France, Italy and United States. In countries with high production volumes for poultry meat and eggs, the production volumes of non-chicken poultry species are between 10-20%. However, in many countries with an average production volume, almost all of the production is made with products obtained from chicken. This situation can be accepted as evidence that quail production will increase much more worldwide in the near future.

Japanese Quail as a Model Animal

Due to the important features mentioned in the introduction section of the paper, Japanese quails are used as model animals in many research areas. Compared to the chicken, it reaches sexual maturity faster, has a short generation interval, is easy to breed, requires less space and feed, and is resistant to diseases. Research tools developed for chickens can be used directly on quail or applied with some modifications. In this study, only poultry science, which is one of the scientific fields in which quails are used as model animals, is emphasized.

Since almost all of the commercial production in chickens and turkeys is carried out with hybrid material, environmental factors are of great importance in these animals raised under confined and environmentally controlled conditions. The improvement of the environment is also essential for the highest level of reflection of the genotype to the phenotype. For example, in studies conducted in the last decades, it has been revealed that lighting made at wavelengths that produce blue and green light close to ultraviolet in broilers increases the slaughter weight by 5-8% (Rozenboim, 2004). When the WOS database was searched, 28 studies were found in which monochromatic lighting applications were carried out using Japanese quails from 2000 to the present. Similarly, studies on environmental practices such as alternative rearing systems, enriched cage systems, litter management, stress management, epigenetic adaptation, thermal manipulation, incubation practices have been investigated in Japanese quails. The effects of the applications made in these studies on the growth, feed efficiency, slaughter carcass characteristics, gene expression levels, blood parameters, behavior and fear of quails were evaluated. It is stated in the conclusion part of many studies that the results obtained from these studies can be adapted to other poultry species.

For many years, scientific studies on animal feeding practices have been carried out by using quails in order to improve various yield characteristics in quails and to reduce the effects of negative factors such as environmental stress. Especially after the use of antibiotics in poultry feeding was banned in many countries, it is seen that the studies in question focused on adding materials that can be an antibiotic alternative to quail rations and drinking water or giving embryos through in-ovo feeding. Various feed additives have been used for different purposes such as improving feed efficiency, improving animal health and welfare, increasing yield level, and improving product quality. These include various organic acids (Soltan, 2008; Swiatkiewicz, 2010), probiotics (Deng, 2012; Wijayanti, 2019), prebiotics (Sarangi, 2016; AbdelHafeez, 2017), synbiotics (Radu-Rusu, 2010; Sarangi, 2016), and plant extracts (Murugesan et al., 2015; Ahsan et al., 2018). Recently, some herbal extracts have attracted a lot of attention due to their bioactive components (Wallace, 2010; Korkmaz and Narinç, 2022) and have shown beneficial effects on quails in antimicrobial, antioxidant, anti-inflammatory and antiparasitic ways (Cheng, 2014). In many studies carried out with quails, plant extracts or oils were evaluated as additives. These include black cumin (El-Bagir, 2006; Yalçın , 2009), thyme (Al-Kassie, 2009; Ghasemi, 2010), sage (Demir, 2008; Rasouli, 2019), rosemary (Yeşilbağ, 2011), olive leaf (Parsaei, 2014), passiflora extract (Landy, 2012; Banisharif, 2016), and grape seed oil (Tekeli, 2016; Salman, 2019).

The quail genome and the chicken genome have a very high similarity. Due to recent advances in next-generation sequencing, abundant sequence data has been generated for the quail genome and transcripts. These sequence data are valuable resources for studying functional genomics using quail, one of the model animals used to investigate gene function and networks. Quail is the best optimized model to study the functional genomics of poultry. In recent years, there have been many studies on this subject (Shin, 2017). Genomic selection could not be applied in Japanese quails, as it was in chicken species, because it is an expensive method and the product obtained is low in value. On the other hand, conventional genetic improvement studies have been carried out for many years and there are lines that have been integrated into commercial production in terms of egg and meat yield as a result of long-term selection studies in different parts of the world. Numerous short-term selection studies have also been carried out for different traits in Japanese quails, which are used as model animals for poultry genetic improvement studies. Many genetic parameters and genetic trends obtained in these long- and short-term selection studies played a key role in the breeding of other poultry species. Due to the specific characteristics of poultry breeding, 8-12 traits for layers and 10-16 traits for broilers are evaluated together in selection lines. Today, although genotyping of these traits can be done easily using SNP chips, it is always a problem how the genetic relationships between traits will be and how the total genetic progress in the next generation will be determined in terms of related traits. In order to prevent problems that may arise in this case, it is examined how the genetic relationships between the traits emerged in previous studies and measures are taken in the indexes according to the situation. Today, the genetics departments of the company that produce broiler and layer chicken hybrids all over the world, whose numbers do not exceed the fingers of both hands, follow scientific studies on genetic parameter estimations in poultry, and the majority of these studies were carried out using Japanese quails.

Conclusions

Commercial production of Japanese quail is increasing worldwide, and genetically improved high-yielding flocks are becoming more common. The concept of "model animal", which has been around for quails globally since the past, has recently evolved into "production animal". However, due to its important advantages, valuable information that can be transferred to other species continues to be produced in many scientific disciplines, especially in poultry genetics and breeding.

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RURAL DEVELOPMENT AND AGRO-ECONOMY

CALCULATION PRICE OF TRACTOR OPERATION BASED ON COSTS

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Abstract

Depending on the annual engagement of the tractor, on average from 400 to 500 hours, the calculation of fixed and variable costs for the MTZ 952 tractor is presented in the paper. Fixed costs refer to the costs of interest, depreciation, insurance, garaging and registration. Variable costs include fuel, lubrication, repair, and maintenance costs. In addition to the mentioned costs, profit margin and gross salary of workers were added, which influenced in formation of price of tractor work services and is shown in the calculation. Based on the total annual costs from 19,140 to 22,855 EUR hourly labor price from 45.71 to 47.85 EUR was calculated according to annual tractor engagement of 500 to 400 hours. With a higher annual engagement of tractor 500 hours, stated costs per hour were lower. In the structure of formation price of tractor work, variable costs are represented by 62.27-64.56%, fixed costs 13.14-15.68%, the salary of worker 6.46-6.76%, and profit margin had a share from 15.59-15.64%. Based on the calculated costs, farmers can realistically form service prices for tractor work. Also, the obtained results help them in rational decision-making to choose what is more profitable for them to purchase a new tractor or to hire a tractor from other farmers.

Keywords: *tractor, labor cost, fixed, variable, cost.*

Introduction

In the costs of production of agricultural products include costs of operating tractors and attachments. Technical and technological development and computerization of agricultural machinery have influenced and increase price of tractors and machines operation. According to research by Gunnarsson (2008), the share of agricultural machinery labor costs in total production costs is about 25%. On farms with a high degree of mechanization of production process, the costs of mechanization have a significant share in total costs.The use of tractors and combines with high engine power and advanced technologies has increased the fixed and variable costs of using machinery (such as, depreciation, spare parts, maintenance, repair, fuel and oil consumption, etc.) which is researchs confirmed by Sopengo et al. 2016, Najafi et al. 2015. The increased cost of tractor operation increases total production costs (Von Pentz, 2011), so the rational use of machinery and with small savings can improve the profitability of production (Zimmer et al., 2018). The authors also point out that on farms, fixed costs are higher for new tractors due to depreciation, insurance, interest and garage than for used tractors.

Farmers who own modern and expensive mechanization, and do not engage it enough on their farm, are economically interested in providing services to other producers. Practical experience shows that farmers form the prices of machine work at a flat rate, in direct contact and agreement with recipient of services, without taking into account the actual costs. Very often, prices of services are far higher than the real prices, because they are determined by market conditions and depend on the number of farmers who provide and seek the services of machines. Due to lack of labor in agriculture, demand for the provision of mechanical services has increased from year to year. Many agricultural producers in Serbia face these problems, which is also the case with producer association Eko Agrar from Čajetina

The aim of the research is to point out methodology of calculating fixed and variable labor costs of tractors and other agricultural machinery, in order to help farmers to realistically form the prices of machinery in the provision of their services. Also, by comparing the costs of owning agricultural machinery with the prices of service engagement of tractors and other machinery, taking into account the example of rementioned association, farmers will make rational economically correct decisions.

Material and Methods

The data were collected in the association Zlatiborski Eko agrar from Čajetina, was calculated and shown on the example of the MTZ 952 tractor. Based on the criteria of time variability and volume of use, the costs of agricultural machinery are divided into fixed (cost of ownership) and variable (available costs). The basic parameters taken to calculate fixed and variable costs of the new MTZ 952 tractor showen in Table 1.

Table 1. Basic parameters for cost calculation for MTZ 952 tractor

Parametres	Value
Nominal power of tractor	66 kW
Engine power utilization rate	80%
Specific fuel consumption	238 g/kWh
Ratio between kg and L of fuel	1kg = 1.176 L
Price of fuel*	1.6 €
Purchase value (Vo)	27,800 €
Interest rate (Ks)	3% od Vs
Number of years of tractor use (n)	12
Annual engagement of tractor work	400 and 500 hours
Expected liquidation value (Vn)	7,000
Mean value of the tractor (Vs)	17,400
Insurance costs	1% od Vs
Garage costs	1 % od Vo
Repair costs	0.8% od Vo

* Calculation of fuel price is for 2021.

The following methods were used in the calculation of tractor operating costs

Annual depreciation is calculated by the linear method according to formula, assuming an optimal service life of 12 years when tractor should be replaced with a new one.

$Ap = (Vo - Vn)/n$,

The mean value of tractor (Vs) is calculated according to formula: $Vs= Vo+Vn/2$

The annual amount of interest (Ki) for funds engaged for the purchase of tractors is calculated:

$Ki= Vo+Vn/2 \times Ks$, Ks - Interest rate on borrowed funds

Fuel consumption (Pg) was calculated using the formula: *Pg= Sw x Si x Spg x k*

(Pg - Fuel consumption (L); Sw- Tractor PTO power (kW); Si-degree of engine power utilization in% (80%) Spg-Specific fuel consumption (g / kWh), k- consumption conversion factor fuel from kg to L (1kg = 1.176 L).

Profit margin was calculated based on the formula Pm = (Ft + Vt) / 100 x 20,

(Pm-Profit margin; Ft-Fixed costs; Vt-Variable costs)

The above methods for calculating costs were applied for hiring the MTZ 952 tractor in 2021. According to the annual engagement of tractors for 400 and 500 hours, a comparison of the movement of total direct material costs in € and per working hour € / h is shown.

Results and Discussion

Fixed costs are calculated each year and include the following costs: depreciation, interest, insurance, garaging, repairs and maintenance, and registration. Fixed costs do not change on an annual basis, regardless of the volume of production and the degree of use of machinery. Also, fixed costs can be estimated based on specific, mathematical methods and formulas. According to Ranogajec (2009), Ćejvanović et al. (2016), and Koprivica et al. (2022) depreciation is the part of the value of fixed assets, and value of tractor, when used in the foreseen period of time, is gradually reduced and transferred to new products. A new tractor can be purchased from the depreciation costs of tractor, when service life has expired, if there has been no change in the purchase price. The annual amount of depreciation base calculated using the linear method is evenly distributed for each year of the planned use of the machine and amounts to € 1,733.33, regardless of the volume of work engagement of the tractor. In relation to purchase price of the new tractor, expressed as a percentage, depreciation is 6.24%. For Altintas and Ozcelik (2014), who calculated depreciation for tractors engaged on larger farms, it amounts to 5.8% of the purchase price of new tractor. The total amount of annual interest expenses under bank terms of loan for the purchase of a tractor is 3% of the average value and amounts to € 522, or € 1.31 / h and € 1.04 / h, depending on the annual engagement for 400 and 500 hours (Table 2).

Table 2. Calculation of annual fixed costs for MTZ 952 tractor depending on engagement (in €)

Annual fixed costs	400 hours	500 hours	Share in %
Depreciation costs	1,733.33	1,733.33	57.59
Interest costs	522	522	17.34
Insurance costs	174	174	5.78
Garage costs	278	278	9.24
Repair costs	222.4	222.4	7.39
Registration costs	80.0	80.0	2.66
Total annual fixed costs	3,009.73	3,009.73	100
Fixed costs per hour of work	7.52	6.02	/

According to the methods of the authors Khairo (2009), Zimmer (2018), Molenhuis (2020) and Edwards (2015), based on the purchase price of the tractor by 1%, garage costs were calculated and amount to € 278. If the actual insurance costs are not known, they are calculated from the average value of the tractor by 1%, which is € 174 per year (Table 2).

Repair costs are difficult to predict, due to their variability and tendency to increase over life of the machine, due to unforeseen failures. Annual repair costs refer to major repairs, such as engine overhaul are calculated on the basis of 0.8% of the purchase value of the tractor and amount to € 222.4. The cost of tractor registration is € 80 and includes technical inspection and

registration services. The total amount of annual fixed costs is € 3,009.73 or 10.83% of purchase value of the tractor. The results are in line with amount fixed costs stated by Edwards (2015), and lower than costs stated by Zimmer (2018) for new 75 kW tractors is € 5,909, which refers to tractors of higher power than the tested one.A higher volume of annual tractor operation will reduce fixed costs per hour of operation from 7.52 € / h to 6.02 € / h for 400 and 500 hours of annual engagement. Variable costs are fuel, lubricant, maintenance and repair costs. Variable (operating) costs vary depending on degree of engagement of machinery in production process. The average fuel consumption of tractor depends on engine power, the operating mode of tractor, and working conditions (and it is calculated according to the formula given in the part material and method). With a fuel consumption of 14.78 L / h at fuel prices of € 1.6, fuel costs for 400 hours of tractor operation were calculated € 9,459 and for 500 hours of operation € 11,824.
Fuel costs have the largest share in variable costs (Altintas and Ozcelik 2014), which was confirmed in our research, and also other authors cited in the paper. The costs of regular annual maintenance and repair (costs of oils, lubricants, filters), make up 20% of fuel costs and amount to € 1,892 for € 400 and € 2,364 for 500 hours of tractor operation. According to Lazarus (2021) the cost of lubricating oil is 10% of cost of fuel. In case of minor breakdowns in the regular use of tractor, 2% of the purchase value of tractor is planned for repair costs, which amounts to € 556 (Table 3).

Table 3. Calculation of variable costs for tractor MTZ 952 depending on engagement (in €)

Annual variable costs	**400 hours**	**%**	**500 hours**	**%**
Fuel costs	9,459	79.45	11,824	80.19
Lubrication costs	1,892	15.89	2,364	16.03
Repair and maintenance costs	556	4.66	556	3.78
Total annual variable costs	**11,907**	**100**	**14,744**	**100**
Total variable costs per hour worked	29.77	/	29.49	/

Labor costs can have a fixed and variable component depending on the number of working hours, in this case are not classified as either fixed or variable costs. Based on the data from the research for 2021, labor costs amounted to 3.10 € / h (Table 4). Also, the owner of the tractor realizes a profit margin that makes 20% of the total (fixed plus variable) costs of the machine and amounts to 7.46 € / h and 7.10 € / h for 400 and 500 hours of annual work (according calculated by formula in material and method). The author Molenhuis (2020) proposes that the profit margin be 15% of machine cost.

Table 4. Total costs and prices of MTZ 952 tractor services depending on annual engagement (in € / h)

Types of costs	**400 hours**	**%**	**500 hours**	**%**
Fixed costs	7.52	15.68	6.02	13.14
Variable costs	29.77	62.27	29.49	64.56
Profit margin	7.46	15.59	7.10	15.54
Salary of worker	3.10	6.46	3.10	6.76
Price of tractor service (in € / h)	47.85	100	45.71	100
Total annual costs (in €)	**19,140**	/	**22,855**	/

In the structure of total tractor costs, fixed costs are represented by 13.14% to 15.68%, depending on annual tractor engagement of 500 and 400 hours (Table 4).
According to the research of Altintas and Ozcelik (2014), fixed costs on large farms are represented by 16.74% in relation to the total costs of tractors, which is in accordance with calculated fixed costs in our research.
Variable costs are represented by 62.27% to 64.56% in the total costs for tractor engagement 400 and 500 hours per year (Table 4).
The service prices of work of tractors calculated in research range from 45.71-47.85 € / h and are higher than the results obtained by Edwards (2015) of 38.70 € / h for an annual tractor engagement of 400 hours.
The calculated results of tractor operating costs cannot be completely in agreement with the results of other researchers, due to differences in the purchase price of tractors, as well as value of variable costs, because of large differences in fuel prices in individual countries which is confirmed by research Goyal and Singh (2020).
There is no single rule and precise method for determining of cost machines, in practice the “percentage method” can be used as stated by numerous authors Edwards W. (2015), Ćejvanović et al. (2016), Zimmer (2018), and Koprivica et al. (2022).
Also, Krmpotić and Kiš (2015) point out that most reliable source of data for calculating the costs of agricultural machinery is keeping one's own records because it is a good basis for estimating real costs.
Authors Sopengo et al. (2016) use a simple web mobile application (AMACA - Agricultural Machine Application Cost Analysis) to determine the actual operating costs of machines when performing various agrotechnical measures.
In some countries, such as Germany and Slovenia, every year in the catalog of minimum prices for service use of tractors and machines in machine rings, a percentage share of costs is given in relation to the purchase price of machinery used. Based on that, producers can tentatively form the prices of their services.

Conclusion

Depreciation costs of 57.59%, followed by interest of 17.34%, garages of 9.24%, repairs of 7.39%, insurances of 5.78% and registrations of 2.66% have largest share in the calculation of tractor operating costs in fixed costs. Fuel costs 79.45-80.19% have largest share in the calculation of variable costs, followed by costs of lubrication 15.89-16.03% and regular maintenance 3.78% -4.66% for 400 and 500 hours of annual tractor engagement.
Based on the results of research, producers can plan fixed depreciation costs and annually allocate certain amounts of money for the purchase of new agricultural machinery. Also, the results of research will help farmers who provide services to calculate total costs of mechanization (fixed and variable) and on that basis realistically form prices of their services. With the development of modern, precise mechanization, the lack of labor in agriculture, engagement of tractors is increasing and margin of services is decreasing, which affects the prices of service work of tractors and machines when performing work operations.

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STRUCTURAL AND NON-STRUCTURAL CARBOHYDRATES CONTENT OF APPLE POMACE SILAGES

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Abstract

Apple pomace was ensiled without additives and with the addition of 15% dried beet pulp in order to increase the level of DM, 15% of sunflower meal and 15% dry beet pulp and 1% of NPN substances, and each of these treatments with and without inoculant. Apple pomace (AP) was obtained from the factory Vino Župa Aleksandrovac, delivered to the Institute of Forage Crops in Kruševac, location Globoder, on April 2nd 2013 and ensiling was performed on April 3rd 2013. Study treatments in the present research of the apple pomace silage were: added feed (A) and applied inoculant (B): A_1 - ensiled apple pomace 100% participation (a_1b_1 - apple pomace without inoculants; a_1b_2 - apple pomace with inoculants; A_2 - apple pomace 85% + 15% of dry beet pulp (a_2b_1 - without inoculants; a_2b_2 with inoculants); A_3 - apple pomace 85% + 15% sunflower meal (a_3b_1 - without inoculants and a_3b_2 with inoculants); A_4 - apple pomace 84% + 15% sugar beet pulp + Benural S 1% (a_4b_1 - without inoculants and a_4b_2 with inoculants). Results of these investigations showed that apple pomace silage with dry beet pulp had the highest CHO (817.8 g kg^{-1} DM), NFC (358.7 g kg^{-1} DM) and WSC (44.9 g kg^{-1} DM) contents. This study has shown that apple pomace silages, as a by-product of the technological process has significant nutritional value.

Keywords: *apple pomace silage, dry beet pulp, sunflower meal.*

Introduction

The ability to provide an adequate level of energy for highly productive dairy cows depends on how accurately and qualitatively the nutritional composition can be determined. Rumen microorganisms use carbohydrates in order to synthesize microbial proteins and maintain the normal functioning of the rumen. Carbohydrates are the basic source of energy necessary for the maintenance and production of milk. They are also precursors in the synthesis of lactose, fats and proteins. Formulation of meals based on the rate of Neutral Detergent Fiber (NDF) in the dry matter of meals is recommended due to the positive correlation between NDF concentration and rumen capacity, as well as the negative correlation between NDF and nutrient energy value (Mertens, 1994).

The concept proposed by Mertens (1983) shows that a meal containing 35% NDF stimulates maximum dry matter intake, meals with a higher NDF content can limit dry matter intake, and meals with a lower NDF content can also limit dry matter intake due to animal energy needs. Mertens (1994) indicated that the maximum rate of NDF in the rations for dairy cows in the middle and at the end of lactation is 1.2 ± 0.1% of body weight per day. The relationship between non-structural carbohydrates, readily available starch and NDF in the meal is very important for maintaining the normal functioning of the rumen. Poore et al. (1991) indicated that the ratio between NDF from forages and easily degradable starch should be 1: 1 in order to avoid depression in fiber digestion and ensure normal rumen function. Nocek and Russel (1988) reported that maximum milk production is achieved when the ratio between nonstructural carbohydrates and NDF is 0.9 to 1.2.

There are a number of factors that affect dairy cows' fiber needs, including dry matter intake, the amount and type of non-structural and structural carbohydrates in the diet, particle size and nutrient processing, and the rate and extent of fiber fermentation processes. Better knowledge of these factors is necessary in order to achieve maximum energy intake in early lactation. The National Research Council (NRC,2001) recommends that the rate of NDF and Acid Detergent Fiber (ADF) in the meal should be 25-28% and 19-21%, respectively, and that 75% of NDF should come from forage feeds.

Apple pomace has significant nutritional value, given that it is a rich source of simple sugars and fiber and contains certain amounts of minerals and vitamins. Therefore, the use of this by-product in animal nutrition is one of the important ways to use it. Nutrient deficiency is a relatively low protein content and the presence of pectin and tannins as antinutritive factors that can negatively affect nutrient utilization in animals (Zhong-Tao et al., 2009).

Previous research has largely taken into account the possibility of using apple pomace to feed ruminants. Fresh, ensiled or dried apple pomace represent the main energy content of ruminants. According to the NRC (2001), a meeting of dairy cattle, the metabolic energy of apple pomaces is 80% of the metabolic energy of maize. Macgregor (2000) states that fresh or dry apple pomace contain about 80% of total digestible nutrients, such as this form, which can be used in the diet of dairy cows in the amount of 13.5 kg per day. Research has shown that apple pomace is more suitable for cattle nutrition than for young dairy cows nutrition (Knežević et al., 2005). According to Walker (2000), the introduction of apple pomace should be carried out with a certain amount of caution, given the availability of simple sugars. The same author states that apple pomace can generally be used in the diet of cattle in the amount of up to 25% of the dry matter of the meal. Fresh apple pomace can be used in animal nutrition only at the local level, in the vicinity of the factories where it is produced, so its use in the fresh state is almost negligibly small (Dhillon et al., 2013). For this reason, the apple pomace is most often used in ensiled or dry form in animal nutrition.

Apple pomace is a rich source of sugar, dietary fiber and minerals. There is a real need to examine the possibility of its use as a nutrient in animal nutrition and in the industrial production of animal feed.

The aims of this investigation is to examine the nutritional value of dried apple pomace and the possibility of its use as a silage in animal nutrition.

Materials and methods

Apple pomace (AP) was obtained from the factory Vino Župa Aleksandrovac, delivered to the Institute of Forage Crops in Kruševac (Serbia), location Globoder, on April 2nd 2013 and ensiling was performed on April 3rd 2013. The apple pomace was ensiled in the experimental containers holding 130 dm^3, with three replications. After compaction, silo mass was covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load.

Study treatments in the present research of the apple pomace sillage were: added feed (A) and applied inoculant (B): A_1 - ensiled apple pomace 100% participation (a_1b_1 - apple pomace without inoculants; a_1b_2 - apple pomace with inoculants; A_2 - apple pomace 85% + 15% of dry beet pulp (a_2b_1 - without inoculants; a_2b_2 with inoculants); A_3 - apple pomace 85% + 15% sunflower meal (a_3b_1 - without inoculants and a_3b_2 with inoculants); A_4 - apple pomace 84% + 15% sugar beet pulp + Benural S 1% (a_4b_1 - without inoculants and a_4b_2 with inoculants).

The apple pomace is characterized by an extremely high moisture content (which is why the dry sugar beet and sunflower were added), yellowish colour and pleasant smell.

Benural S was used as the Non-Protein Nitrogen (NPN) substance, which contained 42% of urea, 56% of bentonite, and 2% of sulphur. In contrast to the pure urea, this product comprises bentonite, which allows slower release of ammonia in the rumen, and more efficient utilization by the rumen microorganisms, it binds some gases and toxic substances and contains certain important elements (K, Na, Mg etc.). The sulphur present in Benural S enables efficient synthesis of some essential amino acids that contain this macro-element (methionine, cystine). Dry sugar beet pulp and sunflower meal were purchased commercially and were according to their declaration. *BioStabil Plus* is inoculant which contains homo-fermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of $5x10^{10}$cfu in a gram of product.

Samples were dried to constant weight at 65° C for 48 h and dried samples were ground through a screen size of 1 mm. All analyses were done in duplicate and component concentrations were corrected to a 105° C dry matter basis. Total carbohydrates [CHO = 1000 – (CP + ash + ether extract)] and Non-Fibre Carbohydrates [NFC = 1000 – (aNDF + CP + ash + ether extract)] were calculated according to NRC (2001). Neutral detergent fibre was determined according to the method by Mertens et al. (2002). Acid detergent fibre and Acid detergent lignin were determined according to the method AOAC 973.18. WSC (Water Soluble Carbohydrates – monosaccharides and disaccharides) were determined as total water soluble carbohydrates was determined according to procedures described by Hall et al. (1999). Two stage pepsin-cellulase method was used for *in vitro* DMD - Dry Matter Digestibility (De Boevar et al., 1986).

Data were analyzed using ANOVA in a randomized block design using the Stat. Soft. Statistica 6. The statistical significance of differences tested using LSD-test and significant differences among means were accepted at $P< 0.05$.

Results and discussion

The obtained results for the total carbohydrates, non-fiber carbohydrates, water soluble carbohydrates, cell wall components and dry matter digestibility in ensiled apple pomace with dry beet pulp, sunflower meal and sugar beet pulp wit inoculants and without inoculants are presented in the Table 1.

Results obtained in this study showed that apple pomace silage with dry beet pulp had the highest concentration of CHO (817.8 g kg^{-1} DM), NFC (358.7 g kg^{-1} DM) and WSC (44.9 g kg^{-1} DM). On the other hand, ensiled apple pomace 100% participation was characterized by the highest concentration of NDF (541.7 g kg^{-1} DM), ADF (486.7 g kg^{-1} DM) and ADL (142.3 g kg^{-1} DM). Although the concentration of NDF, ADF and ADL were very high in this treatment, DMD of apple pomace silage was the highest (783.0 g kg^{-1} DM), followed by apple pomace silage with dry beet pulp (772.6 g kg^{-1} DM). Apple pomace with sunflower meal silage was characterized by the lowest concentration of CHO, NFC, WSC and NDF, but on the other hand, this silage was characterized by the lowest DMD (727.8 g kg^{-1} DM). Application of inoculant did not affect the content of NFC, NDF, ADF and ADL, but silage treatments without inoculant

showed higher content of CHO and WSC and higher DMD, compared to treatments with inoculant (Table 1).
According to NRC (2001) data, moist apple trop in dry matter contains 52.5% NDF, 43.2% ADF and 15.4% lignin. In studies conducted by Besharati et al. (2008), Taasoli & Kafilzadeh (2008), Mirzaei-Aghsaghali (2011), Pieszka et al. (2015), Juskiewicz et al. (2015) the content of NDF in the dry matter of apple pomace ranged from 35.3% to 61.2%, and ADF from 28% to 46.7%. In a study by Juskiewicz et al. (2016) ADL content in dried apple pomace was 10.4%, while in a study by Pieszka et al. (2016) obtained value of ADL content of 14.2%.

Table 1. Carbohydrates content and dry matter digestibility of Apple Pomace silages

Treatments		CHO g kg^{-1} DM	NFC g kg^{-1} DM	WSC g kg^{-1} DM	NDF g kg^{-1} DM	ADF g kg^{-1} DM	ADL g kg^{-1} DM	DMD g kg^{-1} DM
A_1	B_1	794.4^{c}	282.8^{de}	42.87^{a}	539.5^{a}	486.4^{a}	145.0^{a}	795.3^{a}
	B_2	782.7^{d}	265.5^{ef}	27.18^{c}	543.8^{a}	486.9^{a}	137.5^{ab}	770.8^{b}
A_2	B_1	825.9^{a}	366.3^{a}	45.24^{a}	499.5^{b}	389.4^{c}	100.2^{d}	775.7^{b}
	B_2	809.6^{b}	351.2^{ab}	44.56^{a}	500.7^{b}	364.9^{d}	84.7^{e}	769.4^{b}
A_3	B_1	665.7^{f}	239.9^{fg}	34.13^{b}	5456.3^{c}	403.7^{b}	124.5^{bc}	733.9^{cd}
	B_2	670.2^{f}	233.1^{g}	24.14^{c}	464.7^{c}	409.9^{b}	124.1^{bc}	721.6^{d}
A_4	B_1	750.5^{e}	296.3^{cd}	41.98^{a}	493.3^{b}	388.8^{c}	104.7^{d}	745.1^{c}
	B_2	743.8^{e}	322.4^{bc}	33.55^{b}	456.4^{c}	380.6^{c}	123.4^{c}	747.3^{c}
Mean A_1		**788.6^{b}**	**274.2^{c}**	**35.0^{b}**	**541.7^{a}**	**486.7^{a}**	**141.3^{a}**	**783.0^{a}**
Mean A_2		**817.8^{a}**	**358.7^{a}**	**44.9^{a}**	**500.0^{b}**	**377.2^{c}**	**92.5^{d}**	**772.6^{a}**
Mean A_3		**667.9^{d}**	**236.5^{d}**	**29.1^{c}**	**460.5^{c}**	**406.8^{b}**	**124.3^{b}**	**727.8^{c}**
Mean A_4		**747.1^{c}**	**309.4^{b}**	**37.8^{b}**	**474.8^{c}**	**384.7^{c}**	**114.0^{c}**	**746.2^{b}**
Mean B_1		**759.1^{a}**	**296.3^{ns}**	**41.0^{a}**	**497.1^{ns}**	**417.0^{ns}**	**118.6^{ns}**	**762.5^{a}**
Mean B_2		**751.6^{b}**	**293.1^{ns}**	**32.4^{b}**	**491.4^{ns}**	**410.6^{ns}**	**117.4^{ns}**	**752.2^{b}**
Mean AB		**755.4**	**294.7**	**36.7**	**494.3**	**413.8**	**118.0**	**757.4**

A_1 – ensiled apple pomace 100% participation; A_2 – apple pomace 85% + 15% of dry beet pulp; A_3 – apple pmace 85% + 15% of sunflower meal; A_4 – apple pomace 85% + 15% of sugar Beet pulp + Benural d 1%; B_1 – silages treatments witout inoculant; B_2 – silages treatments with inoculant; CHO – Total Carbohydrates, g kg^{-1} DM; NFC – Non-Fiber Carbohydrates, g kg^{-1} DM; WSC – Water Soluble Carbohydrates, g kg^{-1} DM; NDF – Neutral Detergent Fiber, g kg^{-1} DM; ADF – Acid Detergent Fiber, g kg^{-1} DM; ADL – Lignin, g kg^{-1} DM; DMD – Dry Matter Digestibility, g kg^{-1} DM; Different letters denote significantly different means ($P< 0.05$).

Due to the high moisture content, when ensiling apple pomace, it is necessary to add dry material as absorbent, such as cereal straw, which prevents nutrient loss due to squeezing silage water (Alibes et al., 1984; Antov et al., 2004). The research they conducted Pirmohammadi et al. (2006) showed that by ensiling apple pomace with the addition of 10% wheat straw in relation to the amount of pomace, can get silage of satisfactory quality, which characterized by low pH value, low concentration of acetic and butyric acid, and high lactic acid concentration. According to Antov et al. (2004), very good silage quality was obtained by combined ensiling of apple pomace with sugar beet pulps and beer tropic in a ratio of 25:50:25. The same authors also state that apple pomace can be successfully ensiled in different combinations with fresh beet pulps, as well as in combinations with up to 35% corn and up to 35% chopped oat straw.

Conclusions

According to the results obtained in this investigations we can conclude that the inoculant application in apple pomace silages did not have significant effect on the NFC, NDF, ADF and ADL content. The higher content of CHO and WSC was determined in the treatments with inoculant application. Application of inoculant influenced significantly higher DMD of investigated silages. Results of these investigations showed that apple poace silage with dry beet pulp had the hihest CHO (817.8 g kg^{-1} DM), NFC (358.7 g kg^{-1} DM) and WSC (44.9 g kg^{-1} DM) contents, but this treatment was characterized by the lowest ADF content (377.2 g kg^{-1} DM) and lignin content (92.47 g kg^{-1} DM). On the other hand, the highest DMD was determined in ensiled apple pomace – 100% participation. These studies have shown that apple pomace silages, as a by-product of the technological process has significant nutritional value as a nutrient in animal nutrition, and can be successfully used in industrially produced substitutes for other nutrients. In this way, it can significantly contribute to the economy of production, conservation of natural resources and environmental protection. Future research should provide even more precise answers on the possibilities of using apple pomace in other areas of application.

Acknowledgments

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TENDENCIES AND PREDICTION OF MAIN ANIMAL PRODUCT PRICES AND PRICE PARITY IN SERBIA

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Abstract

In this paper, subjects of research were prices and price parities of important kinds of animal products in Serbia – fattening pigs, cow’s milk and fattening chicken. The main goals, based of quantitative analysis, were to find out tendencies in prices and prices parities movements, and to predict it in future period. The absolute prices are analyzed in the period 2002-19. The analysis of the relative prices, which are prices parity of certain animal product in relation to maze, was conducted for the period 1994-2019. The aim of this analysis was to formulate the relative changes of the economic position of certain animal product in relation to maze. Prediction of the prices was made for a five-year period, 2020-2024. ARIMA models were used for predict of price parity. Change rates were used to forecast absolute prices. Quantitative analysis was performed by using descriptive statistics method, and prediction period was 2020-24. Average year price of pigs was 1.65euro/kg. Price was changing in interval between 1.05 and 2.40euro/kg. Annual change rate of pig price in Serbia was minus 0.21%. Average year price of milk was 0.24euro/l. Price was changing in interval between 0.17 and 0.29 euro/l. Annual change rate of milk price in Serbia was 2.57%. Average year price of chicken was 1.04 euro/kg. Price was changing in interval between 0.72 and 1.87 euro/kg. Annual change rate of chicken price was minus 1.63 %. The average price parities of animal products for the period 1994-2017 will have next forecast for year 2024: pig’s price parity will decrease from 14.25 to 13.29; milk price will change insignificantly, from 1.85 to 1.86; chicken price will decrease from 11.85 to 4.40. Absolute prices of animal products predict in 2024 on the next level: pigs 1.58euro/kg, milk 0.31euro/l and chicken 0.75 euro/kg.

Key words: *animal products***,** *prices, prediction***,** *Serbia.*

Introduction

Production of animal products depends of natural (agro-ecological) and economics conditions required for their production, and of state agrarian policy. The Republic of Serbia has favorable natural conditions to provide enough fodder for animal. Fodder plants posses more than 200.000 hectares in the Republic of Serbia. Also, corn is number one plant with about 1 million hectares, about 6.5 million tons. Area of meadows and pastures is about 670.000 hectares.

In this paper, subjects of research were prices and price parities of important kinds of animal products in Serbia. In analysis and prediction was taken most important animal products for Serbian agriculture: fattening pigs, cow’s milk and fattening chicken.

The main objectives, based of quantitative analysis, were to find out tendencies in prices and prices parities movements, and to predict it in future period.

There are numerous examples of applying quantitative methods in analyzing, modeling, forecasting and planning of economic characteristics of agricultural products and inputs in agriculture (Ivanišević et al. 2016; Mihajlović et al. 2018; Mutavdžić et al. 2007; 2010; 2017, Novković et al. 2006; 2020; 2021).

Materials and methods

The research methods applied in this paper were selected based on the described subject and aim of the research. The statistical methods included descriptive statistics and time series analysis.

The absolute prices and the price parities of the most important animal products in Serbia were analyzed by means of descriptive statistics for the period 2002–2019. The analysis of the relative prices, which are price parities of certain animal product in relation to maze, was conducted for the period 1994-2019.The analysis included the basic statistical indicators: average value, extreme value (minimum and maximum), coefficient of variation and average annual rate of change (r).

Prediction of the prices for the industrial crops was carried out using of the average annual rate of change (r):

$$r = (G - 1)$$

$$G = \left(\frac{\mathrm{Y}_n}{\mathrm{Y}_1} \right)^{\frac{1}{n-1}}$$

where:

r = the average annual rate of change,
G = the average annual index of change,
Y_1 = the absolute value of the first member of the time series,
Y_n = the value of the last number of the time series,
n = the length of the series (the number of years).

ARIMA models were used for predict of price parity, while average year change rates were used to forecast absolute prices.

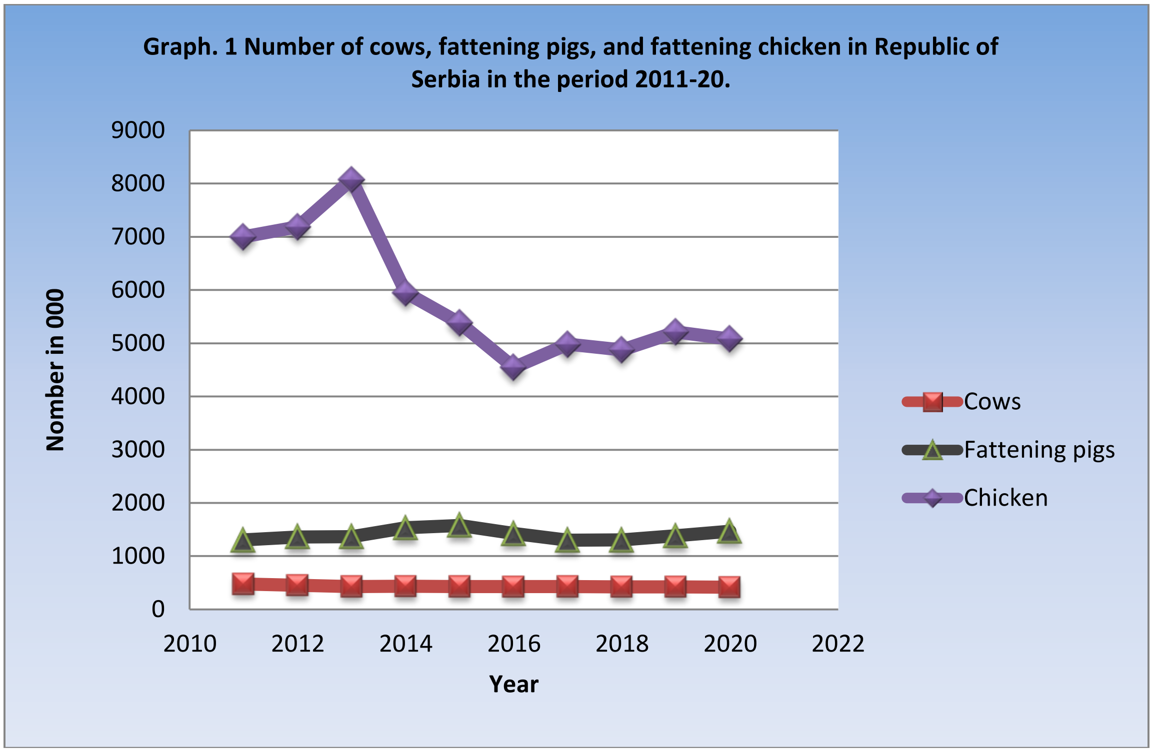
The average annual prices of the animal products were converted into euro per ton to enable comparison with foreign countries and to reduce the factor of domestic inflation. Conversion of the prices into euro was carried out according to the average annual exchange rate of euro based on the data of the National Bank of Serbia. The prediction was made for the period 2020–2024.

The data used in the analysis refer to the prices in Serbia. The price series in this paper are either taken from or formed on the basis of statistical publications of the Institution of Statistics of the Republic of Serbia.

Results and discussion

<u>Animal production in the Republic of Serbia</u>

Graphic number 1 shows number of cows, fattening pigs, and fattening chicken in the Republic of Serbia in the period 2011-20.



Source: Statistical institution of Serbia

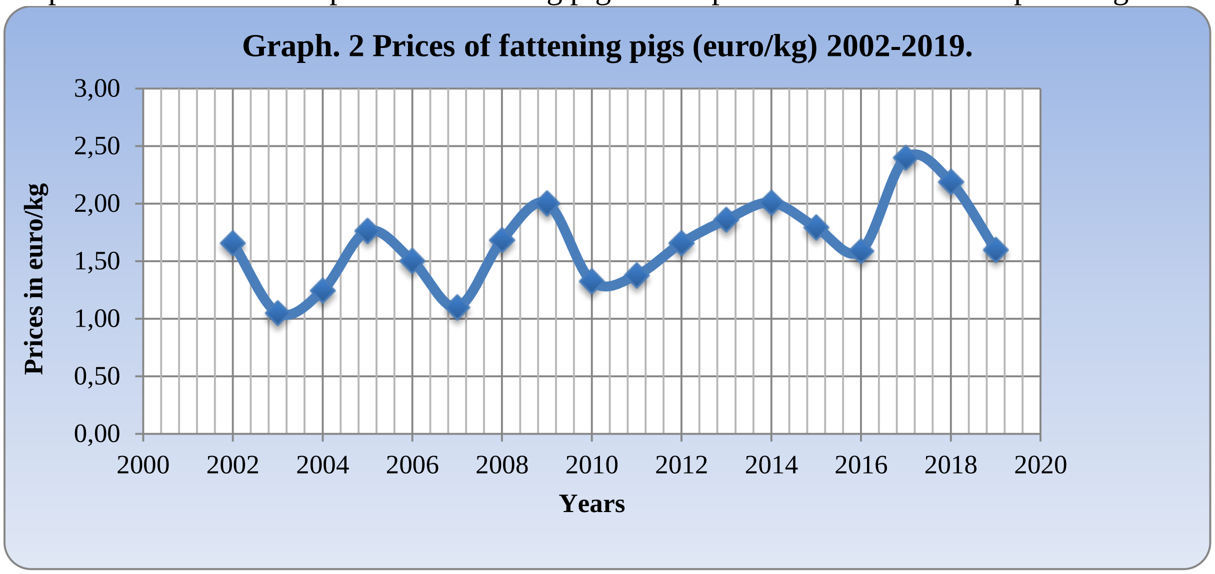
Fattening pigs in Serbia ranged from 1.3 to 1.58 million in analyzed period. The minimal pig number was in 2017, and maximal in the 2015. Descriptive analysis of the prices of fattening pigs show that average price in analyzed period (2002-2019) was 1.65 euro per kg. The price ranged from the minimum price of 1.05 EUR/kg in 2003, to the maximum price of 2.40 EUR/kg in 2017. The coefficient of variation was relatively high, but not the highest of all analyzed animal products, amounting to 21.81%. The price of fattening pigsshows a tendency of slight decrease at an average annual rate of minus 0.21%.

Number of milk cows in Serbia in analyzed period decreased in the interval from 477. 000 herds in first year, to 417.000 in last year (2020). The average annual price of cow's milk was 0.24 euro per litter. The annual price of milk varied in the interval from 0.17EUR/l in 2003 to 0.29EUR/l in 2008. Price of cow's milk had a lowest coefficient of variation of 18.95%. The milk price showed tendency of increase at an average rate of 2.57% per year.

In Serbia, number of fattening chicken decreased from 7 million in 2011, to 5.1 million in 2020. Maximal number of fattening chicken, 8.1 million was in 2013, while minimal of 4.5 million was in 2016. Descriptive analysis of the prices of fattening chicken show that average price in analyzed period (2002-19) was 1.04 euro per kg. The price ranged from the minimum price of 0.72 EUR/kg in 2010, to the maximum price of 1.87 EUR/kg in 2003. The coefficient of variation was highest comparing with other analyzed animal products, and it was 28%. The price of fattening chicken shows a tendency of decrease at an average annual rate of minus 1.61%.

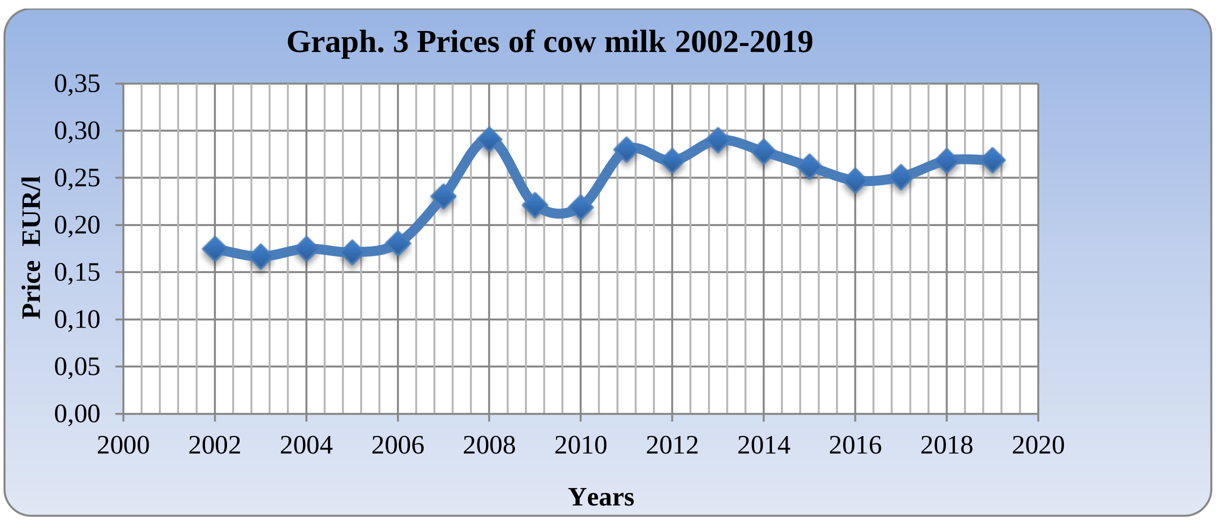
Animal products price

Graphic number 2 shows prices of fattening pigs in the period 2002-19 in euro per kilogram.



Source: Statistical institution of Serbia

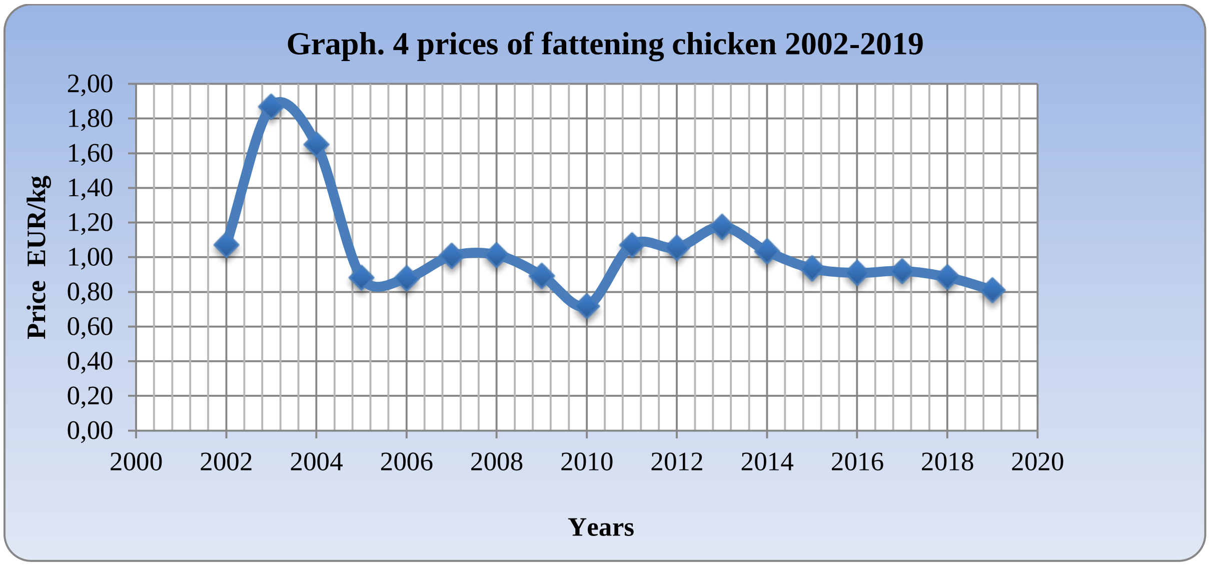
The average price of fattening pigs was 1.65 EUR/kg. It ranged from 1.05 EUR/kg in 2003 to 2.40EUR/kg in 2017. The coefficient of variation of fattening pig price was 21.81%. Price shows low negative tendency. The average year change rate in analyzed period was minus 0.21%.
Graphic number 3 shows prices of cow milk in the period 2002-19 in euro per litter.



Source: Statistical institution of Serbia

The average price of cow milk in observed period was 0.24 EUR/l. It ranged from 0.17 EUR/l in 2003 to 0.24 EUR/l in 2008. The coefficient of variation of milk price was 18.95%. Milk price shows positive tendency. The average year change rate cow milk price was minus 2.57%.
Graphic number 4 shows prices of fattening chicken in the period 2002-19 in euro per kilogram.The average price of fattening chicken was 1.04 EUR/kg. It ranged from 0.72 EUR/kg in 2010 to 1.87 EUR/kg in 2003. The coefficient of variation of fattening chicken price was the

highest, 28%. Price shows negative tendency, much stronger than to fattening pigs. The average year change rate in analyzed period was minus 1.63%.



Source: Statistical institution of Serbia

Predicted prices of analyzed animal product are presented in table 1.

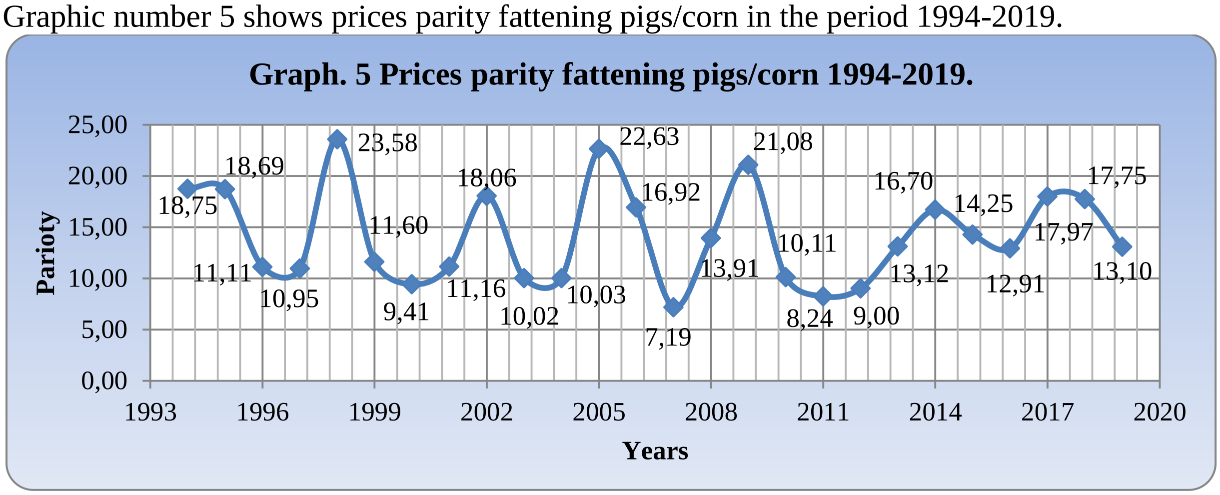
Table 1. Predicted prices of animal products in Serbia (euro/t; euro/l) for the period 2020-2024

Animal product	*Year*				
	2020	*2021*	*2022*	*2023*	*2024*
Fattening pig	1.59664	1.593287	1.589941	1.586602	1.58327
Cow milk	0.276939	0.284056	0.291357	0.298844	0.3065252
Fattening chicken	0.796797	0.783809	0.771033	0.758465	0.746102

Source: Author's calculation

Animal products price parity

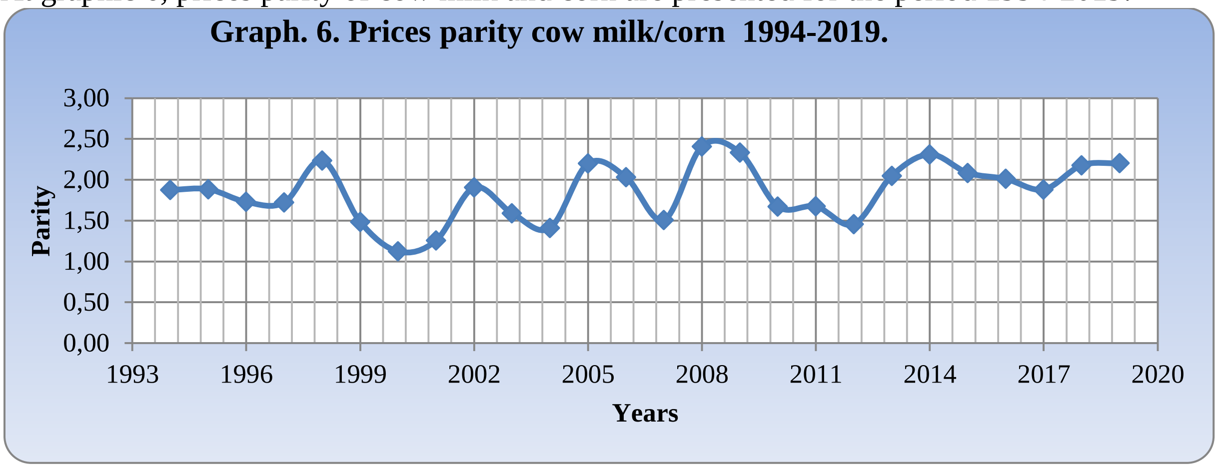
Graphic number 5 shows prices parity fattening pigs/corn in the period 1994-2019.



Source: Statistical institution of Serbia

The average relative price of fattening pig, i.e. fattening pig/corn price parity was 14.25. This means that one kg fattening pig was worth as much as 14.25 kg of corn. The price parity varied in the interval from 7.19 in 2007 to 23.58 in 1998. The coefficient of variation was 21.62%. The fattening pig /corn price parity had a negative tendency. Average change rate of parity was minus 1.43%.

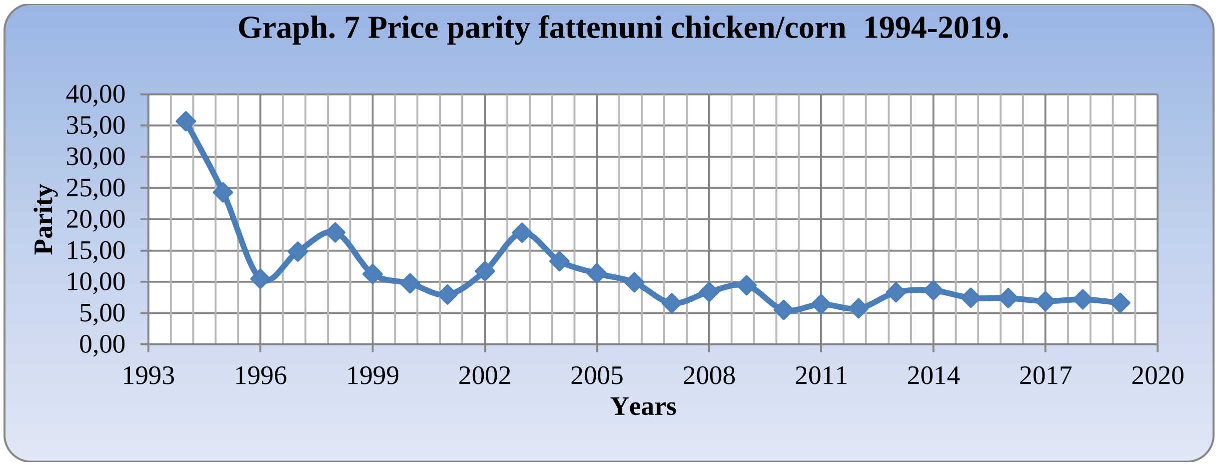
At graphic 6, prices parity of cow milk and corn are presented for the period 1994-2019.



Source: Statistical institution of Serbia

The average relative price of cow milk, i.e. cow milk/corn price parity was 1.85. This means that one litter of cow milk was worth as much as 1.85 kg of corn. The price parity varied in the interval from 1.12 in 2000 to 2.41 in 2008. The coefficient of variation was 20.57%. The cow milk/corn price parity had a low positive tendency. Average change rate of parity was 0.65%.

Graphic number 7 shows prices parity fattening chicken/corn in observed period. The average relative price of fattening chicken, i.e. fattening chicken/corn price parity was 11.85. This means that one kg fattening chicken was worth as much as 11.85 kg of corn. The price parity varied in the interval from 5.49 in 2010 to 35.69 in 1994, first year of analyzed period. The coefficient of variation was extremely high (because of price parity in first year of observed period, 67.54. The fattening chicken /corn price parity had a high negative tendency. Average change rate of parity was minus 5.50%.



Source: Statistical institution of Serbia

Using ARIMA models, prices of analyzed animal product parity with corn price are aer predict. Results of ARIMA prediction are present in table 2.

Table 2. Predicted prices parity of animal products in Serbia for the period 2020-2024

Animal product	*Year*				
	2020	*2021*	*2022*	*2023*	*2024*
Fattening pig/corn	11.811	14.692	15.231	13.469	13.286
Cow milk/corn	1.902	1.851	1.865	1.861	1.863
Fattening chicken/ corn	6.002	5.552	5.137	4.752	4.396

Source: Author's calculation

Conclusion

Conclusions of this research is:

- The prices of fattening pigs and chicken have tendency of decrease, while cow milk price increase. All prices are very unstable.
- The same situation is with price parity. So, in general, relatively price position of animal products is not good, and it will be even worse.
- If state don't change agrarian policy, animal production will be in much more worse position than it is now.

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DEVELOPMENT OF BEEKEEPING AND HONEY PRODUCTION AS A RESULT OF THE POLICY MEASURES APPLIED IN BULGARIA AFTER THE MEMBERSHIP TO THE EUROPEAN UNION

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Abstract

The diverse and abundant vegetation in Bulgaria creates excellent conditions for beekeeping. The development of beekeeping in Bulgaria is related to the development of the sector in European countries and in Russia. The first major steps in the development of the sector are the delivery of the first frame beehives in 1884, the establishment of the first beekeeping organization in 1889 and the creation of the first Beekeeping Testing Station with departments in Vidin and Smolyan in 1951. Although Bulgaria occupies 0.08 % of the world's territory, the country provides over 1 % of global honey production and accounts for about 2 % of world trade in bee products. Support to the sector is carried out through the National Beekeeping Program (NBP) as a part of the Common Agricultural Policy of the European Union (EU). NBP, developed with the wide participation and cooperation of beekeeping organizations in accordance with the requirements of European legislation, has a positive impact on the development of the sector. Despite the overall results, the sector continues to need the introduction of new technologies, better control of the health status of bee colonies and measures for solving the issues of personnel training and qualification, protecting bees from diseases and poisoning and promoting the development of bee products. The main objective of the paper is to evaluate the overall impact of the implementation of policy measures targeted to stimulate the beekeeping and honey production in Bulgaria and to identify the main problems faced by the sector.

Keywords: *beekeeping, apiculture sector, honey production, agricultural policy, Bulgaria.*

Introduction

Beekeeping[2] is one of the most ancient crafts known to man and one of the oldest methods of food production. There is archaeological evidence that it has been known to man since ancient times. At that period honey was collected with no attempts for saving the nest or the bee colony. Even in ancient times, honey was valued as food and a medicine (Crane, 1999). The first attempts for domesticating wild bees occurred when artificial hives were created from hollow logs, wooden boxes, pottery vessels, and baskets. But beekeeping as we know it began at the late 1700s with the introduction of the modern hive. Inventions and discoveries made in the 19th century gave a significant impetus to the development of modern beekeeping. With the

[2] Two terms are used for beekeeping - beekeeping and apiculture, and, despite the specifics of their use, for the purposes of current paper, I will use them as synonyms

invention of the first beehive with movable frames in 1814 and of the honey extractor (centrifuge) in 1865. Frame beekeeping became a highly productive branch of agriculture in many countries (Gupta et al., 2014; Crane, 1999).

In Bulgaria, even in the first years after its establishment, significant amounts of honey and beeswax were produced and there was an intensive trade with bee products. During different historical periods, some state and monastery taxes were paid in kind with honey and beeswax, as well as various fees and fines. At first, development of beekeeping in Bulgaria is related to the development of the sector in European countries and in Russia. The first major steps for its progress are linked to the delivery of the frame beehives in 1884, the establishment of the Bulgarian beekeeping organization in 1889 and the creation of the Beekeeping Testing Station with departments in Vidin and Smolyan in 1951. The diverse and abundant vegetation of the Balkan Peninsula and respectively in Bulgaria, as well as the appropriate natural, climatic and ecological conditions create excellent preconditions for beekeeping. Bulgaria occupies 0.08 % of the world's territory, but is a net exporter of honey and bee products. The good quality parameters of Bulgarian honey make it largely export-oriented (AR, 2021).

The development of the sector is also favored by the long traditions in the production of honey and bee products and the strong demand from consumers in connection with the irreplaceable health benefits of these products (AR, 2018). Despite the reported positive trend of consolidation of agricultural farms, production remains predominantly small (Koteva,2019). Beekeeping is still an alternative employment for the population in underdeveloped rural areas, providing additional income (AR, 2021). The sector continues to need the introduction of new technologies, better control of the health status of bee colonies and measures for solving the issues of personnel training and qualification, protecting bees from diseases and poisoning and promoting the development of bee products.

Support to the sector is carried out mainly through the National Beekeeping Program (NBP) as a part of the Common Agricultural Policy of the European Union (EU). NBP, developed with the wide participation and cooperation of beekeeping organizations in accordance with the requirements of European legislation, has a positive impact on the development of the sector. For the period 2006 – 2020, the total amount of funds allocated to support beekeeping in Bulgaria are 47,679,936 BGN distributed between a total of 17,888 contracts (ARs, 2006-2021). The main objective of the paper is to evaluate the overall impact of the implementation of policy measures targeted to stimulate the beekeeping and honey production in Bulgaria and to identify the main problems faced by the sector.

Materials and methods

The paper is based on a thorough review of reports, programs, strategies, policies and official documents, provided by the Bulgarian Ministry of Agriculture and the EU, and statistical data, related to the development of agriculture and respectively, beekeeping, at national level. The change in the number of bee colonies, the number of bee farms, honey production, the average yield and the average number of bee colonies per farm, as well as the amount of funds allocated and the number of contracts for beekeeping support per year for the period 2005 – 2020 are considered as a priority for the current study.

During the period 1944 – 1989, agriculture in Bulgaria and beekeeping in particular were developed in two main directions - state and amateur. Amateur agriculture offers alternative occupation and produces mainly products intended for household consumption, while state agriculture is included in the planned state economy through the Labor Cooperative Agricultural Farms and Agricultural Institutes (Shumkova and Balkanska, 2019; Koteva, 2019). In the early 1990s, the land was shattered between small owners. This led to a drastic decline in revenue and a reduction in productivity per unit area. After the Great Economic Crisis in 1997 and the completion of restitution of agricultural lands, many people either leased their lands to cooperatives, or returned to rural areas and began to process their land without having any previous experience or education in agriculture. At that time, there was no actual access to external funding so the owners were not able to develop their farms.

Results and discussion

Considering the importance of agriculture for Bulgarian economy, the traditions in the sector and the favorable natural conditions for its development, in 1999 the first National Agriculture and Rural Development Plan 2000-2006 (NARDP) was developed (Preliminary assessment of the RDP 2007-2013, MAF). In 2003, Bulgaria adopted the Law on Beekeeping (SG 57, 24.06.2003), which regulates public relations in regard to beekeeping, production of and trade with bee products and the protection of bees and honey plants in the country. Since 2008, the country has been developing and implementing 3-year National Beekeeping Programs (NBP) in accordance with EU legislation and co-funded by the EU. The main goal of the program is to improve the conditions for production and trade in honey and bee products, increase the efficiency of production, quality and competitiveness of Bulgarian honey and bee products, protection of the population of Bulgarian honey bees, sustainable development, ensuring better employment and higher incomes of beekeepers (NBP 2020-2022).

The financial support under the NBPs is provided for investments, reimbursement of part of the costs incurred or for financing of projects under 6 individual measures: (a) technical assistance for beekeepers and beekeepers' associations; (b) control of aggressors and diseases in the hive, in particular against varroasis; (c) rationalization of mobile beekeeping; (d) measures to support beekeeping analysis laboratories in order to help beekeepers sell their products and increase their value; (e) measures to support the renewal of hives in the Union;
(f) cooperation with specialized bodies for the practical implementation of applied research programs in the field of beekeeping and bee products.

The funds allocated for beekeeping for the 2006 – 2020 period (Fig. 1) were constantly increasing by 2013. During the implementation of the first NBP (2008 – 2010), most of the financial resources were directed to the second year of the program (2009), when, as can be seen from the figure below, was the first peak of funding. Although the budget of NBP 2011 – 2013 increased, during 2013 there were no funds allocated for Activities 1 and 2 of Measure A - Technical Assistance and also for 2012 and 2013 there was no funding for Measure C, Sector 2 – Support for laboratories for the purchase of laboratory equipment (NBP 2011-2013). The drastic decrease in 2014 is related to the start of the third period of the NBP and the second programming period (2014 – 2020) for Bulgaria as EU member. After 2014, the financial support and, accordingly, the number of concluded contracts remained at relatively stable levels, as the fluctuations were a result of the budget allocated in the Program for the relevant year and the number of applications submitted and approved for funding.

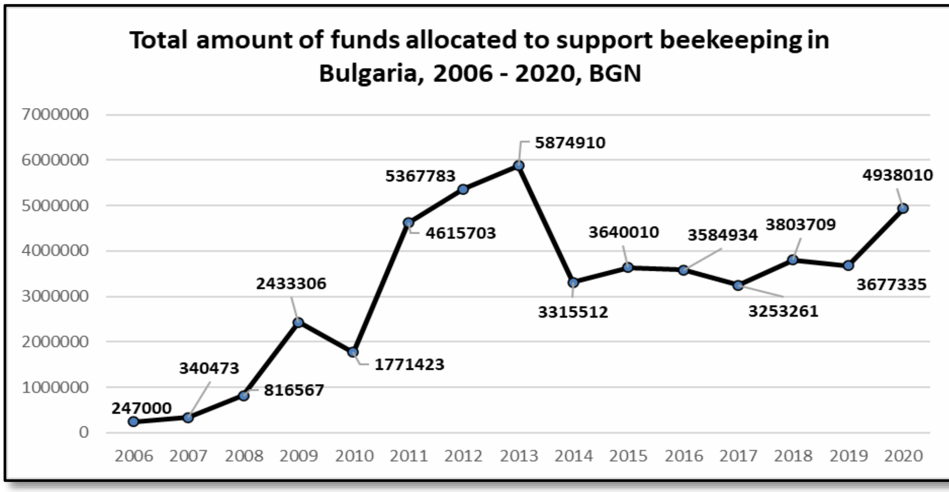

Figure 1: Funds allocated for beekeeping support in Bulgaria, 2006 – 2020, BGN
Source: Based on data from Agrarian Reports 2007-2021, MAF

For the 2005 – 2020 period, the number of bee colonies has increased by almost 30 % (Fig. 2) – from 663,367 bee colonies in 2005 up to 863,283 in 2020. The average number of bee colonies in one farm has also increased for the 2006 – 2020 period more than 4 times – from 17.9 in 2006 to 74.3 in 2020 (ARs, 2007-2021). At the same time, the number of bee farms for the 2007 – 2020 period (Fig. 3) has decreased by more than one-third. The sector is still characterized by a large number of small producers. There is a tendency to reduce the share of farms with less than 9 and with 10 – 49 bee colonies at the expense of increasing the share of farms with 50 – 149 and more than 150 bee colonies but beekeeping in Bulgaria is still extensive and fragmented. For most of the farms, beekeeping is not the main source of income, but rather an additional activity, while the number of professional beekeepers with 150 and more bee colonies remains small.

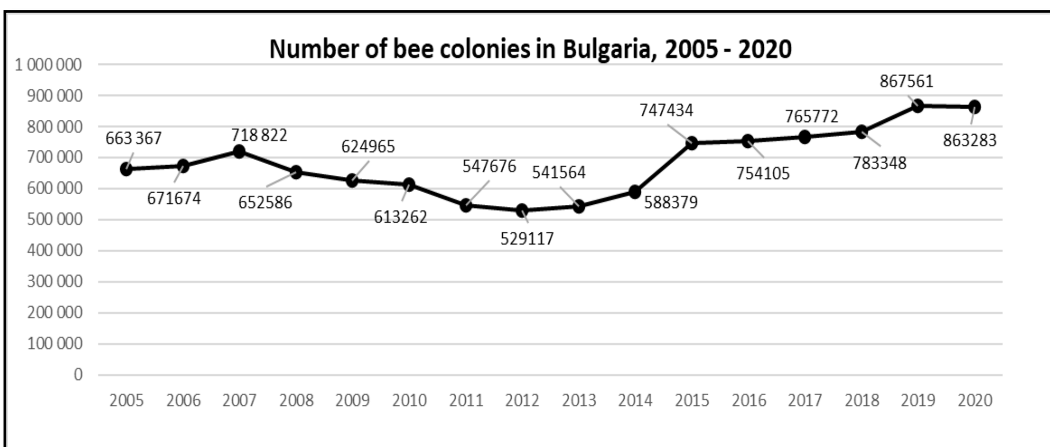

Figure 2: Bee colonies in Bulgaria, 2005 – 2020, number
Source: Based on data from Agrarian Reports 2006-2021, MAF

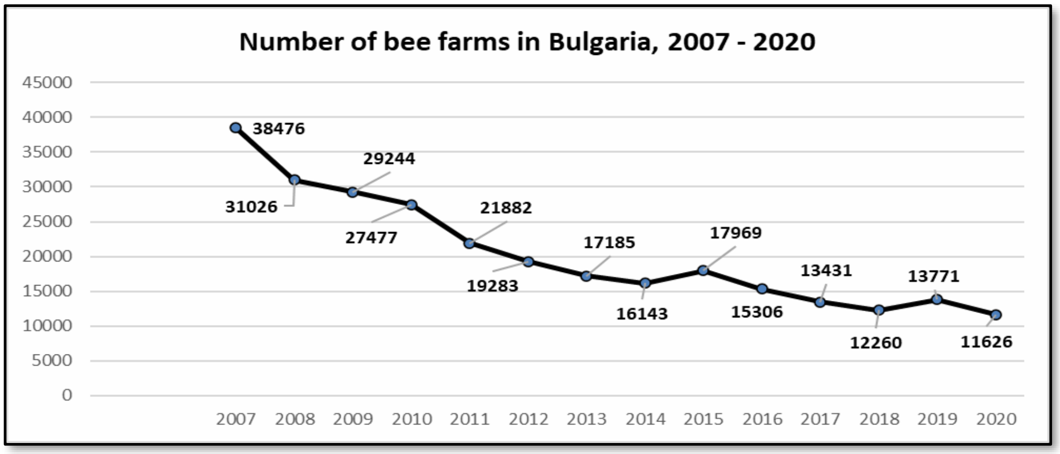

Figure 3: Bee farms in Bulgaria, 2007 – 2020, number

Source: Based on data from Agrarian Reports 2008-2021, MAF

The share of farms practicing mobile beekeeping is relatively constant, but still at very low levels – about 3 % of all beekeepers (ARs, 2007 – 2020). Among the main reasons for this is that beekeeping is not the main activity of many of the beekeepers, so they spend less time and resources on it. Another reason is the risk of poisoning the bee colonies if they are located near treated agricultural areas – poisoning, together with diseases and starvation, is still one of the main reasons for bee colonies loss.

Despite the state's efforts and measures to protect bee colonies, thousands are still destroyed every year due to disease, famine, poisoning and other causes (Fig. 4). One of the most common diseases in Europe, including Bulgaria, is varroasis, and about 99% of bee colonies are treated against it every year. Its invasion significantly affects the yields, and treatment measures against it – the quality of honey production.

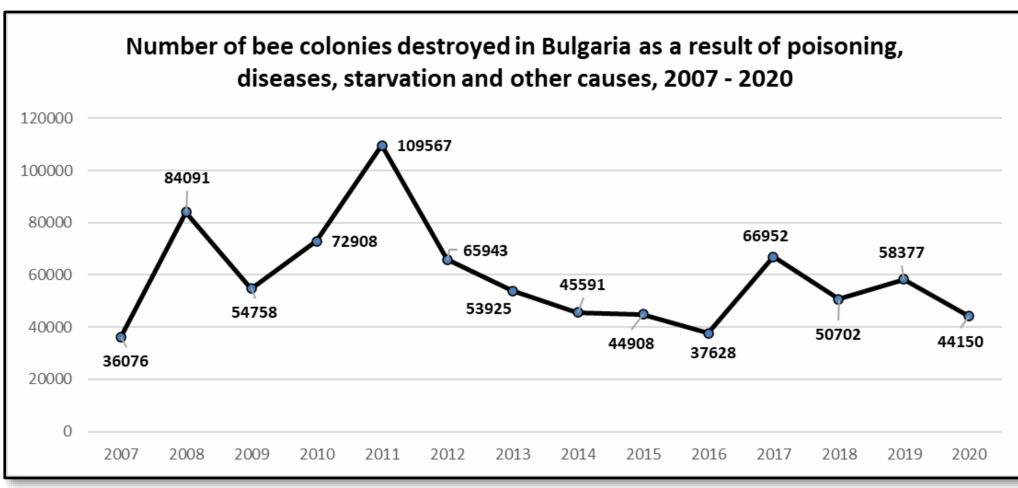

Figure 4: Bee colonies destroyed in Bulgaria as a result of poisoning, diseases, starvation and other causes, 2007 – 2020, number

Source: Based on data from Agrarian Reports 2008-2021, MAF

As beekeeping is strongly dependent on climate conditions and drought and high temperatures have a strong negative impact on the amount of bee products produced, during years with unfavorable conditions there is a lower average and total yield of honey and bee products. With the exception of these years, the yield from one bee colony is relatively stable - and remains between 17 and 20 kg per bee colony (ARs 2005 – 2020) and the total amount of honey produced – about 10 000 tons per year (Fig. 5).

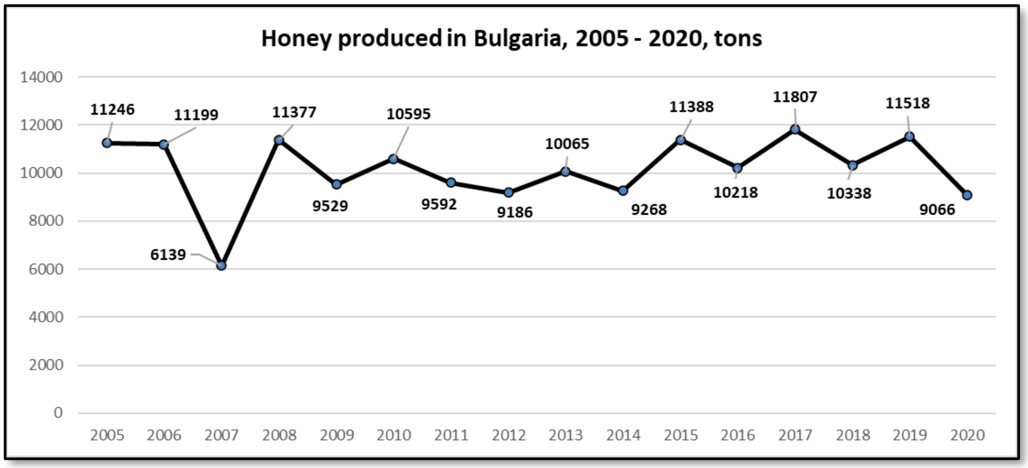

Figure 5: Honey produced in Bulgaria, 2005 – 2020, tons

Source: Based on data from Agrarian Reports 2006-2021, MAF

Conclusion

Beekeeping and honey production have long traditions in Bulgaria and could be dated back to the establishment of the country. The development of the sector is also favored by the strong demand from consumers in connection with the irreplaceable health benefits of these products. However, after the restitution of agricultural lands, a large part of agricultural farms, including beekeeping farms, remained small and fragmented.

For the 2006 – 2020 period, the financial support to the sector is almost 48 mln. BGN (or € 24 mln.). The funds are provided mainly through the National Beekeeping Program (NBP) and the support is allocated in individual measures for technical assistance, improving the health conditions of bee colonies, rationalization of mobile beekeeping, renewal of hives, supporting laboratories and research activities. Measures should also be taken for increasing labor productivity, more rational use of honey-bearing vegetation and for further stimulation of mobile beekeeping, which is set at relatively constant, but still at very low levels.

As a result of measures taken, for the 2005 – 2020 period, the number of bee colonies has increased by almost 30 %. The average number of bee colonies in one farm has also increased for the 2006 – 2020 period more than 4 times. At the same time, the number of bee colonies destroyed every year due to disease, famine, poisoning and other causes is still very high.

For the 2007 – 2020 period, the number of bee farms has decreased with more than one-third and there is a tendency to reduce the share of farms with less than 9 and with 10 – 49 bee colonies at the expense of increasing the share of farms with 50 – 149 and more than 150 bee colonies. On the other hand, for most of the farms, beekeeping is not the main source of

income, but rather an additional activity, while the number of professional beekeepers with 150 and more bee colonies remains small.
Despite the efforts of the state, beekeeping is still seen more as an alternative employment for the population in underdeveloped rural areas, providing additional income and is still extensive and fragmented. The agrarian sector as a whole and beekeeping in particular, continues to need the introduction of new technologies, better control of the health status of bee colonies and measures for solving the issues of personnel training and qualification, protecting bees from diseases and poisoning and promoting the bee products.

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ATTITUDES ON SOCIAL SOLIDARITY ECONOMY IN A REGIONAL CONTEXT: CASE OF THE REGION OF WESTERN MACEDONIA IN GREECE

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Abstract

Government policies have strengthened entrepreneurship in a variety of ways by addressing the negative factors of the last decade such as economic distress, the banking crisis and the pandemic. People, by creating new businesses or producing innovative products, are in turn coping with these circumstances. In this environment, efforts are being made to restore cooperative entrepreneurship to a new and more flexible form, appropriate to the conditions of the current era. The social economy is essential and holds an important place in the business world. Cooperative organisations create new jobs and employ people belonging to socially vulnerable groups. They also provide benefits to their members, to the local community and their achievements support areas remote from major urban centres. The Region of Western Macedonia in Greece, due to de-lignification, was marked by unemployment and economic decline, and as a result, the attitudes and opinions of citizens regarding the forms of Social Economy were explored in this paper. It is for this reason that the region was chosen as the field of research, and the survey was conducted in 2021. According to the findings of the survey, most citizens of the Region of Western Macedonia do not know what a Social Cooperative Enterprise is, unlike other forms of cooperative organizations that are more popular and well-known in the region. Whereas, the majority thinks the benefits that the Social Economy offers to its members and to the local community, such as the introduction of innovations and price stability in supplies and products, contribute dynamically to local economic development.

Keywords: *Cooperative Organizations, Women’s Cooperatives, Social Cooperative Enterprise, Entrepreneurship.*

Introduction

Governments and people are taking action to deal with the changing conditions of the last decade in ways they see as being appropriate in each case, given the current circumstances. Investing in social capital is one way of managing today's complex situation. It is an important factor leading to social cohesion, the progress of society and the strengthening of the positions of individuals and businesses working with cooperative organisations. (Oudeniotis & Tsobanoglou, 2022). The third sector of the economy, because of its enormous contribution, has been the subject of interest to many international bodies such as the International Cooperative Alliance, COPA-

COGECA[3], the European Economic and Social Committee (EESC) and CIRIEC International[4] (Adoracion & Domingo, 2022). Cooperative enterprises of all kinds are seen as effective levers that create economic and social value for their members and the whole community in which they operate (Jokiel & Jokiel, 2021).
The social economy is central to government policy-making because it offers sustainable solutions especially to contemporary social, environmental, political and economic problems that have arisen (Avagianou, et al., 2022). The legacy of these kinds of businesses is the provision of goods and services using profits to achieve social goals. Social impact is also reflected in cases of providing goods or services to vulnerable people or employing disadvantaged people (Monteiro, et al., 2022). The needs met by the social economy are absent from both the private and public sectors. They are enterprises based on democracy and autonomy as well as on the free and voluntary participation of their members (Sdrali, et al., 2015).
Some of the strategies of companies to deal with the economic crisis, which led to the reduction of wages and staff, are the management of the number of production factors, their different uses and finally the geographical area to which the products produced are addressed (Kontogeorgos, et al., 2015; Chakravarthy , 1982). In addition, the Greek social enterprises in response to the COVID-19 pandemic increased and expanded their activities and employed more members. Furthermore, changes in the organizational structure, the shift to digital entrepreneurship and the change of direction towards the local markets helped to manage unforeseen crises (Loukopoulos & Papdimitriou, 2022).
The conditions that humanity is experiencing, starting with the economic crisis and ending with the pandemic, have had negative effects on both the business world and society as a whole. In Greece, the decline in the gross domestic product (GDP) and the increase in unemployment are elements that mark both of the above cases. Moreover, the de-lignification of the Region of Western Macedonia is causing negative effects to local society and the rest of Greece (Sapardani, et al., 2021). Businesses, households and governments are trying to tolerate in this unfavourable climate. The return of co-operative entrepreneurship to a more flexible form has begun to emerge in recent years. According to data from the Ministry of Rural Development and Food in Greece, from 2018 to 2022 there is a significant increase from 608 to 1,114 cooperatives, i.e. 508 more cooperative organizations of various forms (agricultural/women's). While in the Region of Western Macedonia, the number of cooperatives increased from 2018 to 2022 with the creation of 11 new cooperative enterprises (Ministry of Rural Development and Food, 2022).
According to Law 4430/31-10-2016, "social innovation" is the creation of products or services based on collectivity and equality rather than competition. Whereas, "collective benefit" is considered to be the equitable use of production relations and the creation of new jobs (Legislation Information Bank, 2016). Social Cooperative Enterprises are divided into various categories, aiming at social integration, social care, collective and productive activities (Ministry of Labour, 2020). According to the 2019-2020 report the registrations of social enterprises per year showed a significant increase. Specifically, while in 2012 there were only 40 Social Cooperative Enterprises, in 2018 they have risen to 403 (Ministry of Labour, 2020). According to the General Register of Entities (https://kalo.gov.gr) on 31/05/2020 of the total of 1.737 entities of the General Register of Entities, 96% are included in the Social Entrepreneurship

[3] Comité des organisations professionnelles agricoles-Comité général de la coopération agricole de l’Union européenne / Committee of Professional Agricultural Organisations-General Confederation of Agricultural Cooperatives
[4] International Centre of Research and Information on the Public, Social and Cooperative Economy

Register and 4% in the Special Register of Other Entities K.AL.O. (General Sectetariat of Work, 2022). The aim of this research is to explore the attitudes and opinions of citizens regarding the various forms of the Social Economy and its overall importance.

Material and Methods

The region of Western Macedonia was chosen as the field of research and the survey was conducted in 2021. An appropriately structured questionnaire was prepared for the research needs. The questionnaires were completed with face-to-face interviews by researchers from a total of 137 citizens. The Statistical Package for Social Sciences (SPSS) software program was used for statistical processing of the survey data. Statistical tests were performed for the control of the hypotheses, namely, the statistic of X^2, the control of Kruskal - Wallis Test, the control of two Mann - Whitney Test and finally the non-parametric correlation coefficient of Spearman.
137 citizens participated in the study, of which 50.37% are men and 49.63% are women. The educational level of the sample is 64.2% higher education graduates followed by secondary and primary school graduates at 27% and 8.8% respectively. Also, the highest percentage of the sample 38.8%, has a family monthly income in the range of 1,201 to 3,000 euros, while 7.5% of respondents has a monthly income up to 600 euros. A high family income of 2,001 euros and above is observed in 17.2% of the sample. In terms of occupational status, 38.7% are public employees, 31.4% private employees and 14.6% freelancers. Regarding the age range, the largest percentage of the sample is observed in the range from 31 to 50 years old, 33.1% in the age range 51 years and above and finally 7.5% of the sample is up to 30 years old.

Results and Discussion

According to the x^2 statistical test, 2/3 of the sample of respondents, while knowing the general meaning of the term cooperative has not cooperated with any form of cooperative organization (x^2=30.626^{a}, df=4, Sig.=0.000). Also, 44.7% of the respondents know what a cooperative is and believe that the cooperative products/services have a stable quality (x^2=12.290^{a}, df=4, Sig.=0.015). The majority of respondents who recognize the cooperative form as a type of business agree with the view that in rural cooperatives there are problems of mismanagement (x^2=10.713^{a}, df=4, Sig.=0.030) while 76% expect that cooperatives provide investments and create new jobs that contribute to the development of the local community (x^2=11.047^{a}, df=4, Sig.=0.026). In addition, a large percentage of respondents, whether they know an agricultural/women or other type of cooperative or not, particularly prefer the local products produced in Western Macedonia at a rate of 70.8% due to pride related to origin (x^2=17.560^{a}, df=4, Sig.=0.002) and 76.7% due to support they want to offer to the producers of these products/services (x^2=20.934^{a}, df=4, Sig.=0.000). Also, more than half prefer cooperative products or services due to their quality (x^2=15.224^{a}, df=4, Sig.=0.004).

Table 1. Statistical test x^2

	Knowledge of agricultural or women's cooperative or other type of cooperative		
Family monthly income	Yes	No	Total
Up to 600 euros	7.5%		7.5%
From 601 up to 1,200 euros	34.3%	2.2%	36.5%
From 1201 up to 2,000 euros	29.9%	9%	38.9%
From 2,001 up to 3,000 euros	8.2%		8.2%
From 3,001 and above	6.7%	2.2%	8.9%
Total	86.6%	13.4%	100%
$x^2=11.050^a$, df=4, Sig.=0.026			

The variables total monthly family income and knowledge of agricultural/women's or other type of cooperative (Table 1) do not follow a normal distribution so we proceeded with the non-parametric tests (Kruskal-Wallis Test) (Table 2). In this example according to the Kruskal-Wallis Test, p=0.037 i.e. p<0.05 we reject H0 and accept H1. That is, there is no homogeneity in terms of knowledge of rural/women's or other types of cooperative among the 5 categories of total monthly family income. To see which family income categories are differentiated from which we need to test in groups of two (Mann-Whitney test) (Table 2). According to the Mann-Whitney test, p=0.017 i.e. p<0.05 we reject H0 and accept H1. That is, there is no homogeneity between the second and third category of total monthly family income. Thus, there is a statistically significant difference of knowledge of agricultural/women's or other type of cooperative between the second (from 601 to 1,200 euros) and third (from 1,201 to 2,000 euros) categories of total monthly family income. From the above statistical tests, respondents belonging to small family monthly income scale categories of up to about 1200 euros believe in this type of business form and in the power of grouping that it provides to its members. People through the cooperative organization achieves more than if they acted alone. That is, through the cooperative they can achieve both economies of scale and high bargaining power in the market.

Table 2. Kruskal-Wallis Test and Mann-Whitney Test

	Knowledge of agricultural or women's cooperative or other type of cooperative (Kruskal-Wallis Test)	Knowledge of agricultural or women's cooperative or other type of cooperative (Mann-Whitney Test)
Family monthly income	Sig 0,037 Reject the null hypothesis	
From 601 up to 1,200 euros		Sig 0,017
From 1,201 up to 2,000 euros		Reject the null hypothesis

Spearman's correlation coefficient is a non-parametric correlation coefficient, denoted by Ps and takes values from -1 to 1. In the above variables, purchase of cooperative products and consumption of cooperative products and services, the Spearman's correlation coefficient is Ps=0.805** with p=0.000. This means that the correlation is highly positive and statistically significant at the 0.01 level of significance. That is, when the purchase of cooperative products increases, the consumption of cooperative products and services is expected to increase because the respondents are satisfied with these products or services.

Also, the Spearman's correlation coefficient between the variables regarding the existence of problems such as mismanagement in cooperatives and the difficulties faced by new and flexible cooperatives in the beginning, is Ps=0.808** with p=0.000. This means that the correlation is highly positive and statistically significant at the 0.01 level of significance. That is, if the problems of mismanagement of cooperatives are reduced, the difficulties of new and flexible cooperatives in starting their operations are also reduced.

Table 3. Spearman’s Correlation Coefficient

	Consumption of cooperative products and services	New and flexible cooperatives may face difficulties at the beginning of their activities
Purchase of cooperative products	0,805** Sig 0,000	
In cooperatives there may be poor management issues in many cases		0,808** Sig 0,000

Table 4 analyses the relationship between the variable "knowledge about Social Cooperative Enterprise" and the variable "age". Two new variables related to age were created for the specific needs of the survey. That is, the age variable was categorized into two scales age below 40 years old and age above 40 years old. Specifically, more than half of the sample respondents do not know what a Social Cooperative Enterprise is and 21.8% are under 40 years old.

Table 4. Knowledge of Social Cooperative Enterprise.

Age	Yes	No	Total
Under 40 years old	9.8%	21.8%	31.6%
Over 40 years old	34.6%	33.8%	68.4%
Total	44.4%	55.6%	100%
x^2=4.471^a, df=4, Sig.=0.034			

Conclusions

In recent times, with the various negative changes in the economy and public health issues with the COVID-19 pandemic, the importance of earning a living and the sustainability of any business has come to the forefront. Neither Greece nor the Region of Western Macedonia is exempt from these conditions. The ever-changing environment and the period of delignification accelerated the process of entering these different circumstances. States stepped up their efforts to respond and manage the crises with policies to support businesses and households. There was also a need to create a new form of enterprise and to reintroduce cooperatives in a new and more flexible form adapted to the demands of the times.

According to this study, a large percentage of respondents have knowledge about cooperatives in a general sense and especially about agricultural and women's cooperatives. While, less popular is the form of Social Cooperative Enterprise, especially among the under 40 age group. Most of the respondents tend to agree with the existence of problems in agricultural cooperatives due to mismanagement. In addition, if the problems of mismanagement in cooperative enterprises are reduced, the difficulties of new and flexible cooperatives in starting up are also reduced. It is also worth mentioning that respondents belonging to categories with low family monthly income invest and support the cooperative business form because they believe in the power of association it provides to its members.

Cooperative organisations generally create new jobs for specific groups, such as the unemployed or people with special skills, thus contributing to the improvement of the economy and the local community. Finally, the human capital-based form of this type of enterprise offers economic, social and cultural benefits to its members and to society in general.

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VEGETABLE GARDENS EFFECTS ON HOUSHOLDS’ SUSTAINABILITY IN THE GERT SIBANDE DISTRICT, SOUTH AFRICA: A MESMIS MODEL APPROACH

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Abstract

The Gert Sibande district (Mpumalanga Province) is impaired with a high poverty rate of 46.5%. As a result, the Agricultural Research Council in partnership with the Department of Agriculture, Land Reform and Rural Development signed a Service Level Agreement to improve communities in nine provinces of South Africa through the development of a vegetable garden strategy- The Agricultural Paraprofessional Development Programme (APPDP). The objective of the APPDP was to create and support sustainable rural development through improved food production, food security and household incomes. Even so, beneficiaries of the project recorded the following challenges: lack of infrastructure, loss of interest in gardening and water scarcity. The study aimed to analyze the effect of vegetable gardens on households’ sustainability in Gert Sibande. Semi-structured questionnaires were administered to 54 purposively selected vegetable gardening households. The descriptive statistics, Management Systems Assessment Framework Incorporating Sustainability Indicators Model (MESMIS) and Pearson correlation coefficient were applied to meet the study’s objectives. Data were analyzed using the Statistical Package of Social Sciences Version 25. The study revealed that 63% of the households generated income through vegetable gardening. Output sold was negatively correlated with number of household members, at 0.270 correlation coefficient. The higher the household members, the less output sold and the less food available to support social initiatives. In addition, households were not aware of environmental issues such as water recycling, maintenance of local biodiversity and soil management. Overall, the study concluded that the vegetable initiative has great potential to enhance the livelihoods of rural communities.

Keywords: *Vegetable gardens, Households, Sustainability, Gert Sibande District, Mpumalanga Province.*

Introduction

The combined area of home food gardens in rural areas of South Africa amounts to almost 200,000 ha, and the crops grown include maize (*Zea mays*), sorghum (*Sorghum bicolor*), sweet potatoes (*Ipomoea batatas*), cabbage (*Brassica oleracea var. Capitata*), squashes (*Cucurbita sp*.) and spinach (*Spinacia oleracea*) (Carstens et al., 2021). *Figure 1* demonstrates that own consumption and direct sales make up 42% of the produce distribution in South Africa. This shows the willingness of community members to consume and buy home-grown vegetables from households.

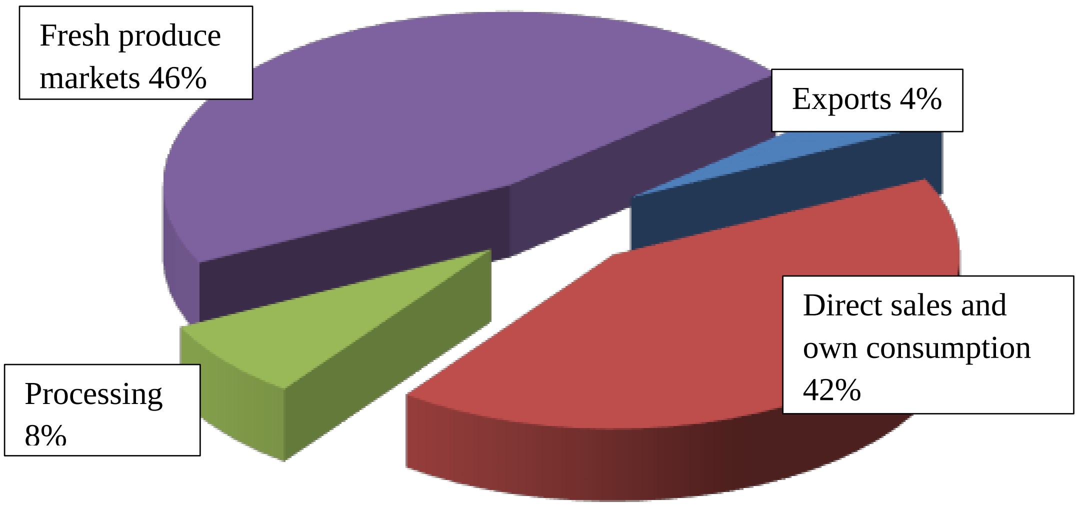

Figure 1: Vegetables produced locally in South Africa

Source: Department of Agriculture, Land Reform and Rural Development, 2021

According to Statistics South Africa (2001), any household that earns income from either formal employment in the agricultural sector, or as a skilled agricultural worker, or from sales or consumption of home produce and livestock, is defined as an agricultural household (Pauw et al., 2005). Approximately 80% of the agricultural households in Mpumalanga province derive more than 50% of their household income from employment in the agricultural sector and about 5800 households depend solely on subsistence farming for main source of food (Scheepers et al., 2009).

The Gert Sibande is the largest district covering almost half of the Mpumalanga Province's geographic area, and has the largest agricultural sector (CGTA, 2020). The district is dominated by Black Africans who constitute 92% of the total population, and the remaining 8% is comprised of Whites, Coloureds, and Indians. Children aged between 0-19 years of age constitute 39% of the total population, and those aged between 20-59 years of age constitute a further 53% of the population (CGTA, 2020). The poverty rate in Gert Sibande district stands at 46.5%, making it the second highest of the three districts in the province, together with a youth unemployment rate of 38.4% (CGTA, 2020).

The Agricultural Research Council (ARC) and Department of Agriculture, Land Reform and Rural Development (DALRRD) signed a Service Level Agreement (SLA) to improve communities in all nine provinces of South Africa through the development of a vegetable production garden strategy- The Agricultural Para-Professional Development Programme (APPDP). The objective of the APPDP is to create and support sustainable rural development through improved food production, food security and household incomes, as well as the provision of production inputs, infrastructure, mentorship, and technical support (DALRRD, 2015).

Furthermore, according to the World Health Organization (WHO) (Jowell, 2011), each person should consume at least five portions of fruit and vegetables daily (400g collectively) to maintain a healthy diet with enough micro-nutrients. The World Health Organization also emphasizes that households engaged in vegetable production in their homestead gardens appeared to achieve

greater food security and nutritional status compared to non-farming households of the same socio-economic status. Home food gardens have the potential to alleviate household expenditure on food and generate a substantial income through the sale of vegetables. This income “saving” enables families to purchase more energy-dense foods and improve their nutrition (Jowell, 2011). A study by Oldewage-Theron et al. (2008), revealed that a vegetable garden project can on average generate an income of about R300 per month. Home food gardens are also considered the most important form of food production for majority of people in rural communities, because of their low input requirement and investment for a nutritional return (Carstens et al., 2021). Output is achieved on small plots of land, using less fertilizer, pesticides and irrigation (DALRRD, 2015).
However, the following challenges existed among beneficiaries of the vegetable project: lack of infrastructure for protection from animals, loss of interest in gardening and water scarcity. Drought is a major factor in agriculture that hampers production and reduces efforts made by government agencies in improving the livelihoods of rural farm households. The research study aims to analyze the effect of vegetable gardens on households’ sustainability in the Gert Sibande district of Mpumalanga province. The following are the objectives of the study:

i. Identify and compare socio-economic characteristics of vegetable gardens households.
ii. Assess the economic, social, and environmental sustainability of vegetable gardens households.
iii. Determine the correlation between socio-economic and sustainability variables.

Materials and Methods

Study area

Gert Sibande District Municipality is one of the three district municipalities that form part of the Mpumalanga Province. It is bordered by the Ehlanzeni district to the north-east and Nkangala district to the north, the KwaZulu-Natal and Free State Provinces to the south, Swaziland to the east and lastly Gauteng Province to the west (CGTA, 2020). The district is also the largest of the three districts, covering 40% of the Mpumalanga Province’s land mass (IDP, 2020/2021). It consists of the following seven municipalities: Chief Albert Luthuli, Dipaleseng, Dr Pixley Isaka Ka Seme, Govan Mbeki, Lekwa, Mkhondo, and Msukaligwa. Data collection occurred in the Gert Sibande district.

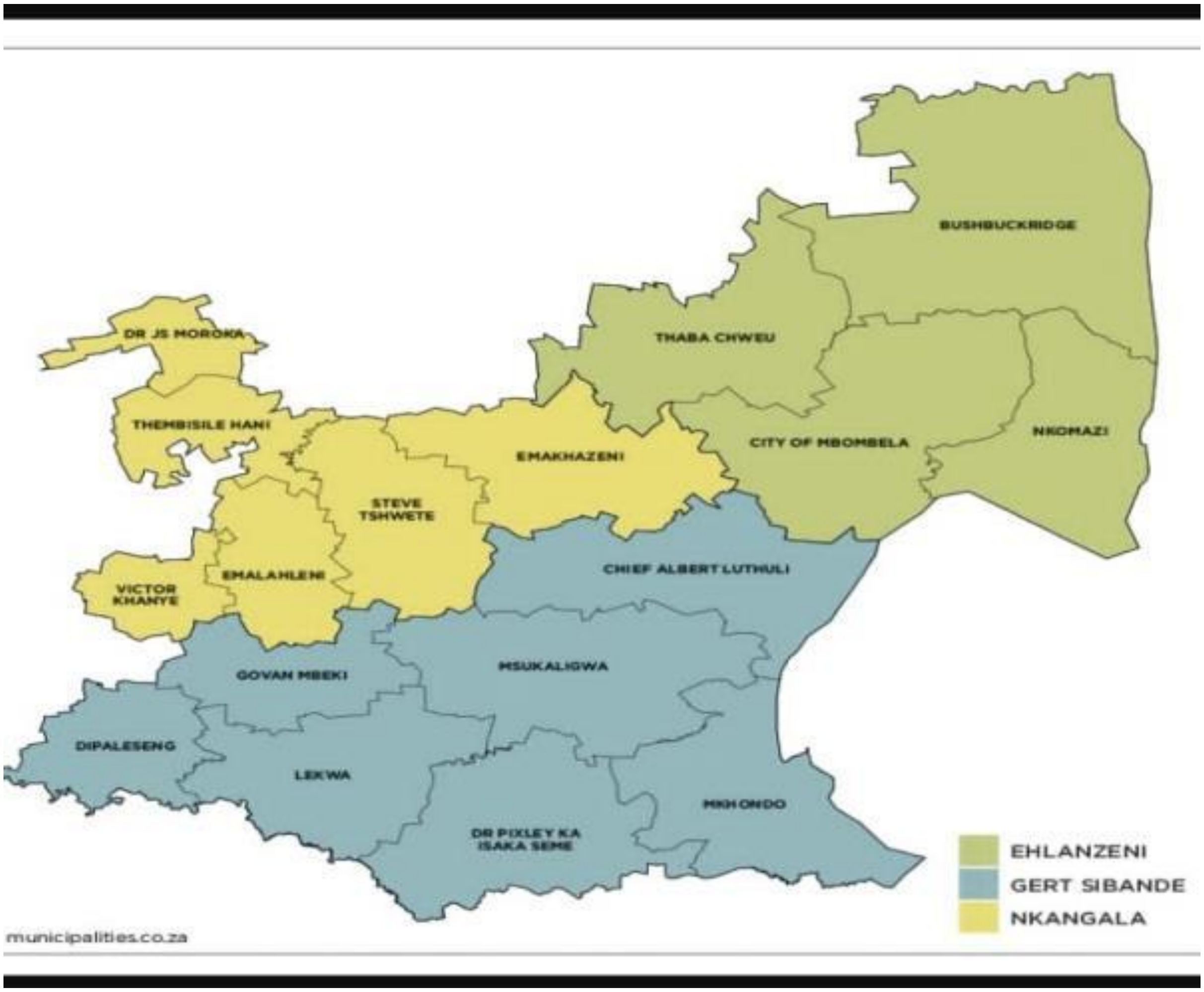

Figure 2: A Map showing the 7 local municipalities in Gert Sibande District

Source: https://municipalities.co.za/provinces/view/6/mpumalanga

Study design

Semi-structured questionnaires were administered to a total of 54 vegetable garden households. A semi-structured questionnaire gives the research a meaningful result as it allows respondents to provide their personal opinions on a set of questions provided. A rule of thumb was applied to determine the sample size, that is, 10% of the population. The research used both quantitative and qualitative methodological tools to retain more detail and understanding when interpreting data. The questionnaires were developed and collected by researchers at the Agricultural Research Council (ARC) working to develop communities. The primary data were collected through interviews, site observations, focus groups, and government reports. Prior to the survey, the questionnaires were pre-tested. The pilot tests were made by distributing the questionnaires to three household heads in each district, to determine whether the instruments applied were appropriate and suit the study.

A purposive sampling technique was used to select the beneficiaries of the APPDP vegetable initiative. Purposive sampling is a form of non-probability sampling technique in which researchers rely on their own judgement when choosing which members of the population should participate in the surveys. The main aim of the Agricultural Para-Professional Development Programme is to support community members, especially youth. Therefore, the household selected for this study were predominantly youth.

Analytical techniques

Descriptive statistics

Descriptive statistics were applied to identify and compare the socio-economic characteristics of vegetable gardening households in Gert Sibande district. The technique provides an easy way of summarising large quantitative data into a clear and understandable manner (Knupfer and McLellan, 1996) through tables, graphs and measures of central tendency. The data were quantitatively analyzed using the Statistical Package for Social Sciences (SPSS) Version 25.

MESMIS Model for assessing the sustainability of vegetable garden households

Sustainability in the context of this study refers to the ability of food production initiatives to meet the needs of the poor on a long-term basis and ensuring that food initiatives do not compromise the ability of future generations to meet their own (Madlala, 2012). It requires that one not only look at income generation, food production, and land distribution but also consider environmental issues such as climate change, seasonality, and water scarcity (Speth, 1994). Thus, sustainability evolves around the three pillars: Economic, Social and Environmental.

The Management Systems Assessment Framework Incorporating Sustainability Indicators (MESMIS) Model was used to assess the economic, social, and environmental sustainability of vegetable gardening households. The model characterises the system, it identifies critical points and selects specific indicators for the economic, social and environmental dimensions of sustainability (Cruz et al., 2018). Some of the sustainability indicators selected for this research include household income generated from the vegetable garden, selling of produce in the local community, expansion of the production scale, increase in income, vegetable garden supporting social initiatives, and household environmental awareness etc. The indicators were then integrated through mixed techniques (qualitative and quantitative) and the analysis of the multi-criteria.

Pearson correlation

Correlation measures the relationship between two variables. The relationship between the two variables is one which either (a) as the value of one variable increases, so does the value of the other variable; or (b) as the value of one variable increases, the other variable value decreases (Schober et al., 2018), wherein the former is referred to as a positive correlation, and the latter a negative correlation. Therefore, the change in the size or magnitude of a variable is associated with a change in the size of another variable, either in the same or opposite direction.

There are four measures of correlation, namely, the Pearson, Kendall rank, Spearman, and Point-Biserial correlation.

The Pearson correlation was applied for this research to determine the relationship between socio-economic and sustainability variables. The Pearson correlation coefficient ranges from -1 to +1; a value around ±1 indicates a perfect degree of correlation, and a value closer to 0 indicates a weak correlation (Schober et al., 2018), where 0 indicates no monotonic association between variables.

Results and Discussion

The results from Table 1 indicated the socio-economic characteristics of households in the Gert Sibande district. The vegetable government strategy is aimed at supporting community members, especially youth producing vegetables by providing them with production inputs, infrastructure, and training. As a result, there were more young households less than 35 years of age producing vegetables, constituting 70% compared to those aged between 36 and 45 years, constituting only 30%. The young household population was dominated by females than males, with percentages of 87 and 13, respectively. Furthermore, the highest level of education attained by households in the district was Grade 12 at 39%, followed by Grade 11 at 33%, the rest of the respondents were scattered across different lower educational levels and post-matric qualifications. The family size ranged from 1 to 5 household members (56%), 6 to 10 (27%), and >10 (17%). The vegetables were mostly produced in homestead gardens, as shown in Table 1.

Table 1. Distribution of selected households according to their socio-economic characteristics of the Gert Sibande District, Mpumalanga, South Africa

Variables	**Households**	**Households (%)**
Gender		
Female	47	87
Male	7	13
Total	54	100
Age		
Less than 35 years	38	70
36 - 45 years	16	30
46 - 55 years	0	0
> 56 years	0	0
Total	54	100
Highest education of the respondent		
Grade 8	0	0
Grade 9	3	6
Grade 10	7	13
Grade 11	18	33
Grade 12	21	39
Technician/Diploma	2	4
University degree	3	5
Other post-matric qualifications	0	0
Total	54	100
Number of household members		
1 to 5	30	56
6 to 10	15	27
>10	9	17
Total	54	100
Land acquisition		
Household garden	40	74
Inheritance	5	9
Permission to occupy/communal land	9	17
LRAD/government lease	0	0
Other	0	0
Total	54	100

Household income		
<R500	14	26
R500-R1000	17	32
R1001-R2500	19	35
>R2501	4	7
Total	54	100
Type of income		
Social grants	38	71
Employment	11	20
Other	5	9
Total	54	100
Household head		
Child	4	7
Female	33	61
Male	17	32
Total	54	100

Source: ARC Data
LRAD (Land Redistribution for Agricultural Development)

About 74% of the Gert Sibande households had their own homestead gardens, and only few have inherited or occupied communal land. Moreover, there were no households who received land through the Land Redistribution for Agricultural Development (LRAD), which is designed to provide grants to black South African citizens to access land specifically for agricultural purposes to improve nutrition and incomes of the rural poor who are interested in farming on any scale. The source of income dominant among households in the district was social grants, with 32% and 35% of the households having an income ranging from R500-R1000, and R1001-R2500, respectively. In addition, households spent more of their income on electricity, food and transport as depicted in *Figure 3*. About 35% of households spent more than R601 on food, and 59% spent less than R200 on electricity. Moreover; 61% of females headed their households, with only 32% and 7% headed by males and children, respectively.

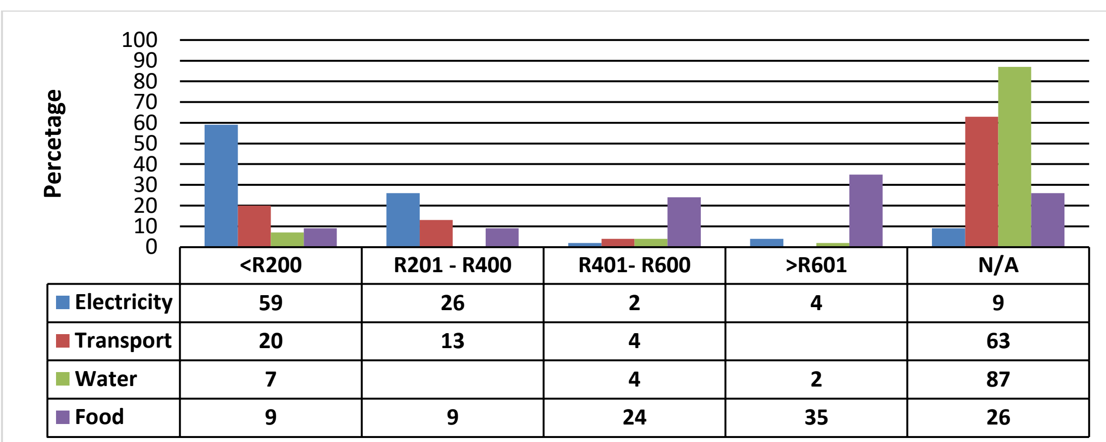

Figure 3. A Bar graph indicating household expenditure per month in the Gert Sibande District, Mpumalanga, South Africa.

Source: ARC data

Economic sustainability

The results from Table 2 show that the vegetable garden government strategy had a positive economic effect on households in the Gert Sibande district. Almost 90% of the households agreed that the vegetable garden assisted in feeding their families and helped reduce their expenditure on food. Additionally, 63% recorded that the initiative also generated income through the direct sale of vegetables. However, it was difficult to determine whether income was consistently going up as 70% of the households stated that they did not keep record of financial statements/accounts of the homestead food garden. Nonetheless, 93% intended on expanding the scale of production.

Table 2. Economic sustainability indicators in the Gert Sibande District, Mpumalanga, South Africa

Variables	**Households**	**Households (%)**
Do you have a vegetable garden?		
Yes	51	94
No	3	6
Total	54	100
Does the vegetable garden assist to feed household?		
Yes	48	89
No	6	11
Total	54	100
Does the vegetable garden generate income?		
Yes	34	63
No	20	37
Total	54	100
Do you record income generated from the garden?		
Yes	12	23
No	38	70
Sometimes	4	7
Total	54	100
Is income consistently going up?		
Yes	13	24
No	18	33
Not applicable	23	43
Total	54	100
Do you intend to expand the production scale?		
Yes	50	93
No	2	3
Not Sure	2	4
Total	54	100

Source: ARC Data

Social sustainability

As indicated in Table 3, 50% of the vegetable garden households agreed that through the intervention, households were able to support other initiatives through the provision of home-grown fresh vegetables. Thus, improving the food security status of community members in the district. However, there was little support received from members of the community regarding the production of vegetables. Only 33% received voluntary support from local members. Furthermore, 50% of the households sold their produce to generate income, the remaining produce was for home consumption.

Table 3: Social sustainability indicators in the Gert Sibande District, Mpumalanga, South Africa

Variables	**Households**	**Households (%)**
Is the vegetable garden supporting other social initiatives?		
Yes	27	50
No	27	50
Total	54	100
Do you receive voluntary support from community members?		
Yes	18	33
No	26	48
Sometimes	10	19
Total	54	100
Are you selling some of the produce within the local community?		
Yes	27	50
No	27	50
Total	54	100

Source: ARC Data

Environmental Sustainability

Majority of the households in the district were not aware of environmental issues such as climate change, pollution, environmental and resource depletion. Households should be trained on how to conserve the environment through, for example, water recycling, use of waste nutrients to prepare the ground for vegetable planting, controlling shade, and maintaining local biodiversity.

Correlation between socio-economic sustainability variables

Land acquisition and availability of a vegetable garden

The study revealed a positive correlation between land acquisition and availability of a vegetable garden with a Pearson correlation coefficient of 0.290 at 5% significance level. This implied that the correlation between the two variables was weak because the coefficient value was closer to 0. The two variables moved in the same direction and magnitude, that is, *the more vegetables planted, the more land acquired, and the less vegetables planted, the less land acquired*. When the coefficient of correlation is squared it becomes the coefficient of determination (Schober, 2018). This implies that a coefficient of correlation of 0.290 will result in a *coefficient of determination* of 0.08 or 8%. This tells us that 8% of the change in land acquired was associated with the change in vegetables planted. About 90% of the variability was yet unexplained, thus;

there are other relevant factors that affected the acquisition of land such as socio-economic characteristics, cultural, and political systems. If land can be made available through government policies, more households will be engaged in vegetable production.

Household head and land acquisition

Household head was also positively associated with land acquisition with a correlation coefficient of 0.316 at 5% significance level. As established in the descriptive statistics, majority of the female headed households owned home gardens as a form of land acquisition for vegetable production. The ability for females to acquire communal land is still an on-going problem. According to Teagle (2021), women own just 13% of private farmland in South Africa against a world average of 20%.

Table 4: Pearson correlation in the Gert Sibande District, Mpumalanga, South Africa

	AVG[1]	**VGGI[2]**	**GEN[3]**	**AGE[4]**	**HER[5]**	**NHM[6]**	**LA[7]**	**HEF[8]**	**HH[9]**	**SOLC[10]**
AVG[1]	1	-.186	.147	.020	-.019	.018	**.290***	.030	.039	.081
VGGI[2]	-.186	1	-.068	.258	-.111	-.113	.024	-.140	.012	.230
GEN[3]	.147	.068	1	.250	-.045	-.093	.146	.063	.126	.055
AGE[4]	.020	.258	-.250	1	-.094	.066	-.097	**-.281***	-.131	.162
HER[5]	-.019	-.111	-.045	-.094	1	.039	-.076	.180	-.041	-.168
NHM[6]	.018	-.113	-.093	.066	.039	1	-.195	-.145	-.083	**-.270***
LA[7]	.290	.024	.146	-.097	-.076	-.195	1	**.368****	**.316***	-.171
HEF[8]	.030	-.140	.063	-.281	.180	-.145	.368	1	.088	.093
HH[9]	.039	.012	.126	-.131	-.041	-.083	.316	.088	1	**-.354****
SOLC[10]	-.081	.230	-.055	.162	-.168	-.270	-.171	.093	-.354	1

Source: ARC Data
Correlation is significant at 0.05 level* (2-tailed)
Correlation is significant at 0.01 level** (2-tailed)

Availability of vegetable garden, Land acquisition, Age, Household expenditure on food, Number of household members, Household head, Selling output in local community

Table 5: Description of socio-economic sustainability variables

NO.	**Variable**	**Description**
1	AVG	Availability of a Vegetable Garden
2	VGGI	Vegetable Garden Generate Income
3	GEN	Gender
4	AGE	Age
5	HER	Highest Education of the Respondent
6	NHM	Number of Household Members
7	LA	Land Acquisition
8	HEF	Household Expenditure on Food
9	HH	Household Head
10	SOLC	Selling Output in Local Community

Source: ARC Data

Household expenditure on food and land acquisition

As indicated in Table 4 an increase in household expenditure on food also motivates households to acquire more land/vegetable gardens to assist in feeding their families and generate extra income through the sale of these vegetables. The two variables were positively correlated at 1% significance level.

Selling produce in local community and household head

In contrast, there was an inverse relationship between output sold within the local community with household head, with a negative Pearson correlation coefficient of -0.354 at 1% significance level. According to Ndobo et al. (2013), female headed households are more likely to face moderate-to-mild as well as severe food insecurity forms than those headed by males.

Age and household expenditure on food

Age was negatively associated with household expenditure on food with a Pearson correlation coefficient of -0.281 at 5% significance level. This implied that middle aged household members (ages 36 to 45) spent less on food compared to youth (less than 35 years of age). Thus, vegetable gardens were more likely to have a positive economic and social effect on older household groups than youth. Garcia and Grande (2010) studied the determinants of food expenditure patterns among older consumers: The Spanish case which revealed that spending declines with increasing age. On one hand, calorie requirements decrease with age and older consumers purchase smaller amounts of food. Additionally, many older people depend only on old-age grants which are lower than those of whom have retired from paid employment, this in turn reduces food consumption due to lower overall income.

Selling output in local community and number of household members

There was also a negative correlation between output sold with number of household members, with a Pearson correlation coefficient of -0.270 at 5% significance level. A study by Maijama (2019) found that high unemployment is caused by population growth. Therefore, with self-employment through the vegetable garden intervention, household members can focus more on improving their well-being and quality of life. Moreover, when population increases there will not be enough surplus vegetables to be sold to community members, production will be mainly for consumption purposes. This ultimately makes it difficult to achieve an economic and socially sustainable community.

Conclusion

It can be concluded that the vegetable garden intervention has great potential to improve and maintain the economic and social well-being of households in the Gert Sibande district, Mpumalanga, South Africa. The study established that vegetable gardens were a source of income for many rural households in the district. The household gardens did not only assist to feed their families, but also support social initiatives such as orphanages in local communities. Households were also motivated to expand the scale of vegetable production.

It was difficult to determine whether income was consistently going up due to the absence of financial records. The APPDP intervention should provide training to households, especially those with secondary and post education on how to record financial activities and the role of each financial statement (Balance sheet, Income and Cash-flow statements) in business. Financial planning should also be prioritised in the training programme, this will help households to be in control of their income, expenses, and investment such that they can achieve their business goals. The study also recommends that community engagement be encouraged to address issues such as poverty and malnutrition. Communities should work together to achieve sustainable goals. In addition, it is strongly recommended that nutrition education be an integral part of the programme to ensure communities have a good understanding of the principles of a balanced diet and its effect on productive families.

The community development programme and other relevant stakeholders should have a clear monitoring and evaluation system to measure the overall effect of the garden initiative on households’ sustainability in the long run. Through monitoring and evaluation, the programme can be evaluated as being judged as a “success” or “failure” and identify lessons learnt for future improvement.

The study also revealed that the programme was inconsistent in the provision of production inputs. This could implicate the programme’s efforts in improving the livelihoods of households. Mentors should keep record of all households who received inputs monthly to avoid duplications or leaving out other households. Infrastructure such as shade nets to protect the plants during hot conditions should be prioritized to prevent production loss. There was a negative correlation between the number of household members and the sale of output. The higher the number of household members, the less output sold and the less food available to support social initiatives. Output produced is mainly for consumption and not for sale, therefore reducing the efforts made in achieving sustainability goals. It is recommended that households be made aware of the importance of family planning to anticipate and attain their desired number of children and the intervals between their births.

Land acquired was positively associated with the availability of vegetable gardens. If government could make more land available for gardeners, the vegetable garden initiative will have an even greater effect on households’ sustainability. Production will be increased, more output sold leading to higher income levels.

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SOCIO-ECONOMIC AND FOOD SECURITY STATUS: A CASE STUDY OF THE SELECTED HOUSEHOLDS IN THE GERT SIBANDE DISTRICT, SOUTH AFRICA

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Abstract

A report by the Food and Agriculture Organization (FAO) emphasizes that the world is not on track to eradicate hunger as envisioned in the 2030 Sustainable Development Goals (SDG’s). The share of the population below the poverty line in Gert Sibande District has also worsened (46.5%), making it the second highest of three districts in the Mpumalanga Province. This study evaluates the socio-economic and food security status of the selected households in Gert Sibande District. The study used a purposive sampling procedure to select 54 sample households, which were interviewed using semi-structured questionnaires. The descriptive statistics were used to evaluate the socio-economic and food security status of households using qualitative and quantitative methods. The data were analyzed using the Statistical Package of Social Sciences Version 25. The results found that 13% and 87% of the households were food secure and food insecure, respectively. Moreover, of the households who were food insecure (FI); 34% were mildly FI, 51% moderately FI and 15% severely FI. Most of the food insecure households were younger females less than 35 years of age with a household composition ranging from 1 to 5. It was also established that dark green leafy vegetables and ‘other’ vegetables were the most accessible and consumed food groups. The study concluded that household participation in the vegetable project can potentially improve their farming knowledge, increase production and income, and ultimately flatten the food insecurity status. The paper proposed that government agencies should continue investing in agricultural infrastructure, farm knowledge and education.

Keywords: *Socio-economic status, Food security status, Households, Gert Sibande District, Mpumalanga Province.*

Introduction

Issues of household food and nutrition security have received worldwide attention due to the climate change impact and dire economic conditions which put new and additional stress on food systems (STATSSA, 2019). A report by the Food and Agriculture Organization (FAO) (in collaboration with FAD et al., 2018) emphasizes that the world is not on track to eradicate hunger as envisioned in the 2030 Sustainable Development Goals (SDGs) and National Development Plan (NDP) of South Africa. According to the Integrated Food Security Phase Classification (IPC) (2021), about 16% of the population in South Africa face high levels of severe food insecurity and require immediate action to reduce food gaps and improve livelihoods. In addition, out of the nine provinces of South Africa, eight provinces are classified in a ‘Stressed Phase’ and in need of urgent government intervention; namely, Mpumalanga, Limpopo, Gauteng, Northwest, Free State, Northern Cape, Eastern Cape, and Western Cape; while the Kwa-Zulu Natal Province is classified in a ‘Crisis Phase’ (IPC, 2021).

Notable efforts have been made to promote food security by the South African government. The Agricultural Research Council (ARC) and Department of Agriculture, Land Reform and Rural Development (DALRRD) signed an agreement to improve communities in all nine provinces of South Africa through the development of a vegetable production garden strategy- The Agricultural Para-Professional Development Programme (APPDP). The objective of the APPDP is to create and support sustainable rural development through improved vegetable production in their respective homestead gardens to increase food security and household incomes. A strong horticultural sector can become the driving force for economic growth in the rural communities of South Africa (DALRRD, 2015).
According to Galhena et al. (2013), home food gardens are an important supplemental source contributing to food and nutritional security and livelihoods. The production of food on small plots next to human settlements is the oldest and most enduring form of cultivation. Home food gardens are found in both rural and urban areas in mainly small-scale subsistence agricultural systems. They provide various social, economic, and environmental benefits (Galhena et al., 2013), namely: 1) Improving food and nutritional security; 2) Improving health; 3) Empowering the status of women; 4) Preserving indigenous knowledge and building integrated societies; 4) Generating income, enhancing livelihoods, and household economic welfare; 5) The use of environmentally friendly approaches for food production while conserving biodiversity and natural resources. Thus, the collection, coding, and analysis of data on food and nutrition security has become more important to study the impact of, and to inform decisions on government initiatives towards reducing food and nutrition security in South Africa. The study also found that the share of the population below the poverty line in Gert Sibande has worsened over the years to 46.5% in 2019, making it the second highest of three districts in the province. Govan Mbeki Local Municipality has the highest number with 111 815 persons below the poverty line out of the seven local municipalities of the district (Cooperative Governance and Traditional Affairs (CGTA, 2020). The aim of the study is to determine the socio-economic and food security status of the selected households in the Gert Sibande District of Mpumalanga Province.

Materials and Methods

Study area

This research study focused on the Gert Sibande district in Mpumalanga. Gert Sibande District Municipality is one of the three district municipalities that form part of Mpumalanga Province. It is bordered by the Ehlanzeni district to the north-east and Nkangala district to the north, KwaZulu-Natal and Free State Provinces to the south, Swaziland to the east and lastly Gauteng Province to the west (CGTA, 2020). The district is also the largest of the three districts, covering 40% of the Mpumalanga Province’s land mass (IDP, 2020/2021). It consists of seven municipalities, namely: Chief Albert Luthuli, Dipaleseng, Dr Pixley Isaka Ka Seme, Govan Mbeki, Lekwa, Mkhondo, and Msukaligwa.

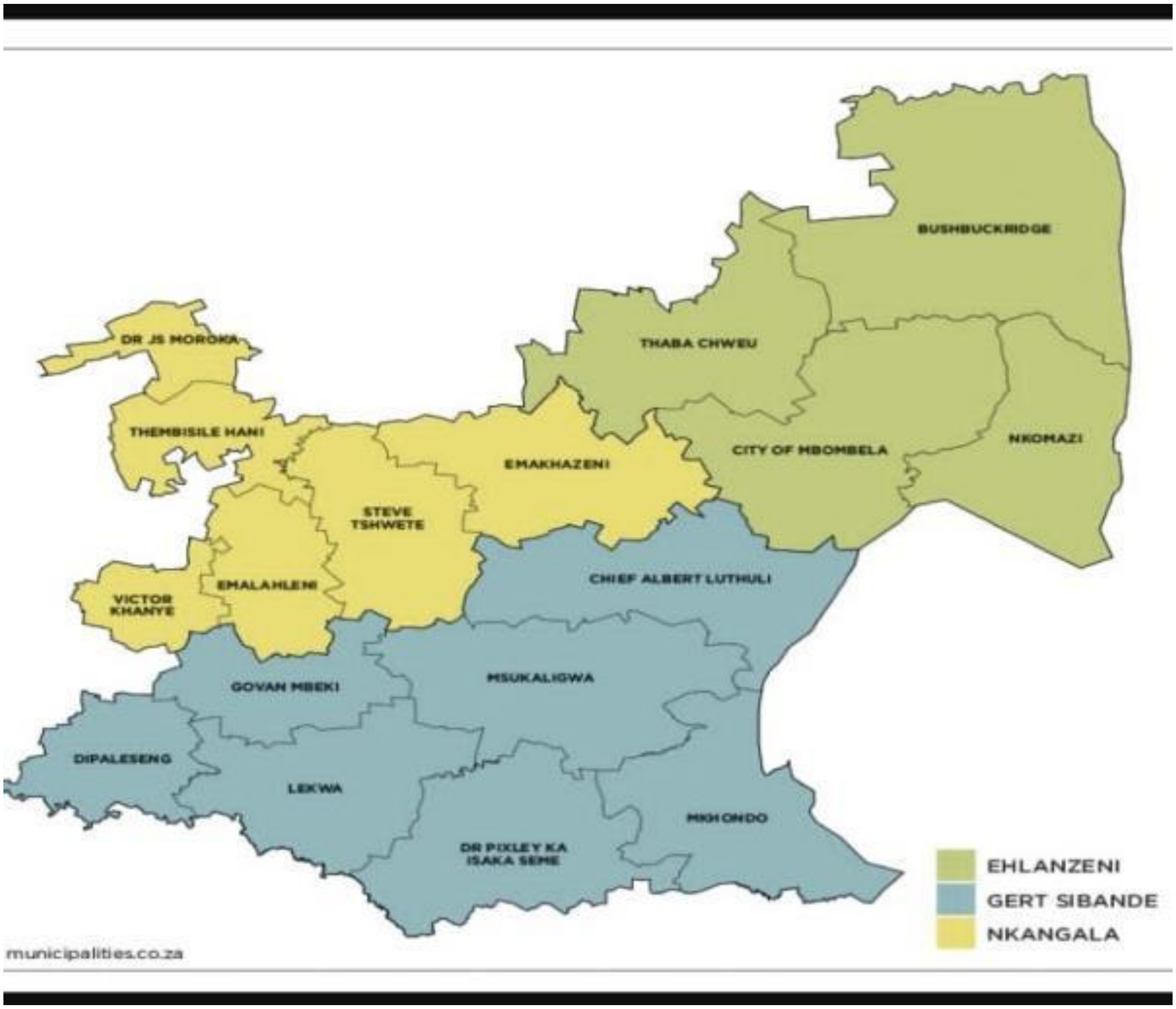

Figure 1: A Map showing the 7 local municipalities in Gert Sibande District
Source: https://municipalities.co.za/provinces/view/6/mpumalanga

Study design

Semi-structured questionnaires were administered to 54 Gert Sibande households. A semi-structured questionnaire gives the research a meaningful result as it allows respondents to provide their personal opinions on a set of questions provided. A rule of thumb was applied to determine the sample size, that is, 10% of the population. The research used both quantitative and qualitative methodological tools to retain more detail and understanding when interpreting data. The questionnaires were developed and collected by researchers at the Agricultural Research Council (ARC) working to develop communities. The primary data were collected through interviews, site observations, focus groups, and government reports. Prior to the survey, the questionnaires were pre-tested. The pilot tests were made by distributing the questionnaires to three household heads to determine whether the instruments were appropriate and suit the study. A purposive sampling technique was used to select the beneficiaries of the APPDP vegetable initiative. Purposive sampling is a form of non-probability sampling technique in which researchers rely on their own judgement when choosing which members of the population should participate in the surveys. The main aim of the Agricultural Paraprofessional Development Programme is to support community members, especially youth producing vegetables by providing them with agricultural inputs, and training. Therefore, the household heads selected for this study were predominantly youth benefiting from the vegetable strategy.

Analytical techniques

Descriptive statistics were applied to analyze the socio-economic characteristics and food security status of households in Gert Sibande district. The technique provides an easy way of summarising large quantitative data into a clear and understandable manner (Knupfer and McLellan, 1996) through tables, graphs, and measures of central tendency. The data were quantitatively analysed using the Statistical Package for Social Sciences (SPSS) Version 25.
According to Clapp et al. (2022), food security is composed of six dimensions; namely, food availability, food accessibility, food utilization and diversity, stability, agency, and sustainability. This study focused on the following dimensions: food availability, food accessibility, food utilization and diversity. Food availability refers to the physical existence of food at national and household levels, as well as food production and distribution (STATSSA, 2019). Furthermore, food accessibility refers to the ability of households to obtain food in sufficient quantities and good quality for a nutritious diet. Food utilization and diversity on the other hand relates to digestion of the food consumed, which is influenced by health status, water sanitation and the chemical safety of the food.
The households were further categorized using categories as reported by Maponya et al., (2022): 1) Food secure: these are households who did not worry about food access; they rarely experienced anxiety about not having enough food and were able to have a full meal three times a day without running out for the past four weeks. 2) Mildly food insecure: households were anxious about not having enough food and consumed inadequate diet or food that they did not prefer. These households experience food insecurity once or twice in the past 30 days. 3) Moderately food insecure: in this category, households start compromising on the quality of food by consuming inadequate diet and less preferred food. Food insecurity is experienced 3 to 10 times in the past 30 days. 4) Severe food insecurity: the condition of reducing meal sizes and the number of meals a person normally consumes daily. The three most sever conditions of going a whole day without eating, going to bed hungry, or running out of food in the past 30 days occurred "often". Households experienced food insecurity more than 10 times a day.

Results and Discussion

The descriptive statistics in Table 1 indicate the socio-economic characteristics of households. Females constituted almost 87% of the total vegetable garden household population compared to males with only 13%. The main aim of the vegetable garden intervention is to support community members, especially youth partaking in vegetable production by providing them with production inputs, infrastructure, and training. As a result, youth (less than 35 years of age) constituted at-least 70% of the population, followed by households ranging from 36 to 45 years of age constituting only 30%. There were no household members between the ages of 46 to 55, and above 56 years of age. The highest level of education attained amongst households in Gert Sibande district was Grade 12, making it simple for them to be trained and mentored, and ultimately mentor others. Furthermore, there were about 56% of households with household members ranging from 1 to 5, compared to those with household members ranging from 6 to 10, and greater than 10 with percentages of 27 and 17, respectively. Approximately 74% of the households had their own household garden (*Figure 5* below demonstrates quality produce harvested from household's garden). Only 9% inherited land, and 17% received permission from the government either to occupy or share land. The South African Child support Grant is about R460 per child therefore a female household head with more than 3 children will have a household income ranging from R1001-R2500. About 35% of households in the district received an income ranging from R1001-R2500 as shown in Table 1. Only 4% of the households received an income higher than R2501, these were perhaps members who received income through employment. In addition, about 71% of households in the district received their income through social grants compared to 20% who received their income through employment. There were also more households headed by females compared to males, with percentages of 61 and 32, respectively. Only 7% of the households in the district were headed by children. It is evident that females played a dominant role in ensuring food security in their respective households.

Table 1: Distribution of selected households according to their socio-economic characteristics in the Gert Sibande District, Mpumalanga Province

Variables	**Households**	**% Households**
Gender		
Female	47	87
Male	7	13
Total	54	100
Age		
Less than 35 years	38	70
36 - 45 years	16	30
46 - 55 years	0	0
> 56 years	0	0
Total	54	100
Highest education of the respondent		
Grade 8	0	0
Grade 9	4	6
Grade 10	7	13
Grade 11	18	33
Grade 12	21	39
Technician/Diploma	3	4
University Degree	1	2
Total	54	100
Number of household members		
1 to 5	30	56
6 to 10	15	27
>10	9	17
Total	54	100
Land acquisition		
Household Garden	40	74
Inheritance	5	9
Permission to occupy/communal land	9	17
LRAD/government lease	0	0
Total	54	100

Household income		
<R500	15	26
R500-R1000	18	32
R1001-R2500	19	35
>R2501	2	4
Total	54	100
Type of income		
Social Grants	38	71
Employment	11	20
Other	5	9
Total	54	100
Household head		
Child	4	7
Female	33	61
Male	17	32
Total	54	100

Source: ARC data
LRAD (Land Redistribution for Agricultural Development)

Food availability revolves around the supply of food made available through domestic production, net imports, food reserves, donations etc (FAO, 2006). The availability of food varies between urban formal and informal areas, as well as rural and tribal authority areas. The latter lies outside the main food distribution networks and is characterized by small food outlets such as general dealers, spaza shops, and street vendors which stock a limited amount of food (Maponya et al., 2022). Majority of the households (44% sometimes; 28% often; 22% always; 6% never) indicated that their food runs out before they can get money to buy more, as depicted in Table 2. More than 70% of the households did not know where the next day's food is going to come from. The food that they buy was also not enough to feed their families (43% always; 29% often; 19% sometimes; 9% never). It is evident that the food security status in Gert Sibande district is worrying as households do not have economic access to grow and purchase their own food, food also do not last until the end of the month. As a result, households often feel hungry (52% sometimes; 20% often; 15% always; 13% never), they eat less than they should (46% sometimes; 26% often; 17% never; 11% always) and children go to bed feeling hungry (50% never; 43% sometimes; 7% often).

Table 2: Households Food availability in the Gert Sibande District, Mpumalanga Province

An impression of Food Availability	**N**	**S**	**O**	**A**
			%	
My food runs out before I get money to buy more	6	44	28	22
I do not know where the next day's food is going to come from	6	74	18	2
The food that I buy is not enough to feed my family	9	19	29	43
I am often hungry	13	52	20	15
I eat less than I think I should	17	46	26	11
I do not have enough money for food	11	43	11	35
I cannot afford to feed my children	15	52	13	20
My children are not getting enough food to eat	24	50	24	2
My children go to bed feeling hungry	50	43	7	0
I go to bed feeling hungry	57	43	0	0

I know where tomorrow’s food is going to come from	28	61	4	7
I can afford to eat enough everyday	59	25	2	13
I have enough money for food	69	22	3	6
I have enough food to last until I get money to buy more	67	28	0	5
I still have food in the house the day before someone gets paid	65	20	9	6
N-Never; S-Sometimes; O-Often; A-Always				

Source: ARC data

Food security exists when all people, at all times, have physical, social and economic *access* to, sufficient, safe and nutritious food which meets their daily dietary needs and food preferences for an active and healthy life (Statistics Division, 2007). Table 3 demarcates food accessibility of households in Gert Sibande district. About 49 out of the 54 sampled households highlighted that they were not able to eat the kinds of foods they preferred due to lack of resources such as capital required to purchase inputs for vegetable production and land to grow food. This in turn resulted in households having fewer and smaller amounts of meals in a day because there was not enough food. Moreover, a total of 26 of the households had to sleep at night hungry and approximately 22 would go the whole day and night without eating anything. Only few of the households in the district were food secure and were able to have a full meal three times a day in the past four weeks.

Table 3: Households Food Accessibility in the Gert Sibande District, Mpumalanga Province

An impression of Food Accessibility	**R**	**S**	**O**	**T**
In the past four weeks, did you worry that your household would not have enough food?	16	24	7	47
In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of lack of resources?	16	17	16	49
In the past four weeks, did you or any household member have to eat a limited variety of foods due to lack of resources?	11	16	18	45
In the past four weeks, did you or any household member have to eat some foods that you/they really did not want to eat because of lack of resources to obtain other types of foods?	9	20	14	43
In the past four weeks, did you or any household members have to eat a smaller meal than you felt you needed because there was not enough food?	9	26	11	46
In the past four weeks, did you or any household members have to eat fewer meals in a day because there was not enough food?	7	22	8	37
In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?	8	20	1	29
In the past four weeks, did you or any household members go to sleep at night hungry because there was not enough food?	15	11	0	26
In the past four weeks, did you or any household members go a whole day and night without eating anything because there was not enough food?	8	13	1	22
R-Rarely; S-Sometimes; O-Often; T-Total household’s members who selected Yes				

Source: ARC data

The Household Dietary Diversity Score is the number of different groups consumed over a given period (Swindale and Bilinsky, 2006). The study employed the 16 food groups shown in Figure 2 above, proposed by FAO, World Health Organization (WHO), and the Food and Nutrition Technical Assistance (FANTA) (Ngema et al., 2018). The respondents were asked whether they consumed a food item listed to one of the food groups at least in the last 7 days. Looking at *Figure 2*, households had access to the following food groups: cereals; Vitamin A rich vegetables and tubers; dark green leafy vegetables; other vegetables; flesh meats; eggs; fish; legumes/nuts and seeds; oils and fats; sweets; spices/condiments/beverages. Dark green leafy vegetables and other vegetables (cabbage, tomato, onion, cauliflower) were the most accessible and consumed food group with percentages of 93 and 96 respectively (*Figure 4* below indicates a farming enterprise of fresh leafy vegetables that households consume and sell to the community and other local markets). This may be due to the vegetable initiative implemented by the ARC in collaboration with DALRRD which provides households the opportunity to grow their food in their respective home gardens. The initiative also monitors the homestead gardens, provides technical support and agricultural inputs as depicted in *Figures 6 and 7* below. Contrary, white tubers and roots; Vitamin A rich fruits; organ meat (iron rich); milk and milk products were the least accessible and consumed food groups with only 52%, 50% and 52% having eaten it in the last 7 days, respectively.

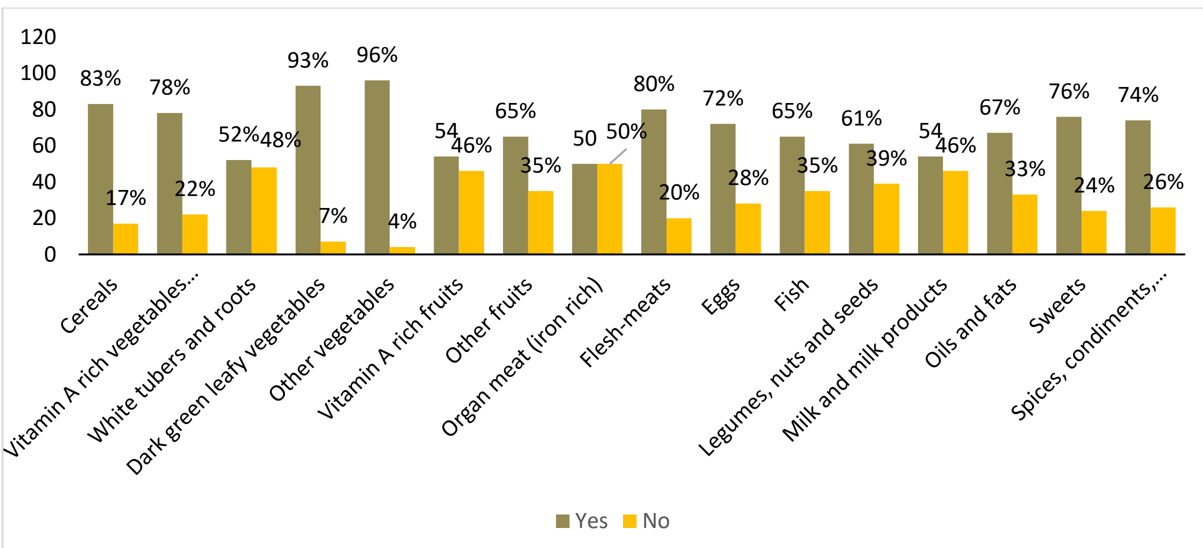

Figure 2: A Bar graph indicating the 16 Food Groups consumed by Households in the Gert Sibande District, Mpumalanga, South Africa.
Source: ARC data

The results below summarise the food security status of households in Gert Sibande district. The food security status in the district is alarming in that almost 90% of the households were food insecure (FI), while only 13% were food secure, as indicated in Table 4. And of those who were food insecure, 34% were mildly FI, 51% moderately FI and 15% severely FI.

Table 4: Households food security levels and extent of food insecurity in the Gert Sibande District, Mpumalanga, South Africa

Variable	**Category**	**Household**	**%**
Food Security level	Food secure	7	12.96
	Food insecure	47	87.03
Extent of food insecurity	Mild	16	34.04
	Moderate	24	51.06
	Severe	7	14.89

Source: ARC data

Selected households' pictures

Figure 4: Demonstration of farming enterprise where households can sell their vegetables to the community and other local markets in Gert Sibande District, Mpumalanga, South Africa.

Figure 5: An example of quality produce from household food gardens in Gert Sibande District, Mpumalanga, South Africa.

Figure 6: Agricultural Research Council official monitoring the household's garden in Gert Sibande District, Mpumalanga, South Africa.

Figure 7: Agricultural Research Council official offering technical support (technical advice and agricultural inputs) to the household in Gert Sibande District, Mpumalanga, South Africa.

Conclusion

The results presented in this paper provided evidence that food insecurity and specifically food inadequacy and hunger remain a challenge in rural South Africa in general, including the Gert Sibande District. The socio-economic characteristics indicated that female headed household were more likely to experience hunger and inadequate access to food. Households with a large household composition were also more likely to experience hunger as the limited food available must sustain more hungry mouths. Furthermore, the paper established that social grants were the main source of income. This type of income is not enough for household members to have access to and eat the kinds of foods they prefer.

The findings also confirmed that although households may meet the dietary diversity requirements, they cannot be deemed as food secure because the food available runs out before month end, and they do not have access to preferred food due to limited resources. Households usually eat what is available, what they grow in their home gardens through the vegetable government strategy, and what they can afford at that point in time. Dark green leafy vegetables and other vegetables were the most accessible and consumed food groups because households produce these in their home gardens. Furthermore, the paper established that households who are food insecure were more than those who were food secure. Only few households had access to food and were able to have a full meal three times a day in the past 30 days without food running out.

The study concludes that household participation in the vegetable government intervention can potentially improve households farming knowledge, increase production and income, and ultimately flatten the food insecurity status. The study recommends that immediate action be taken to avoid putting Mpumalanga Province in a crisis with regards to nutrition and food security of households in the province; this includes expanding the family planning education to limit the family size of rural households (Agidew and Singh, 2018), in addition large scale programmes on food and nutrition security must be rolled out that includes increased support to production of good quality nutritious foods integrated with nutrition education. These could include household and school nutrition garden and small livestock, such as poultry and eggs. Since vegetable production was among the main means to procure food by households in Gert Sibande it is recommended that the APPDP should continue investing in agricultural infrastructure, sourcing more vegetable household producers, consistent provision of inputs, investing in knowledge and education, as well as prioritising household food/vegetable producers.

Further research can be done on determining the impact of the APPDP vegetable strategy on households' food security status. The 87% food insecurity status would have dropped significantly had the paper analysed data before the intervention and after the intervention which is what is recommended for future interventions of this nature.

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FACTORS AFFECTING THE PRODUCTION STATUS OF SMALLHOLDER CROP FARMERS IN THE UNION OF COMOROS, SUB-SAHARAN AFRICA

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Abstract

Smallholder farms constitute 80% of all farms in Sub-Saharan Africa. The main activities which households pursue to sustain their livelihoods in the Union of Comoros are agriculture and fishing. Staple crops contribute almost 80% of the country’s agricultural production value. Recent data however shows that poverty in the country is growing with more than 54% of the population living below the poverty line. The rate is higher among farm families. To address such challenges, the government of the Comoros through the South African government requested expert assistance from the Agricultural Research Council (ARC), a South African government organization for supporting and enhancing agricultural practices with adaptive research. The study aimed to determine the factors affecting the production status of smallholder crop farmers in the Union of Comoros. A total of 155 smallholder farmers were selected through a stratified sampling procedure. A closed-ended questionnaire was administered to farmers. The primary data was analyzed quantitatively using the Statistical Package for Social Sciences Version 26. The Descriptive Statistics and Ordinal Logistic Regression model were adopted to meet the objectives of the study. The paper revealed the production status of smallholder farmers as follows: 26% very poor; 8% poor; 55% average; 12% good. It was concluded that the most important factors affecting farmers’ production status were age, land size and the condition of water resources. The probability of an active production status was reduced by 0.00057 if the farmer increased the farm size by an additional hectare. Contrary, middle-aged smallholder farmers who had water resources in good condition were more likely to improve their production status. For an active production status, the study suggests that middle aged farmers whose primary occupation is farming should be the main targets of agricultural projects/incentives in the country.

Keywords: *Factors, Production status, Smallholder crop farmers, Union of Comoros, Sub-Saharan Africa.*

Introduction

The agricultural sector in the Sub-Saharan Africa plays a vital role in contributing to food security. It employs more than half of the total labour force (IMF, 2021) and sustains livelihoods for majority of smallholder farmers. In addition, smallholder farms constitute almost 80% of all farms in Sub-Saharan Africa and employs about 175 million people, in which women comprise half of the labour force in many of the countries (AGRA, 2017). The crop sector in the region has the highest total agricultural production value with an average of 85% (FAO, 2016).

The Union of Comoros consists of three main islands located in the northern part of the Mozambique Channel off the Coast of East Africa. The islands have a total population of approximately 625,000, of which 52% live in Grande Comore, and only 43% and 5% live in Anjouan and Mohéli, respectively (Meyer et al., 2006). Agriculture, fishing, petty trade, and remittances are the four main activities which households pursue to sustain their livelihoods. Furthermore, agriculture and fishing constitute more than 40% of Comoros Gross Domestic Product; wherein vanilla, cloves, coffee, and ylang-ylang are the primary export crops accounting approximately 20% of the production value. This contrasts with the agricultural sub-sector - producing staple crops such as bananas, cassava, taro, and sweet potato, which contributes about 80% of the agricultural production value against a value of only 21% for exportable cash crops (Meyer et al., 2006). It is evident that the agricultural sector plays a critical role in improving food security and sustaining livelihoods as more than half of the food crops produced are mainly for family consumption with only a small percentage exported.

Recent data however shows that poverty in the Union of Comoros is growing with more than 54% of the population living under the poverty line (Meyer et al., 2006). The rate is higher in rural areas and among farm families. The Anjouan Island is leading with an estimated 61% of households living in poverty, followed by Mohéli (56%) and Grande Comore (34%). Moreover, about 41% of the children experience impaired growth due to poor nutrition and inadequate psychosocial stimulation (Meyer et al., 2006). The high poverty rate in the country is driven by an unsustainable agricultural sector-strategy where more forest land is under cultivation, rather than focusing on improving the farm production status and value addition of agricultural products (WB, 2019). About 72% of farmers are smallholder producers with weak market access and production linkages in a rapidly growing domestic demand for food with more households shifting towards vegetables, meat, and dairy (WB, 2019). The labour force, especially educated youth, is also shifting from primary production due to an unbeneficial agricultural sector, however the absence of non-farm opportunities has only led to high youth unemployment rate. Given scarce resources (such as water) and a rapid population growth, the Comorian agricultural sector is threatened. The study aims to determine the factors affecting the production status of smallholder crop farmers in the Union of Comoros. The study included both socio-economic and production factors as determinants of production. The socio-economic results in relation to the production status of farmers will help understand the nature and condition of crop production in the country, which will in turn assist in customising intervention strategies that will address local farmers’ production challenges. The objectives of the study are to:

I. Determine the socio-economic characteristics affecting the production status of smallholder crop farmers.
II. Analyse the factors affecting the production status of smallholder crop farmers in the Union of Comoros.

Agricultural transformation focused on raising farm yield and productivity, strengthening linkages between farms and the rest of the economy and sustainable practices can deliver large food security gains, raise farm incomes, lower food import balance, and attract labour force back into the agricultural sector, therefore leading to an active production status. Economic transformation should not imply an exit from the agricultural sector but requires building on it to improve farming and enhance efficiency levels by linking farmers to other sectors such as manufacturing in the form of agro-processing. Considering the above, it is necessary to establish results and make recommendations to address the challenges faced by smallholder farmers and the agricultural sector in general in the Union of Comoros.

Materials and Methods

Study area

Comoros also referred to as the "*perfumed Island*" because of its fragrant beauty is an archipelago located on the ancient trade routes of the Indian Ocean. This Island nation is home to approximately 800,000 dwellers, densely populated over a total of almost 1800 square kilometres (Caroline et al, 2019). Comoros cultural heritage originates from Africa, the Middle East and Europe and about 98 percent of the population adhere to a form of Sunni Islam. The Comoros population is dominated by young individuals due to high fertility rates. Moreover, women play an important role in society but only get to exercise their voice in their respective Comorian households rather than in the public space (Caroline et al, 2019). Emigration has also always been part of society with one third of the population living outside of the country today. The emigrants send remittances to their home communities, averaging almost 14 percent of the Gross Domestic Product, this makes Comoros one of the highest remittance-recipient countries in Sub-Saharan Africa (Caroline et al, 2019)
As indicated on the map below **(**Figure 1**)**, Comoros is in the Indian Ocean at off Coast of Eastern Africa, Northern side of Mozambique Channel, and between Madagascar and Mozambique. Other countries in proximity of Comoros are Seychelles in the northeast and Tanzania in the northwest (Meyer et al., 2006). The country consists of four islands - Grande Comore, Mohéli, Anjouan, and Mayotte wherein the latter remains under France administration. About half of the population live within the largest Comoros island; Grande Comore which is also home to the country's capital city "Moroni" (Meyer et al., 2006). Data were collected and analyzed from only the three main islands of Comoros, that is, Grande Comore, Mohéli and Anjouan.

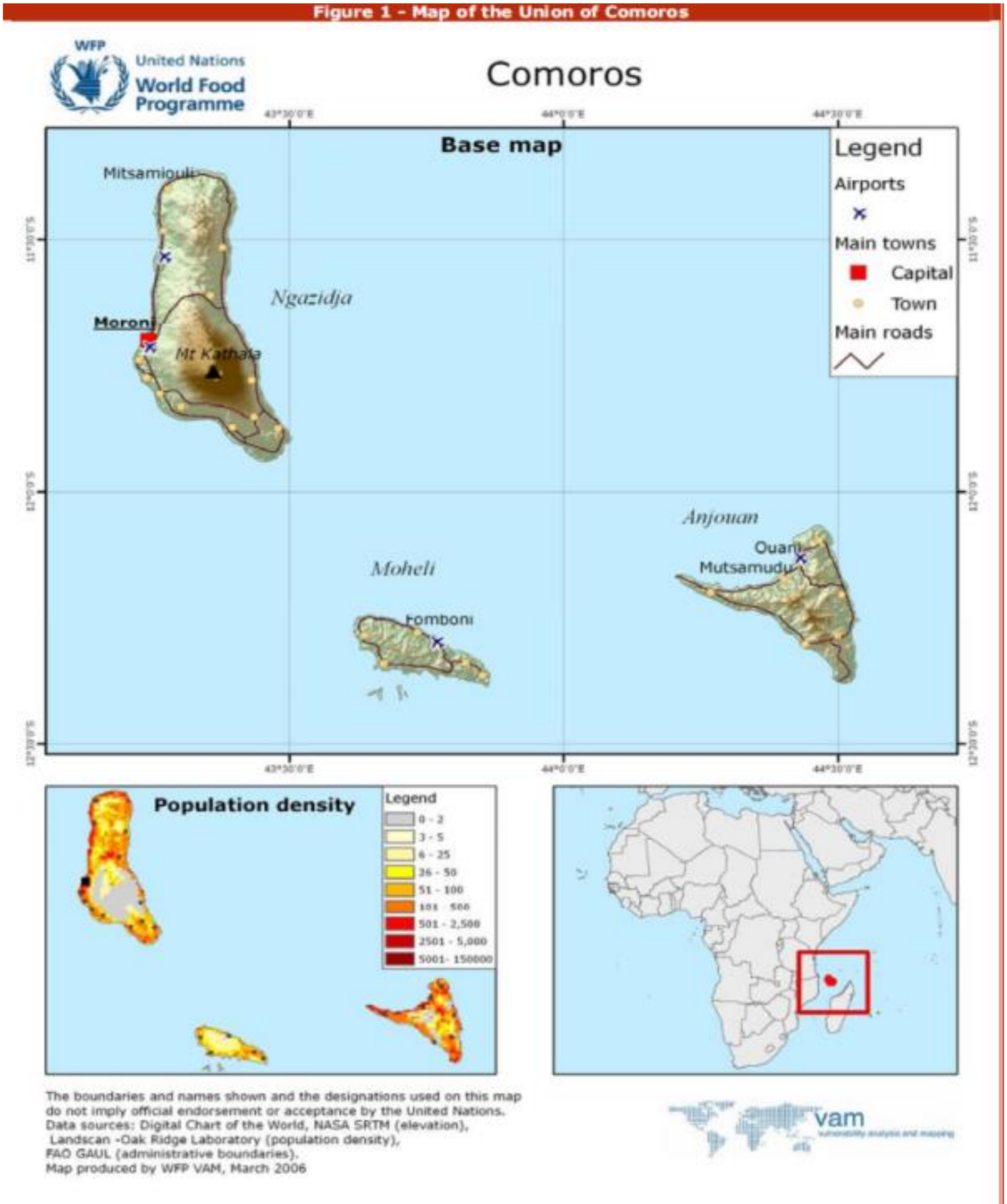

Figure 1: Map of Comoros

Source: World Food Programme (WFP), Vulnerability Analysis and Mapping (VAM) (Meyer et al, 2006)

Study design

A total of 155 smallholder crop farmers were selected through a stratified sampling procedure. According to Pirzade et al. (2011), a stratified sampling technique not only reduces the size of a population but also results in a sample that is a representative of the original population by ensuring that the desired characteristics of an execution are distributed similarly in both the sampled and original trace. A rule of thumb was applied to determine the sample size, that is, 10% of the population.
An approved closed ended/structured questionnaire was then administered to the farmers. Structured questionnaires are easier for the researcher to code and analyze quantitatively, and they reduce the amount of time that a respondent needs to complete the questionnaire. Prior to the survey, the questionnaires were pre-tested. The pilot tests were made by distributing the questionnaires to three smallholder crop farmers to determine whether the instruments were appropriate and suit the study. The questionnaires were developed and collected by researchers at the Agricultural Research Council (ARC) together with the help of local Comoros administrators working to enhance and improve the conditions of production and value addition among the smallholder farmers. The research further used quantitative and qualitative methodological tools to retain more detail and understanding when interpreting data.

Analytical techniques

Descriptive Statistics

Descriptive Statistics were applied to identify and assess the socio-economic characteristics required for efficient crop farming among smallholder farmers. The technique provides an easy way of summarising large quantitative data into a clear and understandable manner (Knupfer and McLellan, 1996) through tables, graphs, and measures of central tendency. The data were quantitatively analysed using the Statistical Package for Social Sciences (SPSS) Version 26.

The Ordinal Logistic Regression Model

The Ordinal Logistic Regression is a statistical analysis model used to determine the relationship between an ordinal dependent variable and one or more explanatory variables that may be either continuous or categorical (Parry, 2020). The model was applied to model the factors affecting the production status of smallholder crop farmers in the Union of Comoros. An ordinal dependent variable is one which there is a clear ordering of the category levels. The model is an extension of logistic regression where the logit of a binary response is linearly related to the independent variables. This is in contrary to the ordinal logistic regression model, which assumes proportional odds: the effect of an independent variable is constant for each increase in the level of the response. Hence the output of an OLR will remain an intercept for each level of the response except one (Parry, 2020).

Mathematical Expression of the Ordinal Logistic Regression: -

Defining the event:

In ordinal logistic regression, the event of interest (ex. Production status) is observing a particular score. For the rating of ***production status of smallholder crop farmer***, you model the following odds (Norušis, 2011):
$\theta 1$ = prob(score of 1) / prob(score greater than 1)

$\theta 2$ = prob(score of 1 or 2) / prob(score greater than 2)

$\theta 3$= prob(score of 1, 2, or 3) / prob(score greater than 3)

$\theta 4$= prob(score of 1, 2, 3 or 4) / prob(score greater than 4)

The last category does not have an odds associated with it since the probability of scoring up to and including the last score is 1.
The Ordinal Logistic regression model for one independent variable is:
$\ln(\theta_j) = \alpha_j - \beta X$ (Norušis, 2011).
The negative sign before the coefficient (β) is placed so that larger coefficients could indicate a larger score. That is, a positive coefficient on a dichotomous factor indicates that higher scores are more likely for the first category, and a negative coefficient indicates that lower scores are more likely. Whilst for a continuous variable, a positive coefficient depicts that as the values of the variable increase, the likelihood of larger scores increases (Norušis, 2011).

Variables of smallholder crop farmers in the Union of Comoros

Production status - the production status of smallholder crop farmers is an ordinal variable, with rankings of very poor, poor, average, good and very good. A ‘very poor’ ranking is an indication of almost no sign of active production, while a ‘very good’ rank is an indication of active production status. The score for production status was obtained through the assessment of existing production on site in terms of the availability of weeds, and pests, as well as crop condition.
Gender - the variable gender was treated as a dummy, where 1 denotes *if farmer is male*, and 0 otherwise. This variable is expected to have either a positive or negative effect on the decision to actively participate in agricultural production. Males are, however, usually the decision makers in a traditional household and are therefore well placed to actively partake in agricultural production.
Age - According to Khatun and Ray (2012), experience on livelihood options and the desire to diversify increase with age; therefore, older farmers are expected to actively participate in crop production to increase income and sustain their families’ livelihoods.
Level of education - Moreover, Sisay (2010) studied the determinants of smallholders’ participation in agricultural production in Ethiopia and established that participation is positively influenced by household size and level of education. The higher the number of household members and the level of education, the higher the participation.
Household size - household size was included as a proxy variable for labour supply. Large household sizes will have more farm labour supply than small sized households.

Land size - Past studies maintain that larger farm sizes are often associated with specialization in agriculture (Khoza et al., 2019). This variable is expected to influence the production status of smallholder crop farmers positively.
Conditions of production resources - availability of production resources were based on the agronomic requirement for optimum crop production. Various agronomic literature provided key tangible resources for optimum crop production such as land, water, and capital (du Plessis, 2003; FAO, 2016; Ceglar, 2013; Khan, 2014). These were measured as ordinal variables.

Results and Discussion

Table 1 revealed the socio-economic characteristics of smallholder crop farmers in the Union of Comoros, as well as the condition of their production resources. Gender distribution among respondents indicated a higher percentage for males (62%) than females (38%). Higher total number of males implies that any developmental strategy for the farmers in the area will benefit them than females. The results also depicted the different categories in which household members fall under; and about 55% of the smallholder farmers fell within the 5 to 9 household size category, followed by the 10 to 14 category with a percentage of 23 compared to other group categories 0-4 (17%), 15-19 (3%) and 20-25 (2%). Large household size may be of advantage to primary production, however, are inconvenient when coming to improving livelihoods due to decisions that must be made, that is, to either sell or retain produce for household consumption. The same view was shared by Randela, (2005), who alluded that household size and dependence values have an influence on marketing as they affect consumption and production. Furthermore, approximately 73% of the respondents did not have any formal education. Only a few numbers of respondents with formal education were scattered across different educational levels. Age of the household head is an important aspect in agriculture because it determines the experience of the farmer. In addition, to a certain extent, age indicates the position of the project in the life cycle. Ngqangweni and Delgado (2003) alluded that household leaders’ experience influences household members’ farming activities. Approximately 34% of the smallholder crop farmers ranged between 45 to 54 years of age, and only 1% fell within the 75 to 84 age group. Middle aged farmers were more prominent in crop production compared to other groups. Additionally, the scale of production, choice of commodities and economic viability in production are often related to the size and the production potential of the land (Chitonge, 2013). The Table summarized the total land sizes owned by respondents in Comoros. The land size was categorized into 0-5ha (52%) and 5-10ha (5%). Almost half of the smallholder crop farmers (43%) did not know their land sizes. It is also notable that most farmers did not own bigger land sizes as about 52% owned only less than 5ha of land. The unknown 43% is probably those farmers without formal education who found it challenging to determine their land sizes.

Table 1: Distribution of selected smallholder crop farmers according to their socio-economic characteristics in the Union of Comoros, Sub-Saharan Africa.

Variables	**Households**	**% Households**
Household characteristics		
Gender		
Female	59	38
Male	96	62
Total	155	100
Age		
Less than 35 years	22	14
35-44 years	45	29
45-54 years	53	34
55-64	26	17
65-74	8	5
75-84	1	1
Total	155	100
Highest education of the respondent		
No formal education	113	73
Grade 1	-	-
Grade 2	-	-
Grade 3	-	-
Grade 4	-	-
Grade 5	5	3
Grade 6	5	3
Grade 7	14	9
Grade 8	-	-
Grade 9	-	-
Grade 10	-	-
Grade 11	3	2
Matric	3	2
Technical diploma	9	6
Other post-matric qualification	3	2
Total	155	100
Household size		
0-4	26	17
5-9	85	55
10-14	36	23
15-19	5	3
20-25	3	2
Total	155	100
Land size		
0-5	81	52
5-10	8	5
Unknown	66	43
Total	155	100
Condition of production resources		
Condition of road to farm site		
Very poor	28	18
Poor	79	51
Average	48	31

Good	-	-
Very Good	-	-
Total	155	100
Condition of farm machinery		
Nothing	155	100
Very poor	-	-
Poor	-	-
Average	-	-
Good	-	-
Very Good	-	-
Total	155	100
Condition of equipment		
Nothing	-	-
Very poor	-	-
Poor	45	29
Average	110	71
Good	-	-
Very Good	-	-
Total	155	100
Condition of water resources		
Nothing	-	-
Very poor	22	14
Poor	42	27
Average	91	59
Good	-	-
Very good	-	-
Total	155	100
Production status of farmers		
Very poor	40	26
Poor	12	8
Average	86	55
Good	17	11
Very Good	-	-
Total	155	100

Source: ARC data

With regards to production resources, 51% of the farmers rated their road condition to be poor while 18% rated it as very poor. Only 31% rated their road as being in an average condition. This entailed that the road condition to farming site was in a poor state, which had a negative implication on the accessibility of the farming site and ultimately affecting the production status of the farmers. The results also showed that Comoros farmers used only hand tools such as spade, rake, hoe, and garden fork for production. Heavy machinery including tractors, ploughs and boom sprayers were not made available to them. The results are alarming considering the vision of the Comoros government to intensify production. Investment in production infrastructure would assist farmers to increase their production output and improve their production status. About 59% of the smallholder crop farmers rated the condition of their water resources as average, and 27% and 14% of the farmers rated the resources as 'poor' and 'very poor', respectively. Of those water resources, 22% of the farmers owned water tanks while only 4% had reservoirs and 1% owned both a water pump and borehole. The farmers do not have adequate water infrastructure for optimum crop production. Even in the presence of fertile soil, inadequate water infrastructure will deem the entire production process ineffective. About 71% agreed that the condition of their farming equipment was on average, and only 29% stated that the equipment was in a poor condition. The production status of smallholder crop farmers was also on average, with a percentage of 55 as indicated in the Table. Further improvements are needed in addressing the needs of rural households and encouraging maximum production of food through various government incentives and strategies in the Union of Comoros.

Table 2: Ordinal Logistic regression results of smallholder crop farmers in the Union of Comoros, Sub-Saharan Africa.

Variables	**Description**	**Coefficient Estimates**	**Significance**
Gender	1 if farmer is male, 0 otherwise	0.262888	0.26
Age	1= >35 2= 35 to 44 3= 45 to 54 4= 33 to 64 5= 65 to 74 6= 75 to 84	0.02102	0.036**
Level of education	No Formal Education Grade 1; Grade 2 Grade 3; Grade 4 Grade 5; Grade 6 Grade 7; Grade 8 Grade 9; Grade 10 Grade 11; Grade 12 Technical Diploma Other post-matric qualifications	-0.0732	0.31
Household size	0-4; 5-9; 10-14 15-19; 20-25	0.017309	0.54
Land size	0 - 5ha; 5 -10ha	-0.00057	0.013**
Condition of road to farm site	0=Nothing 1=Very poor; 2=Poor; 3= Average; 4=Good; 5=Very good	-21.893	0.000
Condition of farm machinery	0=Nothing; 1=Very poor; 2=Poor; 3= Average; 4=Good; 5=Very good	-3.280	0.393
Condition of equipment	0=Nothing; 1=Very poor; 2=Poor; 3= Average; 4=Good; 5=Very good	-17.889	0.991
Condition of water resources	0=Nothing; 1=Very poor; 2=Poor; 3= Average; 4=Good; 5=Very good	2.298	0.068*

Goodness of fit significant values
Pearson 0.096
Deviance 1
Pseudo R-Square values
Nagelkerke 0.585

*Sig at 10%, **Sig at 5%, ***Sig 1%
Source: ARC data

The Pseudo R-square is the proportion of variance in the response variable y that the regression model can explain through the introduction of regression variables. It measures how close the data is to the fitted regression line. As can be seen in Table 2, the value for the Pseudo R-square was 0.585 and this implies that the model explained 58 percent variability of the response data around its mean. The Pearson Correlation Coefficient value was however closer to zero (0.096); a value closer to 0 indicates a weak correlation between the dependent and explanatory variables (Schober et al., 2018).

Significant variables

The probability of the production status of smallholder crop farmers was directly influenced by age, land size, and condition of water resources.
Age

The coefficient of AGE = 0.02102 was found to be positively significant at 5% level, implying that as the smallholder crop farmer ages, his status of production is likely to improve from very poor to very good. The findings concur with Min-Li and Sicular (2013), who found that household-level technical efficiency increases with the age of the household labour force.
Condition of water resources

Similarly, the condition of water resources was also positively significant at 10% level, with a coefficient of 2.298. This entailed that the improvement of water resources was likely to enhance the production status of smallholder crop farmers. The result concurs with Playan and Matose (2006) notions that an increase in water productivity increases crop yield. Thus, any interruptions in water supply could discourage farmers to pursue farming.
Land size

In contrary, the area under production (land size) did not influence the production status of smallholder farmers, with a negative coefficient of 0.00057 at 5% significance level. This finding implied that farmers with a larger production area are less likely to improve their production status. A large farm size requires more production inputs, advanced machinery, irrigation, and intensive labour force which farmers cannot afford to obtain. The result is consistent with Eneyew (2012) whose paper focused on the determinants of livelihood diversification in pastoral societies of Southern Ethiopia. The results also revealed a negative effect of farm size on livelihood diversification.
Consequently, there was no significant relationship between the following explanatory variables, level of education, gender, household size, condition of road to farm site, condition of farm machinery, condition of equipment and the production status of smallholder crop farmers.

Conclusion

The study adopted the ordinary logistic regression model to estimate the factors affecting the production status of smallholder crop farmers in the Union of Comoros. Therefore, it is concluded that the most important factors affecting smallholder crop farmers’ production status were age, land size and the condition of water resources. The probability of an active production status reduced by 0.00057 if the farmer increased the farm size by an additional hectare. On the other hand, smallholder farmers with water resources in a very good condition were more likely to improve their production status by 2.298. Older farmers were also more likely to improve their production status as age translates to experience. The study also revealed that the production status of smallholder farmers was on average.

For an active production status, the study suggests that middle aged farmers whose primary occupation is farming should be the main targets of agricultural projects/incentives in the Union of Comoros. These farmers will in turn be able to train other interested community members in crop production. In addition, the provision of land should be accompanied by production inputs (seeds, fertilizer, pesticides, irrigation), capital, and agricultural training. There is no point in increasing land if farmers do not have access to production inputs. It is also recommended that the crop yield be achieved on less land, using less fertilizer, pesticides, and water. Water is an indispensable input for agricultural production and plays a critical role in food security. The provision of water resources such as boom sprayers and reservoirs are important to encourage active participation in agricultural production.

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GREEN CONSUMPTION IN VIETNAM A CASE STUDY OF YOUNG PEOPLE IN HANOI

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Abstract

Green consumption comes from the desire to protect natural resources for future generations and improve people's quality of life. Vietnam is facing challenges in environmental protection and sustainable development. Population growth leads to higher consumption demands of society. Therefore, the implementation and application of green consumption policies in Vietnam is necessary to encourage sustainable production and consumption. The study aims to assess the current situation and factors affecting green consumption in Vietnam, especially focusing on young people because they make up more than half of the national population. Primary data were collected through interviews with 120 people between the ages of 15 and 35 in Hanoi capital. The results show that 75% of young consumers save water; 77.5% of young consumers save electricity. The reason that motivates them to practice saving water and electricity resources is related to family finances. Moreover, 73.3% of people tend to consume green products and 76.6% of respondents forecast that the trend of consuming green products will increase in the coming time. However, only 54.1% of young consumers have knowledge about green products and 56.6% of the interviewees know where to buy green products. The study also shows that high price is a factor that hinders consumers from looking for green products. Besides, many other factors affect young people’s green consumption behavior such as: educational level, residence status, income, knowledge, etc. Young people have high qualifications, people with higher income, and people with environmental concerns tend to consume more green products than the rest.

Keywords: *Green consumption, Young people, Hanoi, Vietnam*.

Introduction

Green consumption is currently considered the consumption trend of the century when the environment becomes a major concern of many countries around the world (Sylvia & Doris, 2013). During the past decade, many policies and programs have been attempted to successfully transform the industrial structure, making production processes cleaner and more efficient. However, businesses can only reduce the environmental impacts associated with production, but not address the environmental impacts related to the selection, use and disposal of products of people (Maria & Rita, 2013). Therefore, consumption plays an increasingly important role in solving environmental problems; collaboration between producers, consumers and other stakeholders can lead to more sustainable solutions in the production-consumption system (Tania, 2006).

Currently, green consumption is quite popular in developed countries and initiated in developing countries, where personal income and consumption consciousness are increasing. In Vietnam, the concept of green consumption is still quite new, but green consumption is gradually becoming a new trend and consumers' awareness of green consumption is increasing day by day. In addition, the Government of Vietnam has included green consumption as a content in the Green Growth Strategy for the period 2011-2020 and the vision to 2050 of the country. Many green consumption models are supported by the community such as using paper bags instead of plastic bags, using bamboo or paper straws instead of plastic ones (Vietnam MOIT, 2021). The Government considers strengthening of implementation and application of green consumption policies in Vietnam as an effective solution to protect the environment and public health while heading towards sustainable development.

Hanoi is the capital of Vietnam where is residence of more than 8 million people, about 50% of whom are young. Although there are many improvements in green consumption trends, in fact the green consumption awareness and behavior of young people are still limited. Most consumers still prefer the convenience of shopping at a cheaper price at temporary markets and sidewalk shops than buying green brands goods at convenience stores or supermarkets. Therefore, the objective of this article is to provide a picture showing the situation of green consumption of young people in Hanoi, to analyze the factors affecting their green consumption behavior in order to propose some solutions to improve their awareness and behavior about green consumption. The following sections of this paper include material and methods, results and discussion; and conclusion.

Material and Methods

Hanoi is the capital of Vietnam with the population of more than 8 million people (the second most populous city in Vietnam, after Ho Chi Minh City), of which nearly 50% live in the urban area (GSO, 2021). As the political and economic center of the country, Hanoi is a place to attract young laborers from other localities to live, study and work. The diversity of young people's jobs and lifestyles leads to a richness in consumption patterns. Therefore, understanding the real situation of green consumption of young people in Hanoi is necessary to find solutions to guide their green consumption trend in the future.

To analyze the status of green consumption of young people in Hanoi, primary information from 120 consumers in age of 15 to 35 were collected through a survey conducted in May 2022. People with different jobs such as high school students, students, office staffs, salespeople, delivery workers, and shop owners... are selected to ensure the diversity of careers. Information on green consumption of these 120 people was collected through face-to-face interviews using a semi-structured questionnaire. The content of the questionnaire includes demographic information such as age, gender, education level, occupation, and income. In addition, information related to young people's awareness and behavior of green consumption is also mentioned: their understanding about green consumption, their green consumption behavior (frequency of buying green products, amount of money spent on green products, types of green products, places to buy green products...) and their green consumption trends in the future.

Primary data were synthesized and analyzed using descriptive statistics combined with Mann Whitney U-test to see the statistical differences among cases (Dinh, 2011).

Results and Discussion

Description of young people in the survey

This section provides demographic information about the young people including the sex, age, education and their occupation.

Among 120 young people interviewed, 60% were male and 40% were female. This reflects the common status in cities in Vietnam of gender imbalance that the male population is larger than the female population. Regarding age, 20% of young people interviewed are high school students aged 15 to 17; 30% are university and college students aged 18 to 22; and 50% of the interviewees are between the ages of 23 and 35.

Table 1. Statistical description of young people

Demographic Index	Quantity (n=120)	Proportion (%)
1. Gender		
Male	72	60
Female	48	40
2. Age		
15-17	24	20
18-22	36	30
23-35	60	50
3. Educational level		
High school	24	20
University and college	82	68
Postgraduate	14	12
4. Career		
Student	48	40
Self-business	12	10
Civil servant/office staff	36	30
Hired worker (shipper/retailer…)	24	20

*Source: Author's own calculations from survey data in 2022

In terms of educational attainment, the highest proportion (68%) belongs to the group of young people having university degree, the smallest number (12%) is the group of people having post graduated education, the rest (20%) are high school students. Regarding occupation, the student group occupies the highest proportion with 40%; the group of civil servants and office workers occupies 30%; the group of hired workers (delivery staffs, retail staffs in stores, shops or supermarkets) takes 20%; the lowest proportion (10%) belongs to young people who are owners of self-employed shops such as coffee shops, fast food, clothes...

Actual situation of green consumption of young people in Hanoi

Awareness of green consumption

Green consumption can be defined as the purchase, use and dissemination of environmentally friendly products that do not pose risk to human health and do not threaten functions or diversity of natural ecosystem. Green consumption comes from the desire to protect resources for future generations and improve people's quality of life. Currently, consumers around the world are gradually moving towards environmentally friendly green products and consider them as a standard for high quality products and services (Vietnam MOIT, 2021). However, consumers' awareness of green consumption is still limited, especially in developing countries like Vietnam. To find out the perception of young people about green consumption, the study asked the question "how do you understand green consumption?" to the interviewees. There are 4 options for the answer including: green consumption is the consumption of products made from green trees; green consumption is the consumption of environmentally friendly products; green consumption is the consumption of energy-saving products; and green consumption is the consumption of safe and healthy products. The survey results show that the majority of respondents believe that green consumption is the consumption of environmentally friendly and energy-saving products (Figure 1). However, out of 120 interviewed people there are still 10 young people who do not have a clear understanding of green consumption when selecting the first option.

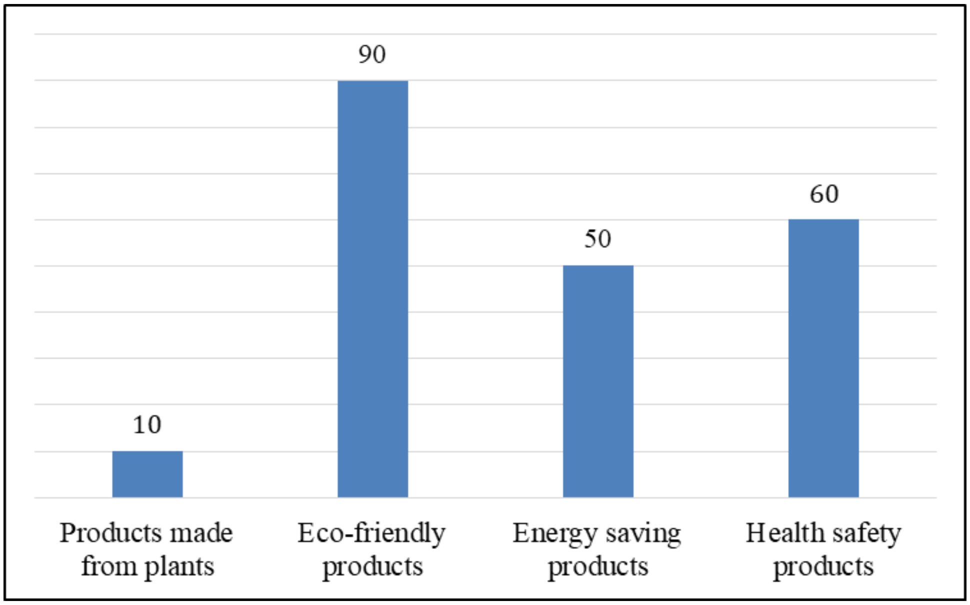

Figure 1. Awareness of young people about green consumption
*Source: Author’s own calculations from survey data in 2022

The approaching to information of young people reflects their awareness about green products. Among the surveyed people, more than 54% answered that they have heard and learned about green products. However, up to 36% of people answered that they have heard about green products but have no time to learn. Similarly, over 43% of young people replied that they do not know where to buy green products (Table 2).

> *“Information about green products usually appear on TV, facebook or Tiktok. In addition, because this issue is concerned by the whole world, I usually follow web information to understand about green product consumption and to be able to select the green products for my family. Moreover, I frequently discuss with my friends about green living trend and gather much knowledge about green product consumption from such discussions (Interviewing of a 19-year-old female student).*

Table 2. Approaching to green products of young people in Hanoi

Index	Quantity (n=120)	Proportion (%)
Heard/learnt about green products		
Heard but not learnt	43	35,8
Never heard	12	10,0
Heard and learnt	65	54,2
Know places to buy green products		
Yes	68	56,7
No	52	43,3

*Source: Author's own calculations from survey data in 2022

Green consumption behavior of young people

- **Criteria to select green products**

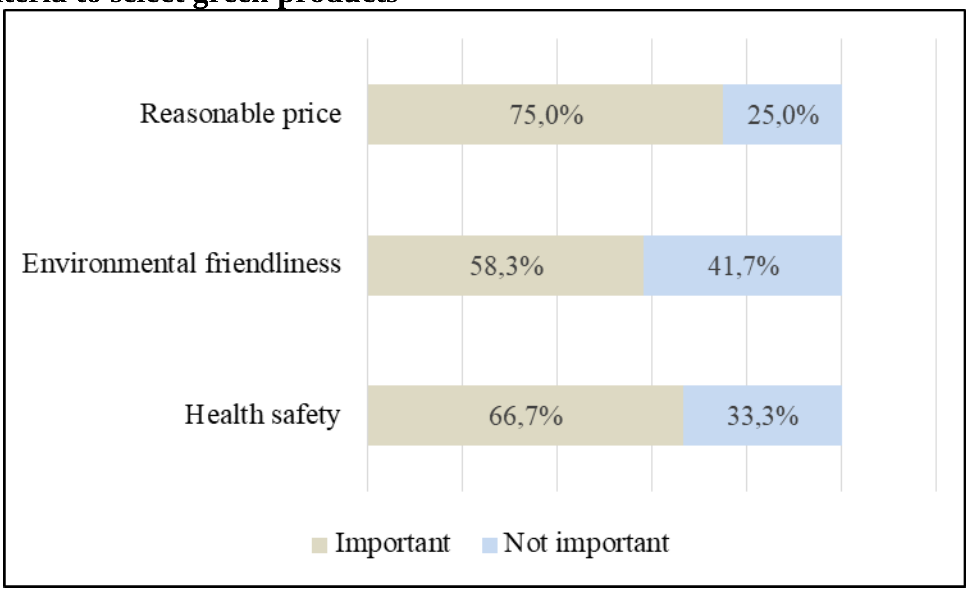

Figure 2 Young people evaluation about the importance of several criteria to select green products

*Source: Authors' own calculations from survey data in 2022

After learning about green products, young people will make decisions related to their green consumption behavior. To make right decisions, they will evaluate the importance of several criteria related to green products including: reasonability of price, environmental friendliness of products, and healthiness of products. Although green products are understood as environmentally friendly ones, this is not the most important criterion for young people to decide to consume green products. Up to 75% of young people think that reasonable price is still the most important factor leading them to green consumption decisions. The second most important factor is the criterion of health and safety with nearly 67% of people rating for it (Figure 2).

Actual situation of green consumption of young people

Table 3 Frequency of green consumption of young people

Index	Quantity (n=120)	Proportion (%)
Using disposable plastic goods		
Daily	60	50
2-3 times/week	48	40
Rarely (or never)	12	10
Using products with green energy label		
Sometimes	36	30
Rarely	24	20
Daily	60	50
Saving electricity, gasoline, gas		
Yes	93	77,5
No	27	22,5
Saving water		
Yes	90	75
No	30	25

*Source: Author’s own calculations from survey data in 2022

To clearly see the green consumption status of young people, the author analyzed their green consumption frequency including: frequency of using single-use plastic; frequency of using green energy labeled products; saving energy (gas, electricity, petrol) and saving water. The results are shown in Table 3.

For the use of single-use plastic, plastic bags, plastic bottles, plastic straws or disposable plastic cups take hundreds or even thousands of years to decompose. It can be easily seen in Hanoi area that plastic bags are commonly used in traditional markets or shops and supermarkets; Plastic cups, plastic straws are used a lot at shops selling drinks, fast food for students, and office workers. In the traditional markets of Hanoi, even few lemons or chilies are all packed in plastic bag. Many sellers and shoppers know that using plastic bags will have an impact on the environment, but they think it is a cost-effective solution because plastic bags are very cheap. Among many types of plastic bags, ultra-thin bags are used a lot but are not collected and recycled. They exist in most landfills and are almost non-decomposable. Up to 50% of young people answered that they use plastic bags every day (Table 3). It can be said that this large amount of consumption causes serious harm to the environment.

For the use of energy-labeled products and equipment such as fluorescent lamps, LED lights, air conditioners, televisions, refrigerators, computers, rice cookers, etc., up to 50% of respondents said that they them daily. The energy label on the product provides full information about the product's energy saving level and energy consumption. Besides, the number of young people with energy saving behavior is quite high (77.5%). The number of young people who efficiently use water is also high with (75%).

Satisfaction of young people about green consumption

Table 4 Young people’s satisfaction and trend of green consumption in future

Explanation	Quantity (n= 120)	Proportion (%)
Satisfaction level when using green products		
Satisfied	70	58,3
Normal	24	20,0
Not satisfied	26	21,7
Green consumption trend in future		
Continuing to use green products	88	73,3
Considering to use	20	16,7
No use	12	10,0

*Source: Author’s own calculations from survey data in 2022

The surveyed results in Table 4 show that 58.3% of young people said they were satisfied with the green products they have been using, and nearly 22% felt unsatisfied after using the products. However, when being asked about the future trend of green consumption, 73.3% of young people said they will consume green products because they know that green consumption will contribute to environmental protection and natural resources for future generations. There are 10% of people who answered that they do not consume green products. They are people who do not have a clear understanding of green consumption and are less able to pay for these products because their income is relatively low.

> *“I have been buying and using green products for quite some time. I buy reputable and branded products. Moreover, those products bring a sense of security when using, so I feel secure and satisfied when using them. Especially after the Covid 19 pandemic, I clearly see the role and responsibility of consumers in protecting the living environment and health. I will continue to consume green products in the future (Interviewing of a 25-year-old female office staff).*

Factors affecting green consumption of young people in Hanoi

- Income

Income is one of the prerequisites that affects the way a person consumes. The higher the income, the greater the spending power. Moreover, the higher the income, the more consumers tend to spend on good quality products that are safe for health and the environment. Especially for young people with high income, green consumption is considered a modern trend.

The income of young people in Hanoi is divided into 4 groups: under 3 million VND/month; 3 to 5 million VND/month; 5 to 7 million VND/month and over 7 million VND/month. The statistics in Figure 3 show that the percentage of young people with incomes between 5 and 7 million is the highest with 33%, the percentage of young people with incomes over 7 million is the lowest with 17%. This can be explained by the fact that young people living and working in the Hanoi have a common age of 23 years and older, they are fresh graduates, so the average salary is usually in the range of 5 up to 7 million VND/month.

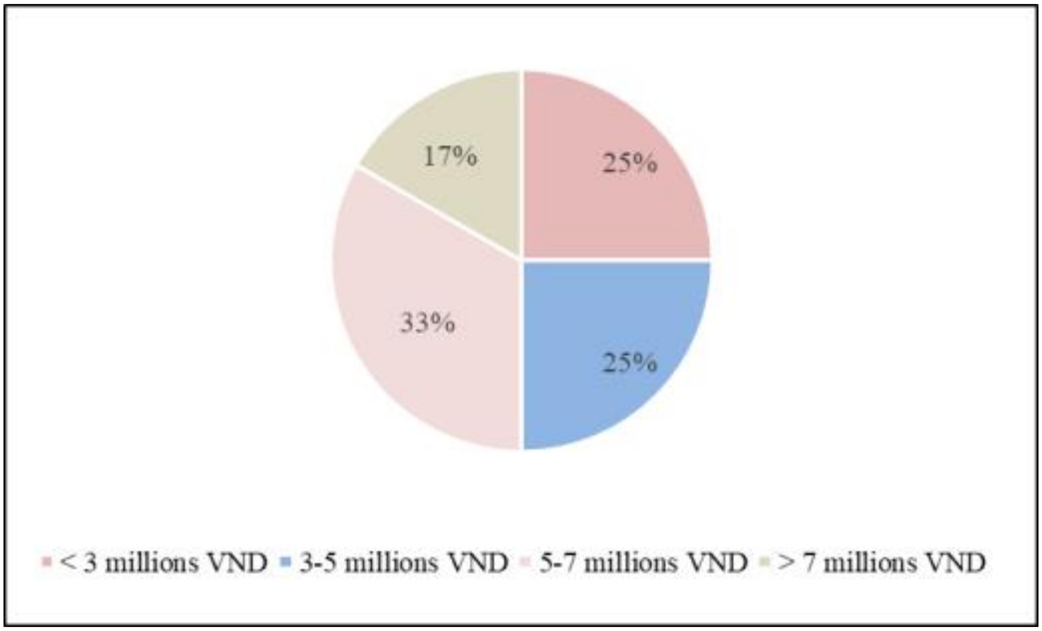

Figure 3. Income distribution of young people in Hanoi
*Source: Author’s own calculations from survey data in 2022

To analyze the influence of income on green consumption, the study collects information of young people about the percentage of spending on green consumption compared to their total spending in one month. The results shown in Table 5 that 75% of young people with incomes below 3 million/month spend on green products less than 20% of total spending. In contrast, 15% of young people with incomes over 7 million/month spend on green products, accounting for more than 15% of total spending. In other words, the percentage of spending on green products is higher the higher the income of young people. This difference is statistically significant across income groups.

> *My husband and I both do business, with a total income of more than 20 millions VND/month. We have a kid, so the need to consume safe and friendly products is always a priority for us. Most of the products in my family are "green" products such as: bamboo straws, bamboo water bottles, etc. I am willing to pay higher prices for products labeled with green standards (Interview with male business owner, 29 years old)*

Table 5 The relationship between income and spending on green consumption

Index	<20% of personal spending (%)	20% to 40% of personal spending (%)	>40% of personal spending (%)
< 3 million VND	75,0	20,8	4,2
3-5 million VND	70,8	23,3	5,8
5-7 million VND	58,3	33,3	8,3
> 7 million VND	50,0	35,0	15,0
Mann Whitney U-test	1.809**	1.419**	0.971*

*Source: Author’s own calculations from survey data in 2022.
*** Confidence 99%; ** Confidence 95%; * Confidence 90%;

- Young people occupation

Occupation and education level are decisive factors for income, so occupation is also a decisive factor in choosing to consume green products. Young people’s occupation influences their buying behavior. Young people with different occupations will have different demand for green products.

To analyze the influence of occupation on young people's green consumption, the study asked the question "if young people are willing to buy green products or are still considering to buy green products". The survey results in Figure 4 show that 75% of students still consider whether to buy green products. In contrast, official staffs often obtain a higher income, so 63% of them answered that they are willing to buy green products. Similarly, 58% of business owners confirm they are ready to shop for green products.

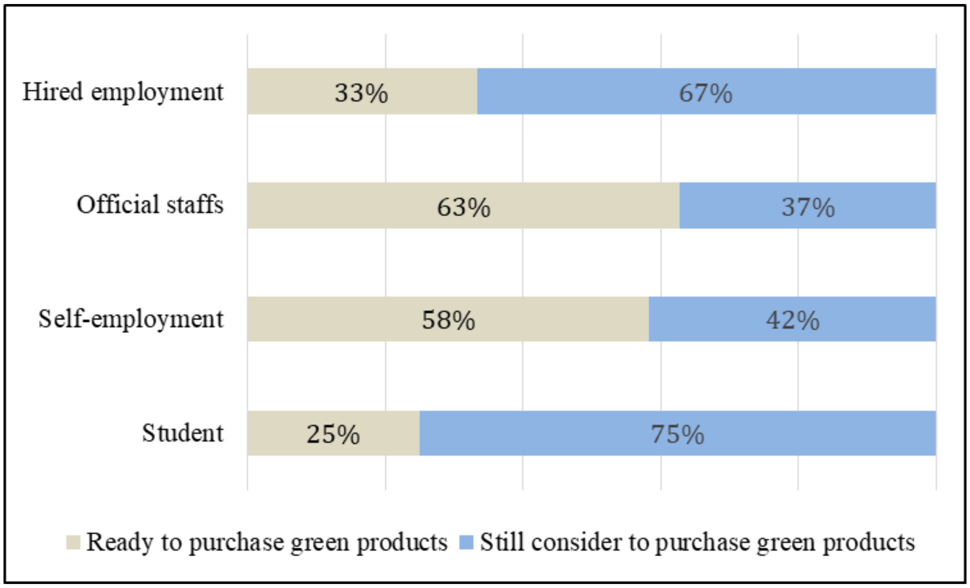

Figure 4 Young people occupation and their willingness to buy green products

*Source: Author’s own calculations from survey data in 2022

- **Gender of young people**

Gender plays a certain role in shaping each person's behavior. Different genders will have different preferences and lifestyles. The statistics in Table 6 show that 55.6% of male consumers say that they are interested in green products, 23.6% think that environmentally friendly products are safe for them normal and up to 20.8% of men are not interested in environmentally products. Meanwhile, 64.6% of women answered that they were interested in consuming green products and just over 10% of young women were not interested in green products.

To see more clearly the relationship between gender and green consumption behavior of young people in Hanoi, the study asks if young people are willing to spend more on green products. As a result, 47.2% of men said they were willing to pay more for safe products, while more than 60% of young women confirmed they were willing to spend more on green products.

Table 6. The influence of gender on green consumption of young people

Index	Men		Women		Mann Whitney U-test
	Quantity (n= 72)	Proportion (%)	Quantity (n=48)	Proportion (%)	
Level of interest in green products					
Do not interest	15	20.8	5	10.4	0.045**
Normal	17	23.6	12	25.0	0.087*
Interest	40	55.6	31	64.6	0.981
The willingness to spend more on green products					
Willingness to spend more	34	47.2	29	60.4	1.121*
Still considering	23	31.9	14	29.2	2.718***
Buy other products with the same use but not green products	15	20.8	5	10.4	0.039

*Source: Author’s own calculations from survey data in 2022
*** Confidence 99%; ** Confidence 95%; * Confidence 90%;

- **The price of green products**

The price of green products is also interested by many young people. Up to 80% of young people answered that they often look at prices to make purchasing decisions. When the prices are much higher than their spending power, young consumers tend not to shop or switch to other types of products instead. On the contrary, there are also young people who think that high product prices mean that product quality is guaranteed, so consumers are still willing to pay for green products with good quality.

Conclusion

Green products are now commonly sold at traditional markets, supermarkets, and convenience stores in Hanoi. In general, the majority of young people have knowledge about green products and intend to consume green. However, the rate of hearing and learning about green products is still relatively low.

Regarding the status of green consumption, it can be seen that the frequency of using plastic bags and disposable plastic items of young people is still quite high. In other words, the use of single-use plastic bags has become a habit of young people in Hanoi. Despite this, a large number of young people confirm that they use energy and save electricity.

When consuming green products, 70% of young people report their satisfaction. In addition, more than 73% of young people declare that they will continue to consume green in the future.

Income factor affects the spending and frequency of using green products of the survey subjects. Besides income, there are other factors such as occupation and gender that also affect the consumption behavior of young people.

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OBSTACLES FOR SOCIETAL PARTICIPATION OF RURAL WOMEN IN DUHOK GOVERNORATE IN KURDISTAN REGION OF IRAQ AND ITS RELATION WITH SOME VARIABLES

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Abstract

Women play a vital role in economic activities. They comprise about 50% of the total global labor engaged in agriculture and food production enterprises. This study is designed to determine the economic participation by rural women in Duhok governorate in Kurdistan Region of Iraq. The number of the respondents in this study was (474) selected by area random sampling. SPSS software was used to analyze the data collected, statistics such as frequency, percentage, mean, standard deviation, correlation, and regression was used. The results shows that the perception level of respondents in economic participation is high. The obstacle facing women in agricultural extension activates, thus the biggest obstacle is "Women training need courses in agricultural fields". The social obstacle of (The prevailing social norms and traditions limit the participation of women in community work) is ranked first, the economic obstacle of (High unemployment rates) is ranked first. The results also show there is a significant relationship between some social, demographic variables and 'economic participation level such as age, number of study years, satisfaction with services in the villages, urban openness and with non-significant living conditions of the family, fathers' occupation or guardian, current job, ability to solve problems. It is recommended that the extension agencies should have a re-orientation of their Programs and activities, where women will be treated equally with their male counterparts. This will ensure more rural and agricultural development in the targeted area.

Keywords: *Rural women, participation, Obstacles, Duhok.*

Introduction

According to Harbi 1997, the people and the government are the main components of the development process of any society. This is done through efforts to achieve economic, cultural, psychosocial and social changes in order to reach of improvement and development goals in their communities.
Women play an important role in the production process through their participation in agricultural activities. Women may directly manage various agricultural activities, particularly when the agricultural aspect accounts high percentage from national income.
In developing countries, women are considered the main labor in agricultural sector. Therefore, women are the main essential variable to reach the economic growth and household well-being in the rural societies (World Bank, 2008).
Work is not only an economic needs to secure the requirements of life for women, but work is a source of self-confidence and character building and at the same time makes women more

integrated in society (CSO, 2016). Therefore, governments and NGOs have become more and focused on women, especially rural women, in conferences and research to achieve development goals recently (Sherif, 2005).

Women have considered half of the rural population and participated in many agricultural and household.it should be given more attention to women who share whole work with man in economics and social activities. This was emphasized in the World Conference on the United Nations' Decade of Women, "which is entitled" Equality, Development and Peace "in 1980 was held in New York. Two-thirds of the work was done by rural women (Adisa, 2013).

The ignorance of rural women makes them unable to keep up with the development of their society. This is due to the customs and traditions prevailing in the society and specific factors economic, social and cultural and the deterioration of the level of health. All these reasons led to the inability to face these challenges (CSO, 2016, Snoor Haydar and Cuma Akbay, 2018). Moreover, the lack of knowledge of the role of women in agricultural production leads to a failure in planning the country in general and rural areas in particular. In other words, this will affect the economy in these rural areas as a result of the lack of information on the various agricultural activities, especially with regard to the contribution of rural women in national income (Das, 1995).

Globally, rural women contribute to almost all agricultural activities both animal and plant production, especially in developing countries. In addition, women carry out various agricultural activities such as agriculture, irrigation and interest in projects such as gardening, ornamental flowers and fish farming, which are successfully managed by rural women (Jonathan, 1998)

However, most of the rural women in Duhok, in particular, are facing many problems, such as the adoption of new transferred technologies, have less opportunities to participate in intra-household, socio-economic and political decision-making processes as well as to the very limited interaction with people outside of the home, high unemployment rates, lack of material support for economic participation by the official authorities, social situation, financial resources for women's organization, education and women's economic dependency. These factors isolate women from development activities as well as from acquiring income, knowledge, skills, etc. This is due to the low knowledge and skills of rural women, which affect to women's participation in extension training activities. This is because lack of training courses in developing behavioral changes in the knowledge, skills and attitudes of rural women Therefore, the objective of this study is to determine the obstacles of societal participation of rural women to overcome these obstacles so that they can effectively participate in all activities and contribute to the process of rural development. The Main Objective of the study is to determine the obstacles of societal participation of rural women in Duhok Governorate, the specific objectives are:

1. To identify the Obstacles of societal participation by rural women in Duhok Governorate / Kurdistan region of Iraq.
2. To identify the Obstacles of social participation by rural women in Duhok Governorate / Kurdistan region of Iraq.
3. To identify the Obstacles of economic participation by rural women in Duhok Governorate / Kurdistan region of Iraq.
4. To identify the Obstacles of agricultural extension participation by rural women in Duhok Governorate / Kurdistan region of Iraq.
5. To identify the relationship between selected characteristics of rural women and the obstacles of societal participation.

Materials and methods

A total of (474) respondents were randomly selected from different rural areas of Duhok governorate in Iraq representing of total rural women who were above 18 years. The data has obtained through face-to-face interview based on a structured questionnaire which has been held from April to June 2018. Three Likert scale was used to determine the Obstacles of societal participation of respondents. The Cronbach alpha value obtained for the scales was (0.876) exceeding. The collected data was subjected to data analysis including statistics such as frequency, percentage, mean, standard deviation, and correlation, using SPSS software.

Results and discussion

1. **To identify the Obstacles of societal participation by rural women in Duhok Governorate / Kurdistan region of Iraq.**

Table (1) shows the obstacles facing the societal participation of rural women which are divided into three categories. Table (1) reveals that 52.954% 251 of the respondents have a moderate degree of obstacles facing the societal participation. The table also shows that 43.882% 208 have a high degree of obstacles facing the societal participation, and only 3.165% 15 of the respondents have a low degree of obstacles facing the societal participation.

Table (1): Distribution of respondents according to the levels of obstacles facing societal participation

Obstacles of Societal Participation	Frequency	Percentage %
(53-87) Few	15	3.165
(88-122) Moderate	251	52.954
(123 or more) Much	208	43.882
Total	474	100

$\bar{X}$=120.742 sd =19.266

2. **To identify the Obstacles of agricultural extension participation by rural women in Duhok Governorate / Kurdistan region of Iraq.**

Table (2) shows the obstacles facing agricultural extension participation are divided into three categories. Table (2) reveals that 50% 237 of the respondents have a high degree of obstacles facing the extension participation. The table also shows that 43.249% 205 have a moderate degree of obstacles facing the extension participation, and only 6.751% 32 of the respondents have a low degree of obstacles facing the extension participation.

Table (3): Distribution of respondents according to the obstacles facing agricultural extension activates

Obstacles of extension Participation	**Frequency**	**Percentage %**
(11-17) Few	32	6.751
(18-24) Moderate	205	43.249
(25 or more) Much	237	50.000
Total	474	100

$\bar{X}$=24.675 sd =4.862

According to table (3), the obstacle of "Women training need courses in agricultural fields" is reported to have the highest mean value with 2.512. This is interpreted that there is a lack of training courses about agricultural fields in the area of study. Table (3) also shows that the obstacle of "The belief that extension activities are not important in society" to have the least mean value with (2.010). This is interpreted that the respondents are aware of the importance of extension activities.

Table (3): Distribution of respondents according to the obstacles items facing agricultural extension activates.

No.	Statements	Degree of obstacles						Mean	Rank
		Big		Medium		Few or Non			
		No.	%	No	%	No	%		
1	Women need training courses in agricultural fields.	285	60.1	147	31.0	42	8.9	2.512	1
2	Lack of awareness and educational programs (civil and political culture of rural women).	233	49.2	157	33.1	84	17.7	2.314	2
3	Lack of time to participate in agricultural extension activities.	202	42.6	207	43.7	65	13.7	2.289	3
4	The low level of women's participation in development programs.	201	42.4	200	42.2	73	15.4	2.270	4.5
5	Man's dominance over the agricultural production process	203	42.8	196	41.4	75	15.8	2.270	4.5
6	Lack of knowledge of types of agricultural extension activities.	182	38.4	228	48.1	64	13.5	2.248	6
7	Lack of extension programs that meet the needs of the local community.	216	45.6	158	33.3	100	21.12	2.244	7
8	Feeling bored.	187	39.5	188	39.7	99	20.9	2.185	8
9	The weakness of agricultural extension institutions.	184	38.8	186	39.2	104	21.9	2.168	9
10	The weakness of women's ability to planning and implementation.	166	35.0	218	46.0	90	19.0	2.160	10
11	The belief that extension activities are not important in society.	146	30.8	187	39.5	141	29.7	2.010	11

3. To identify the Obstacles of social participation by rural women in Duhok Governorate / Kurdistan region of Iraq.

Table (4) shows the obstacles facing the social participation of rural women which are divided into three categories. Table (5) reveals that 74.051% 351 of the respondents have a moderate degree of obstacles facing the social participation. The table also shows that 20.042% 95 have a high degree of obstacles facing the social participation, and only 5.907% 28 of the respondents have a low degree of obstacles facing the social participation,

Table 4: Distribution of respondents according to the obstacles levels facing social participation

Obstacles of Social Participation	Frequency	Percentage %
(32-52) Few	28	5.907
(53-84) Moderate	351	74.051
(85 or more) Much	95	20.042
Total	474	100

$\bar{X}$=71.613 sd =13.439

Table (5) shows that the obstacle of (The prevailing social norms and traditions limit the participation of women in community work) is ranked first with an arithmetic mean 2.409, and that of (Commitment to career) and (Women's unwillingness) occupied the last ranks with an arithmetic mean 1.949 for both.

Table (5): Obstacles of social participation items facing rural women.

No.	Paragraphs	Degree of obstacles						Mean	Rank
		Big		Medium		Few or Non			
		No.	%	No	%	No	%		
1	The prevailing social norms and traditions limit the participation of women in community work.	241	50.8	186	39.2	47	9.9	2.409	1
2	The low education level and illiteracy among rural women	226	47.7	199	42.0	49	10.3	2.373	2
3	Customs and traditions and their impact on the role of women in the political arena.	231	48.7	167	35.2	76	16.0	2.372	3
4	The weakness of the society's attention to the training of women compared to men.	228	48.1	188	39.7	58	12.2	2.358	4
5	Most men do not prefer women to social roles.	224	47.3	195	41.1	55	11.6	2.356	5
6	Low level of social services.	210	44.3	216	45.6	48	10.1	2.341	6
7	The ineffectiveness of women's organizations.	234	49.4	167	35.2	73	15.4	2.339	7
8	Women's poverty.	221	46.6	192	40.5	61	12.9	2.337	8
9	The social upbringing of women in obedience and obedience to men's orders.	221	46.6	190	40.1	63	13.3	2.333	9
10	Customs and traditions prevent women from taking leadership positions.	240	50.6	150	31.6	84	17.7	2.329	10
11	Weak role of civil society institutions and local means.	221	46.6	186	39.2	67	14.1	2.324	11
12	Male relatives (fathers and husbands) are involved in	225	47.5	176	37.1	73	15.4	2.320	12

	women's participation in community issues.								
13	Lack of confidence and conviction in the decisions and views of women.	211	44.5	201	42.4	62	13.1	2.314	13
14	The role of women is concentrated in traditional work (procreation and family care only).	222	46.8	177	37.3	75	15.8	2.310	14
15	The decline of women's motivation towards work.	207	43.7	203	42.8	64	13.5	2.301	15
16	Income inequality between men and women.	213	44.9	184	38.8	77	16.2	2.286	16
17	Feeling tense when facing problems.	201	42.4	196	41.4	77	16.2	2.261	17
18	Limited qualifications and experience of women.	174	36.7	243	51.3	57	12.0	2.246	18
19	Objection guardian husband or father.	201	42.4	183	38.6	90	19.0	2.234	19
20	Weak control of emotions.	182	38.4	217	45.8	75	15.8	2.225	20
21	Lack of laws and legislation for women.	160	33.8	238	50.2	76	16.0	2.221	21
22	Early marriage of women.	187	39.5	200	42.2	87	18.4	2.211	22
23	Weak social responsibility.	159	33.5	251	53.0	64	13.5	2.200	23.5
24	Poor leadership skills of rural women.	183	38.6	203	42.8	88	18.6	2.200	23.5
25	Negative attitudes towards women's participation in public life.	144	30.4	271	57.2	59	12.4	2.179	25
26	Fear of large gatherings.	184	38.8	140	38.0	110	23.2	2.156	26
27	Weak women's ability to learn about their community issues.	148	31.2	237	50.0	89	18.8	2.124	27
28	Feeling frustrated.	151	31.9	197	41.6	126	26.6	2.052	28
29	Women's self-confidence is weak.	139	29.3	219	46.2	116	24.5	2.048	29
30	Lack of confidence in women themselves.	127	26.8	214	45.1	133	28.1	1.987	30
31	Women's unwillingness	101	21.3	248	52.3	125	26.4	1.949	31.5
32	Commitment to career.	137	28.9	176	37.1	161	34.0	1.949	31.5

4. To identify the Obstacles of economic participation by rural women in Duhok Governorate / Kurdistan region of Iraq.

Table (6) shows the obstacles facing economic participation of rural women are divided into three categories. Table (7) reveals that 64.979% 308 of the respondents have a high degree of the obstacles facing the economic participation. The table also shows that 30.591% 145 have a moderate degree of obstacles facing the economic participation, and only 4.430% 21 of the respondents have a low degree of facing the economic participation, Table (6): Distribution of respondents according to the obstacles levels facing economic participation

Obstacles of economic participation	Frequency	Percentage %
(10-16) Few	21	4.430
(17-23) Moderate	145	30.591
(24 -30) Much	308	64.979
Total	474	100

$\bar{X}$=24.453 sd =3.932

Table (7) shows that the obstacle of (High unemployment rates) is ranked first with an arithmetic mean 2.713 and that of (Women's economic dependency) occupied the last rank with an arithmetic mean 2.156.

Table (7): Distribution of respondents according to the obstacles items facing economic participation of rural women

No.	Paragraphs	Degree of participation						Mean	Rank
		Big		Medium		Few or Non			
		No.	%	No	%	No	%		
1	High unemployment rates.	364	76.8	84	17.7	26	5.5	2.713	1
2	Lack of material support for economic participation by the official authorities.	330	69.6	110	23.2	34	7.2	2.624	2
3	The social situation does not encourage economic participation.	265	55.9	179	37.8	30	6.3	2.495	3.5
4	Fear of losing money.	267	56.3	175	36.9	32	6.8	2.495	3.5
5	Weak financial resources for women's organizations.	279	58.9	143	30.2	52	11.0	2.479	5
6	Economic recession.	240	50.6	183	38.6	51	10.8	2.398	6
7	Lack of adequate education on the importance of economic participation.	227	47.9	208	43.9	39	8.2	2.396	7
8	Women's sense of psychological and physical instability.	225	47.5	202	42.6	47	9.9	2.375	8
9	Weak momentum of economic participation.	201	42.4	223	47.0	50	10.5	2.318	9
10	Women's economic dependency.	141	29.7	266	56.1	67	14.1	2.156	10

5. To identify the relationship between selected characteristics of rural women and their opinions about degree of importance obstacles of societal participation.

This section tests the correlation between rural women's opinions about degree of importance of obstacles in general and each of the independent variables (religion, age, number of study years, number of family members, marital status, living conditions of the family, father’s or guardian occupation, current job, ability to solve problems, satisfaction with services in the village, sources of agricultural information, urban openness, and attitude towards extension work).

Table 8: The Correlation between some personal, and socio-economic characteristics of rural women and societal participation obstacles.

No.	Variables	Person’s correlation value	Spearman’s correlation value	Sigma value	Significant
1	Religion		**0.413**	**0.00**	**
2	Age	**0.076**		**0.102**	**ns**
3	Number of study years	**-0.160**		**0.00**	**
4	Number of family members	**0.062**		**0.301**	**ns**
5	Marital status		**0.011**	**0.808**	**ns**
6	Living Conditions of the Family		**0.093-**	**0.044**	*
7	Father’s occupation or guardian		**0.045**	**0.327**	**ns**
8	Current job		**0.082**	**0.074**	**ns**
9	Ability to solve problems	**0.185**		**0.000**	**
10	Satisfaction with services in the village	**0.029**		**0.710**	**ns**
11	Sources of agricultural information	**0.262**		**0.000**	**
12	Urban openness	**0.289**		**0.000**	**
13	The attitude towards extension work	**0.065**		**0.823**	**ns**

Based on the results presented in Table (8), there is a significant correlation between each of (religion, number of study years, living conditions of the family, ability to solve problems, sources of agricultural information, urban openness), and the societal participation of respondents in the study area.

“Religion” has a positive significant relationship with obstacles of societal participation of respondents, the reasons might that the religion of rural women has a great influence on their abilities to take part in societal activities and influences benefit from different agriculture extension, social and economic activities in their local communities.

The result as that “number of study years” which has a negative correlation with obstacles of societal participation of the respondents, the reason may be that education is an important factor to improve their agriculture extension, social and economic, situation, therefore, rural women with low education are trying to compensate this point and achieve self-estimation through active participation in various societal activities in their villages.

The result indicate also that “living conditions of the family” which has a positive correlation with obstacles of societal participation of the respondents, because the greater the number of family members, the more time and space are allowed to participate in all societal activities.

Significant positive relation fond between “ability to solve problems” the obstacles of societal participation of the respondents, may be and opinion with have high ability to resolve problems they will have a positive attitude to participate in societal participation.

“Sources of agricultural information” has a positive correlation with obstacles of societal participation, this means that communication with sources of agricultural information which contributes to increasing the contribution of rural women to societal participation, and the reason may be is that more using of information sources more awareness on the importance of the participation in societal activities.
Another personal characteristic is “urban openness” which shows that there is a positive correlation with obstacles of societal participation, in order to contribute opening of knowledge horizons with neighboring villages and countries and to benefit from many experiences that contribute to raising the level of participation of rural women in societal participation as part of rural development. This implies that the more visit and media contact one have the more aware in societal about obstacles and how to deal with the activities and more participation.

Conclusions

The existence of many obstacles that hinder the societal participation of rural women ranging from medium to high.
The lack of agricultural training is the most important factor hindering the participation of rural women in agricultural extension activities
Community customs and traditions hinder women's participation in social activities
High unemployment and lack of public financial support hinder rural women's participation in economic activities
\The existence of some factors that are related to the extent of rural women's participation in community activities and may limit or increase such participation should be taken into account

Recommendations

The need to work to overcome all the obstacles that hinder the participation of rural women in societal activities by government institutions and civil society organizations.
Activate the role of agricultural extension through the establishment of training courses for rural women in various agricultural topics and encourage them to increase their participation in extension activities
The need to overcome some habits and traditions that hinder the participation of rural women in social activities and awareness in this aspect.
The need to provide job opportunities for rural women and provide loans to enable them to establish small projects and self-reliance and achieve independence.
Taking into account factors that may hinder the societal participation of rural women, especially religion, level of education, and family living status, etc.

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INFLUENCE OF THE ECONOMIC CRISIS TO THE PRODUCTION PRICE OF WINE GRAPES IN THE REPUBLIC OF NORTH MACEDONIA

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Abstract

The grape production together with the wine industry contributes about 20% of the total agricultural GDP in the Republic of North Macedonia and occupation of around 30,000 rural households. Due to the Covid-19 pandemic crisis and the instability of the world situation, the prices of raw materials in agricultural production are constantly increasing. Hence, the main goal of this paper is to assess the impact of the global economic crisis on the production price of wine grape varieties. The main approach of the comparative analysis for the production prices between the reference years 2020 & 2021 and estimated 2022 is based on established normative calculations of grapes within the Institute of Agriculture. The total costs are calculated based on variable and fixed costs of grape production. The investment and inputs costs are collected by commercial companies, suppliers of raw materials and farmers. The findings demonstrate that the average production price of grapes at total costs for 2020 & 2021 accounts in average 0.30 EUR/kg. The results from the estimations of the production price of grapes for 2022 show increase by 20%, which implies that the economic crisis has a large impact on the viticulture and wine industry. It is anticipated that the socio-economic situation of grape producers will be negatively affected. Therefore, the public policies for development of this important sector for the Macedonian agriculture should correspond to the new situation.

Keywords: *socioeconomic situation, world crisis, wine grapes, production price.*

Introduction

The alarmingly high incidence of acute food insecurity and malnutrition starkly exposes the fragility of global and local food systems. Under mounting strain from the increased frequency and severity of weather extremes, the COVID-19 pandemic, increasing conflict and insecurity and rising global food prices. The interconnectedness of drivers is further laid bare by the unfolding war in Ukraine, which not only compromises the food security of those directly affected by the war, but compounds existing challenges faced by millions of acutely food-insecure people worldwide. Some countries facing food crises are particularly vulnerable to the risks to food markets created by the war in the Black Sea area, notably due to their high dependency on imports of food, fuel and agricultural inputs and/or vulnerability to global food price shocks (GFP, 2022). The impact of the COVID-19 pandemic and the related policy measures to curb the pandemic can be assessed from various perspectives. The socio-economic effects might be the most relevant concerning the development of the agricultural sector and the livelihoods of rural households. Among these effects, income from economic activities, other income streams, functioning input and output markets, food and nutrition security, or personal health might be considered. Furthermore, there will be direct effects for the agricultural

producers and rural households and also various indirect effects. (Djanibekov and Herzfeld, 2022).
The war in Ukraine has dealt a major shock to commodity markets, altering global patterns of trade, production, and consumption in ways that will keep prices at historically high levels through the end of 2024, according to the World Bank’s latest Commodity Markets Outlook report (WB, 2022). The World Bank’s Agricultural Price Index gained 11 percent in 2022Q1 (q/q), reaching an all-time nominal high (WB, 2022).
The coronavirus pandemic and military unrest in this period has affected all sectors of the country’s economy and in the social and political sphere. Viticulture and winemaking, as strategic sectors in the Macedonian agriculture and national economy, contributing 17 – 20 % of the agricultural GDP, are not spared from these market disruptions. Vineyards has a share of about 4.6% in the total cultivated land, accounting 23,776 ha in 2021. In the last decade there has been a slight increase in the vineyards area, with a rise of 14% in 2021 compared to the area in 2012 (SSO, 2022). The production of grapes, for the same period, in average amounts to 270,700 tons. In parallel with the increase of the vineyard’s areas, grape production also had a slight upward trend with a 12% increase, hence from 240,461 tons in 2012 to 269,131 tons in 2021. The average grape yields are around 10 tons/ha. Viticulture is most prevalent in the Vardar and Southeast regions of Macedonia (SSO, 2022). Grape growing includes about 30,000 economic entities, out of which about 70% are individual holdings and 30% are agricultural companies. The share of wine grape varieties in the total vineyards area is 70%, out of which 40% are white and 60% are colored varieties. The most frequent wine varieties are Vranec and Kratoshija, which have a longer tradition of growing, and the most frequent white varieties, are: Smedervka, Zhilavka, Chardonnay, Riesling, Sauvignon Blanc etc (MAFWE, 2021). The focus of this study is the production price of the Vranec wine variety, which is represented by 50% of the total vineyards with colored grape varieties, posing base for most of the red wines produced in Macedonia.

Materials and methods

Cost of production (CoP) as an economic indicator represents the average cost of production per one unit of a grape (kg product) and is the minimum selling price of the grape (break-even price) as a break-even point in order to work without losses. The break-even price is the price needed to cover all costs of grape production. The cost of grape production is calculated based on standard definitions and methodology used in different relevant literature (for example, see Ciaian et al., 2013; Kay et al., 2014; FAO, 2016), also customarily applied in the local context (Milanov and Martinovska, 2002; Martinovska *et al.*, 2009).
CoP = TC / Y
CoP - Cost of production (in EUR/kg)[5]; TC - Total cost (in EUR); Y - Yield (quantity of produced grape in kg)
The basis for calculation of CoP are the total costs of production calculated based on the used inputs and resources for producing the grape in typical (normative/normal) production year and market values of the inputs and resources based on their real yearly market value in 2020/2021

[5] All values are presented in EUR. The average exchange rate for 2020/2021 is calculated as average yearly exchange rates in 2020 and 2021 (61.651 MKD/EUR) and average daily exchange rates in 2022 from 1st of January to 15 of June 2022 (61.677 MKD/EUR).

and 2022.[6] The yields are normatively fixed on average yearly expected yields of 15,000 kg grape per hectare. The total costs are calculated based on variable and fixed costs of grape production. The variable yearly costs are calculated based on the direct costs used for materials (fertilizer, pesticides) and resources consumed, irrigation and machinery costs for soil cultivation including the labor for vineyard maintains.

TC = VC + FC

VC - Variable cost (in EUR); FC - Fixed cost (in EUR)

Additionally, the analysis took in consideration the value of fixed costs of production, calculating the costs of depreciation of the assets used for grape production. The fixed costs are calculated based on the value of establishment (cost of investment) the vineyard plantation and value of typical assets (machinery and equipment) used for producing grape on 1 (one) hectare in 2020/2021 and 2022.

D = VA x DR

D – Annual depreciation (in EUR); VA – Value of the asset (in EUR); DR – Depreciation rate (in %, DR = 1 ÷ Years of assets utilization)

Results and discussion

It is evident that the world economic crisis as a result of the combined impact of Covid-19 pandemic and Ukraine war crises has huge impact on increased investment cost for vineyard plantation establishment.

Table 7. Investment in 1 ha vineyard establishment 2020/2021 and 2022 (in EUR)

		Unit measure	Quantity	Price		Total		Change (%)
				2020/21	2022	2020/21	2022	
1	Planting material	seedlings	4,000	0.65	0.81	2,595	3,243	25%
2	Deep ploughing	ha	1	583.93	642.05	584	642	10%
3	Soil cultivation before planting	ha	1	486.61	535.04	487	535	10%
4	Pillars	number	800	1.95	4.86	1,557	3,891	150%
5	Setting pillars	number	800	0.24	0.24	195	195	0%
6	Wire	kg	400	0.97	1.62	389	649	67%
7	Setting wire	kg	400	0.08	0.08	32	32	0%
8	Drip by drip irrigation system	total	1	811.02	1.134.94	811	1,135	40%
9	Planting	days	25	19.46	19.46	487	486	0%
10	Basic fertilization	bags	10	21.09	24.32	211	243	15%
11	Ploughing	number	4	81.10	97.28	324	389	20%
12	Plant protection	number	5	97.32	113.49	487	567	17%
13	Irrigation	total	1	291.97	291.84	292	292	0%
14	Cultivation/digging	number	4	194.64	194.56	779	778	0%
	Total costs 1 year					**9,229**	**13,078**	**42%**
1	Ploughing	number	4	81.10	97.28	324	389	20%
2	Plant protection	number	6	97.32	113.49	584	681	17%
3	Irrigation	ha	1	291.97	291.84	292	292	0%
4	Cultivation/digging	number	4	194.64	194.56	779	778	0%
5	Plantation forming (pruning, etc.)	days	25	19.46	19.46	487	486	0%
	Total costs 2 year					**2,465**	**2,627**	**7%**
1	Soil cultivation	number	4	81.10	97.28	324	389	20%

[6] Difference of market prices in 2020 and 2021 is minor. Modest increase in the value of the fertilizers can be noticed, which does not have huge impact on costs of production and unit price.

2	Plant protection	number	6	97.32	113.49	584	681	17%
3	Fertilization	bags	10	21.09	24.32	211	243	15%
4	Protection/Fertilizer application	number	2	97.32	113.49	195	227	17%
5	Irrigation	ha	1	291.97	291.84	292	292	0%
6	Tying and tangle green shoots	days	10	19.46	19.46	195	195	0%
7	Material for tying	total	1	113.54	113.49	114	113	0%
8	Winter pruning	days	6	19.46	19.46	117	117	0%
9	Pruning and tying green shoots	days	15	19.46	19.46	292	292	0%
10	Removing green shoots	days	2	19.46	19.46	39	39	0%
11	Harvest	days	20	19.46	19.46	389	389	0%
12	Transport	kg	7,500	0.02	0.02	122	182	50%
13	Other variable costs	total	4	19.46	19.46	78	78	0%
	Total costs 3 year					**2,873**	**3,159**	**10%**

Table 8. Value of 1 ha vineyard plantation establishment 2020/2021 and 2022 (in EUR)

Total investment costs (∑ investment costs 1, 2 and 3 year) in EUR	14,568	18,864	29%
Income (yield 3 year, average 7.500 kg/ha) in EUR	2,366	2,638	12%
Total vineyard establishment value (Total investment costs) in EUR	**12,202**	**16,225**	**33%**

The value of establishment of 1 ha vineyard has increased by 33% in 2022, amounting at 16,225 EUR per hectare comparing to the 12,202 EUR in the period 2020/2021.

Main increase of investment costs of 42% can be noticed in the first year of vineyard establishment, amounting at 13,078 EUR/ha in 2022, compared with only 9,229 EUR/ha in 2020/2021. The main impact on investment costs raise is the price increase of the pillars (150%) and wire (67%). Additionally, the rise of the price of drip-by-drip system (40%), seedlings (25%), protection materials (17%), fertilizers (15%) and fuel affecting the cost of land cultivation significantly contribute towards the increase of investment costs. In the second year of establishment the investment cost is expected to increase for 7% (from 2,465 EUR calculated based on the prices in 2020/2021 to 2,627 EUR based on prices in 2022) and 10% in the third year (from 2,873 EUR to 3,159 EUR), calculated based on the hypothesis that the present prices in 2022 will have the same range in next years.

Table 9. Average (normal year) annual cost of grape production 2020/2021 and 2022 (in EUR)

		Unit measure	**Quantity**	**Price**		**Total**		**Change (%)**
				2020/21	**2022**	**2020/21**	**2022**	
1	Ploughing	number	4	81.10	97.28	324	389	20%
2	Plant protection	number	6	97.32	113.49	584	681	17%
3	Fertilization	bags	10	21.09	24.32	211	243	15%
4	Protection/Fertilizer application	number	2	81.10	97.28	162	195	20%
5	Irrigation	ha	1	291.97	291.84	292	292	0%
6	Tying and tangle green shoots	days	10	19.46	19.46	195	195	0%
7	Material for tying	total	1	113.54	113.49	114	113	0%
8	Winter pruning	days	6	19.46	19.46	117	117	0%
9	Pruning and tying green shoots	days	15	19.46	19.46	292	292	0%
10	Removing green shoots	days	2	19.46	19.46	39	39	0%
11	Harvest	days	20	19.46	19.46	389	389	0%
12	Transport	kg	15,000	0.02	0.02	243	365	50%
13	Other variable costs	total	4	19.46	19.46	78	78	0%
	Total variable costs					**2,962**	**3.309**	**12%**
	Yearly depreciation (fixed costs)					**1,534**	**2.088**	**36%**
Total costs in normal year						**4.496**	**5,398**	**20%**
Grape yields		kg	15,000					

CoP (at variable costs)	**EUR/kg**				**0.20**	**0.22**	**12%**
CoP (at fixed costs)	**EUR/kg**				**0.10**	**0.14**	**36%**
CoP (at total costs)	**EUR/kg**				**0.30**	**0.36**	**20%**

The increase vineyard plantation establishment investment and value of vineyard means additional 4,023 EUR fixed costs as a basis for annual depreciation of the vineyard value as an asset which causes annual rise of this costs of 33% or additional 268 EUR comparing annual costs of 819 EUR in 2022/2021 and 1,087 EUR in 2022. In addition, the prices of the basic assets used for grape production (tractor, atomizer, milling machine, trailers, pumps and filters, other small equipment) has increased in average 40%, which results with increasing of the total annual depreciation and fixed costs for 36% or additional 554 EUR per hectare (1,534 rise to 2,088 EUR).

Beside the increase of fixed costs, the annual variable costs of production have increased for 12% in 2022 with increase of additional 347 EUR per hectare (from 2,296 in 2020/2021 to 3,309 EUR in 2022), primarily as result of the increase of fuel price which results with increase of transport costs (50%), ploughing (20%) and protection and fertilization application (20%), but also as result of increased prices of protection materials (17%) and fertilizes (15%). This increases the grape production price calculated based only on variable costs from 0,20 EUR/kg in 2020/2021 to 0.22 EUR/kg in 2022 or additional 0.02 EUR per kilogram grape. At this stage, there is still no evidence of increasing the labor costs. As a result of the economic crisis inflation and impact on increased prices, the total costs of grape production have increased for 20% in 2022 with increase of additional 901 EUR per hectare (from 4,496 EUR in 2020/2021 to 5,398 EUR in 2022). This increases the full grape production price calculated based on total costs from 0.30 EUR/kg in 2020/2021 to 0.36 EUR/kg in 2022 or additional 0.06 EUR per kilogram grape.

The Government has subsidies for the wine grape production in 2020 with 0.03 EUR/kg for the grape sold to the wineries. In 2021, these subsidies increased to 0.05 EUR/kg. Additionally, there are subsidies for producing grape paid per hectare ranging from 649 up to 778 EUR/ha or in average 0.04 and 0.05 EUR/kg calculated based on average 15,000 kg yield. In total, the subsides paid by the Government can be estimated in average to be maximum 0.10 EUR per kilogram of grape, which added to the average buyout price of wine grape of 0.21 EUR/kg increase the average grape price up to 0.31 EUR/kg. It can be noticed that this was sufficient to cover full production costs of grape in 2020/2021 of 0.30 EUR/kg, but it is insufficient to cover the increased production costs and full production price of grape in 2022 of 0.36 EUR/kg. The Government did not plan any additional supporting measures and subsidies for the grape in the new programme for Intervention fund and Programme for agriculture in 2022, which are primary planned for the subsidizing for fertilizers used in production of wheat, barley, sunflower, maize and livestock production (lamb, pigs and laying hens).

Conclusion

It is evident that the world economic crisis and inflation as a result of the combined impact of Covid-19 pandemic and Ukraine war crises has a huge impact on increasing the grape cost of production in North Macedonia. Having in mind that the average buyout price of the wine grape is only 0.21 EUR/kg, which is significantly below the calculated full grape production price of 0.30 EUR/kg before the crises, it is expected that the grape producers will face even higher negative impact with the new calculated and expected production price in 2022 of 0.36 EUR/kg. We can anticipate that some increase in grape buyout price will occur in 2022 as a result of the global trend of inflation. Nevertheless, if the trend of grape buyout prices stays the same as before the economic crisis, the average buyout price of the wine grape of 0.21 EUR/kg will not be sufficient to cover even the variable cost of grape production. This will have a huge negative impact on the wine grape producer's households, but at the same time it'll also have a negative impact on the wine industry in North Macedonia. Although the wine and wine grape are not the most crucial products in the period of world economic and food crisis and can be perceived as luxury, still having in mind that this sector contributes with about 20% of the total agricultural GDP and more or less enables income and contributes to existence of around 30,000 rural entities in the Republic of North Macedonia, it is highly recommended that the Ministry of Agriculture, Forestry and Water Economy (MAFWE) and the Government should take in consideration the negative impact of the world economic crisis, analyze, review and plan future strategic sector development steps and take in consideration additional measures of support the wine and wine grape supply chain.

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PREDICTION OF SUNFLOWER PRODUCTION IN THE REPUBLIC OF SERBIA

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Abstract

The paper, using a quantitative research method, aimed to create an adequate model for predicting the production parameters of sunflower in the Republic of Serbia in the next three years. The selected trend model was used for that, and the analyzed seventeen-year series of data ranged from 2005 to 2021. The results of research obtained using three measures of accuracy of trend selection (*Mean Absolute Percentage Error, Mean Absolute Deviation, Mean Squared Deviation*) show that all three observed parameters of sunflower have a positive tendency to grow with greater oscillations in production. The results of the research can serve the purpose of making rational strategic decisions in the future when it comes to the production of this oilseed.

Keywords: *Sunflower, Prediction, Trend, Republic of Serbia.*

Introduction

Sunflower (Helianthus annuus L.) is one of the most important oilseeds grown Republic of Serbia. It is one of the five main oilseeds in the world, along with soybeans and oilseed rape (Šimić et al., 2008; Seiler et al., 2017). Sunflower is of great economic importance due to the high content of oil in the seeds, and vegetable oils in the core, which are as much as 60%. Sunflower oil is of very high quality, and has a high energy and nutritional value. Sunflower seeds contain on average 43% oil, 18% protein, 26% cellulose, and 3% minerals (Vratarić et al., 2004). All production characteristics of sunflower as well as its possibility of multipurpose use give immeasurable economic significance, and its production stands out as an important segment of agricultural production of a country. In their earlier works, some of the authors primarily dealt with the economic analysis and profitability of growing oilseeds, including sunflower (Knežević and Popović, 2011; Popović et al., 2016; Božić and Nikolić, 2016; Matkovski et al., 2020 etc.). However, as Mutavdžić (2010) concludes in the market economy, successful production, in addition to monitoring and analysis, also depends on the prediction of results and the most important factors that affect it. Given the previously mentioned, the aim of this paper is to create an adequate model for predicting sunflower, i.e. its production indicators.

Material and methods

Descriptive measures of statistics (average, interval of variation, coefficient of variation and rate of change) were used for data processing, while trend analysis related to the seventeen-year-long data series (2005-2021) obtained by looking at the available SO RS data, was used for forecasting. We obtain trend models based on the following expressions:

a) Linear trend: $Yt = \beta_0 + \beta_1 t + e_t$

b) Square trend: $Yt = \beta_0 + \beta_1 \times t + \beta_2 t^2 + e_t$
c) Exponential trend: $Yt = \beta_0 \times \beta^t{}_1 \times e_t$

According to some authors (Markidakis and Hibon, 2000; Goodvin and Lawton, 1999), three measures of accuracy were used to select an adequate prediction model, namely: mean absolute percentage error (MAPE), mean absolute deviation (MAD), and mean square deviation (MSD).
MAPE (*Mean Absolute Percentage Error*) is a prediction method used in time series where periodicity is especially observed and is obtained on the basis of the following statement:

$$\mathrm{MAPE} = \frac{1}{n}\sum \mathrm{I}(\mathrm{yt} - \hat{\mathrm{y}}\mathrm{t})/\mathrm{yt}\,\mathrm{I}100$$

MAD (*Mean Absolute Deviation*) is a dispersion method that is created as a deviation of the modality from the representative parameter and is obtained as follows:

$$\mathrm{MAD} = \frac{1}{n}\sum \mathrm{Iyt} - \hat{\mathrm{y}}\mathrm{tI}$$

MSD (*Mean Squared Deviation*) is the standard deviation that represents the mathematical expectation of how well the arithmetic mean represents the results obtained based on the following expression:

$$\mathrm{MSD} = \frac{1}{n}\sum (\mathrm{yt} - \hat{\mathrm{y}}\mathrm{t})^2$$

Karim et al. (2010) believe that the lowest value of the mentioned measures of accuracy is a correct indicator of the choice of model with minimal errors in prediction.

Results and Discussion

According to FAOSTAT (https://www.fao.org/faostat), the largest producer of sunflower in the world is Russia with a production of 13,314,418 tons in 2020, which was 26.5% of the total world production that year. It is immediately followed by Ukraine with 13,110,430 tons, while the other large producers are Argentina, China, Romania, Turkey, but with far smaller quantities of this oilseed produced. According to the same source, the largest world export in 2020 was realized by Romania with the amount of 1,482,504, while the largest importer was realized by Turkey with 1,206,590 tons.
Regarding the production of sunflower in Serbia, it was on average at the level of 492,142 tons in the observed period, on an average area of over 191,500 ha. Compared to the other two production indicators, sunflower production showed greater variation in the analyzed period measured by the obtained coefficient of variation (cv = 27.3%), but also a higher growth trend (r = 3.49%). The largest produced quantities were realized in 2018 (733,706 t), when the largest areas of sunflower were harvested, while the highest yield was recorded a year later (3.30 t / ha). (Table 1)

Table 1. Dynamics of sunflower production in the Republic of Serbia (2005-2021)

	Average	Interval of variation		Coefficient of variation (%)	Rate of change (%)
		Min.	Max.		
Surface area (ha)	191.507	154.793	239.148	12,76	0,45
Production (t)	492.142	294.502	733.706	27,309	3,49
Yield (t/ha)	2,55	1,80	3,30	17,61	3,03

Source: Auhtors̀ calculation based on data of the Statistical Office of the Republic of Serbia

When analyzing the measures of accuracy of the observed trend models, we notice that the square trend model has the lowest values, and we take it to predict sunflower production in the next three years. (Table 2) A visual representation of the trends of the projected areas under sunflower can be seen in Figure 1, while the actual values of the projected areas can be seen in Table 3.

Table 2. Accuracy measures

	MAPE	**MAD**	**MSD**
Linear	8	14602	310610912
Square	7	12549	226160831
Expon.	8	14253	301738944

Source: Authors̀ calculation

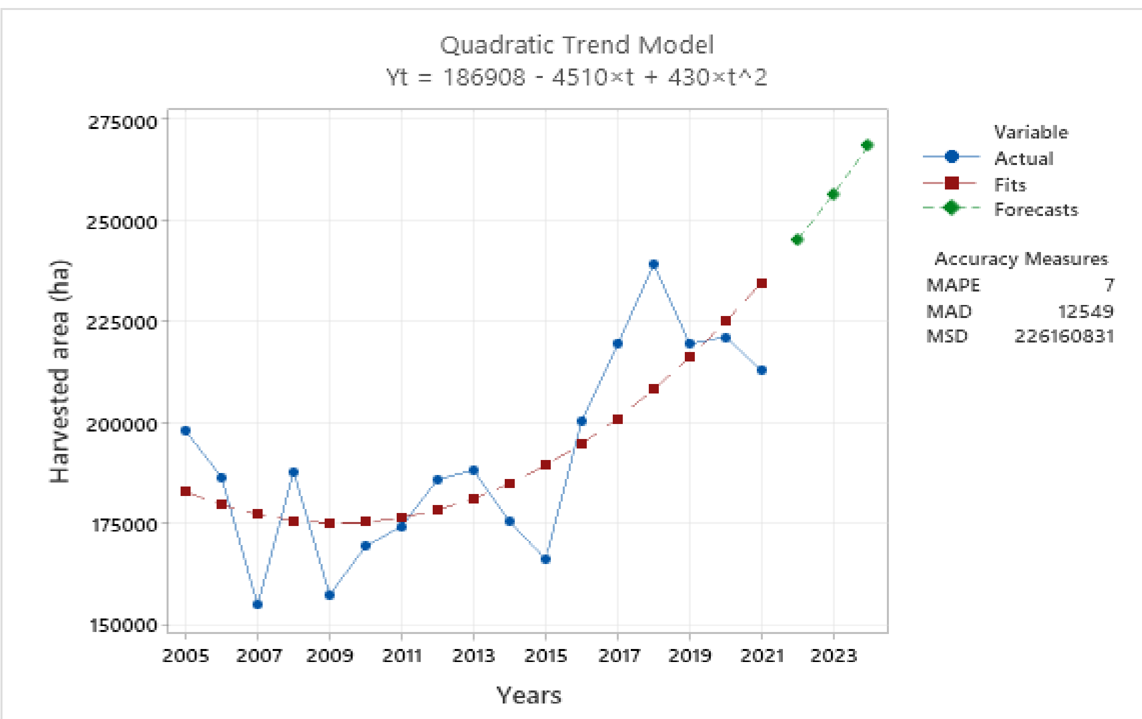

Figure 1. Sunflower surface trends

Table 3. Projected area under sunflower

Years	2022	2023	2024
Surface area	245.166	256.579	268.852

Source: Authors' calculation

As with surfaces, so with production we take the square trend as a more adequate trend model. (Table 4). The visualization of production trends is presented in Graph 2 below, and the realized predictive values of production are visible in Table 5.

Table 4. Accuracy measures

	MAPE	**MAD**	**MSD**
Linear	12	56390	4383046471
Square	11	54367	4178993362
Expon.	11	54550	4249784439

Source: Authors' calculation

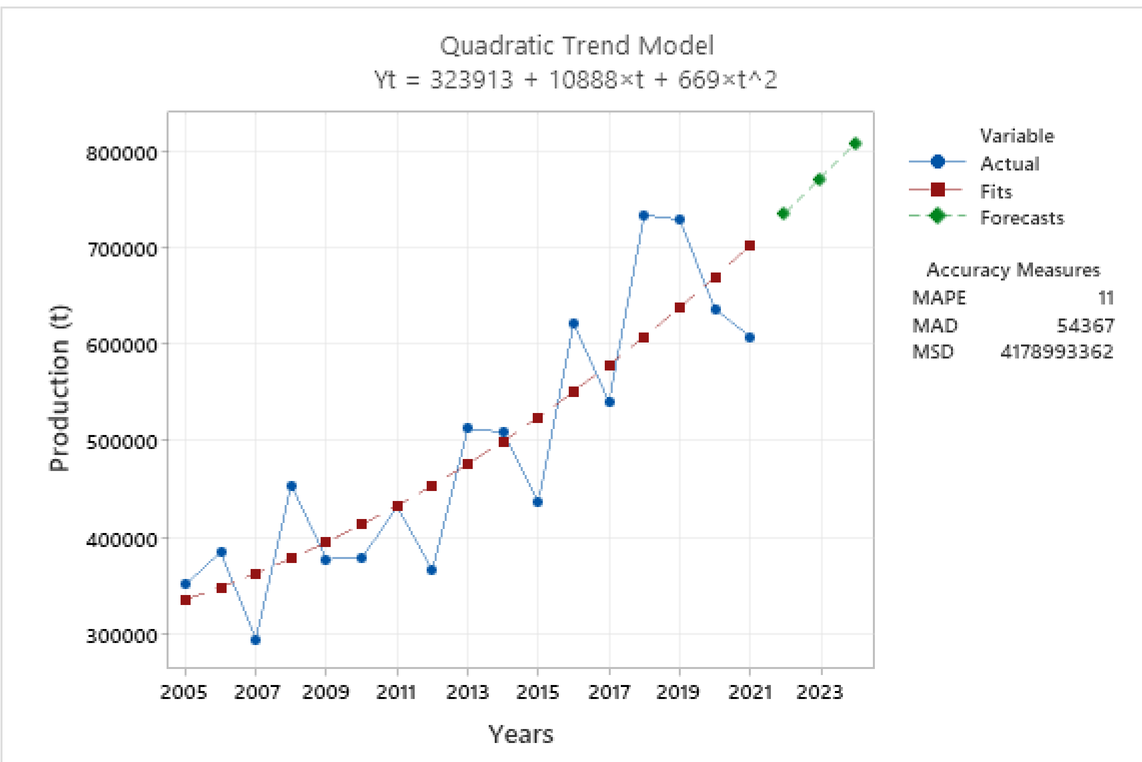

Figure 2. Trends of sunflower production

Table 5. Predicted sunflower production

Years	2022	2023	2024
Production	736.632	772.270	809.247

Source: Authors' calculation

The analyzed measures of accuracy indicate that in terms of yield, the choice of model type is the same as in the previous two cases. (Table 6). The trends and growth of sunflower yield in the analyzed and prediction period is visible in Graph 3, while the following table 7 gives the predicted values of this parameter.

Table 6. Accuracy measures

	MAPE	**MAD**	**MSD**
Linear	8,48101	0,21185	0,05543
Square	7,97622	0,19846	0,05316
Expon.	8,74162	0,21994	0,65861

Source: Authors' calculation

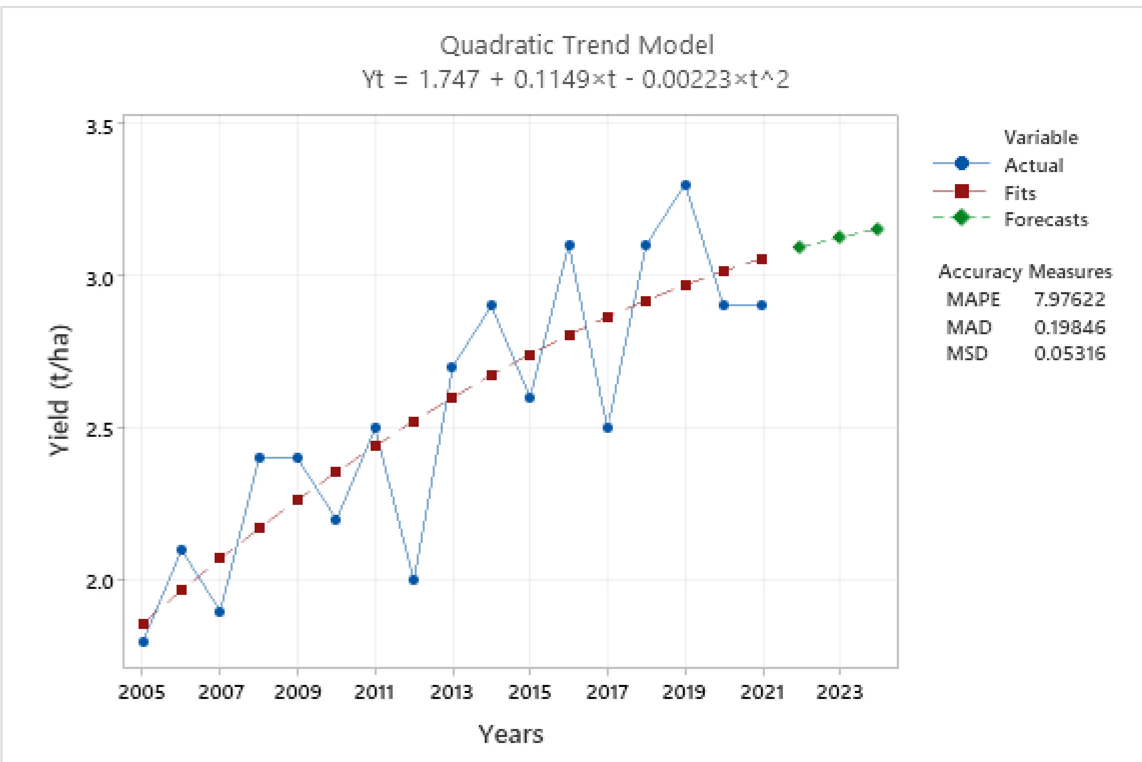

Figure 3. Trends of sunflower yield

Table 7. Predicted sunflower yield

Years	2022	2023	2024
Yield	3,09	3,12	3,15

Source: Authors' calculation

Conclusion

Sunflower is one of the main industrial plants in Serbia with great economic and nutritional significance. In the observed period, slightly over 492,000 tons of sunflower were produced on over 191500 hectares. A slight growth trend of the analyzed indicators of this oilseed crop was observed, with a relatively large variation in production in the seventeen-year-long period.
Based on the used measures of accuracy in obtaining an adequate trend model for forecasting, it was noticed that the observed parameters are constantly increasing in the next three - year period. Namely, according to the obtained results, it can be expected production of 809,247 tons in the last year of prediction (2024) on an area of 268,852 hectares. The projected values of production will be higher by as much as two thirds of the realized average values of production in the previously observed period. In addition, a slight increase in yield is expected, which would amount to 3.15t / ha in 2024.

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FORECASTING TOMATO PRICES ON MARKETS IN THE REPUBLIC OF SERBIA USING THE ARIMA MODEL

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Abstract

Vegetable production is a highly intensive and profitable branch of agriculture, which can have a significant effect on the development of the agricultural sector. In the Republic of Serbia, tomatoes are grown in open fields and in different forms of protected areas (plastic and glass greenhouses). Tomatoes are grown worldwide on over 5,000,000 ha, and in the Republic of Serbia, according to the data retrieved from the National Statistical Office (RZS), tomatoes are grown on about 8.000 ha, being the most commonly grown vegetable crop (together with potatoes and peppers), with an average yield of almost 15 t/ha and total production of 111,639 metric tons (FAOSTAT). Tomato prices differ over quarters in a year, and those discrepancies are not small. Tomato prices are therefore twice/twice and a half times lower in summer than in winter, primarily due to the way of production, based on which it can be concluded that the production season significantly affects the price of this agricultural commodity. Apart from the way and technology of production, the price is greatly affected by the market, and in this case by the import from the neighboring countries. The goal of this paper is the tomato prices' forecast by quarters of 2021, based on the prices from the markets in the Republic of Serbia in the period 2014-2020, recorded and kept within the Market Information System of Serbian Agriculture (STIPS), and by using the ARIMA modelling. Moreover, this analyzes can be used to project the prices for other agricultural commodities and such analyzes can help greatly in current global crisis caused not only by the COVID-19 pandemic, but also by the current war situation in Ukraine.

Keywords: *ARIMA, price, tomato, market, Serbia.*

Introduction

Vegetable production is carried out in almost all regions of the Republic of Serbia, with various crops grown. It is a very intensive and profitable branch of agricultural production (Novković et al., 2012). As such, it can have a significant impact on the development of the agricultural sector, but this production also depends significantly on the level of overall economic development (Paunović, 2016). The tomato (Solanum lycopersicum L.) is one of the most common vegetable crop on the global level, being also one of the most profitable one (Cvijanović et al., 2021). The production of tomatoes, along with peppers is one of the most profitable vegetable productions, of those most represented in the Republic of Serbia (Petrović et al., 2021). Concerning the protected areas in Serbia, , tomatoes occupy the largest area in the structure of production, with a share of over 70% (Zdravković et al., 2012), which has a positive effect on the increase in yields and overall production. The paper used the time series analysis, more precisely ARIMA modeling, in order to forecast the prices for the quarters in 2021, and based on the data of the tomato quarterly prices on the markets in the Republic of Serbia for the 2014-2020 period. The time series means an ordered sequence of observations. Editing is usually done in relation to time in equal time intervals. Forecasting time series, i.e. determining the future course, is one of the most important, if not the most important, goal of time series analysis (Lipovina-Božović, 2014.). Since most of our time series are non-stationary, the model that best describes them is the ARIMA model (which reduces the series to a stationary one by differentiating) which, combined with the arithmetic mean and expert correction, gives the best "recipe" for a quality forecast (Korović, 2015).

Materials and methods

For the purposes of this paper, data on tomato prices on markets in the Republic of Serbia collected through the Market Information System of Serbian Agriculture (STIPS) were analyzed. Prices are collected on a weekly basis, which are used to calculate prices on a monthly and annual basis. Based on the monthly tomato prices on the markets in the Republic of Serbia, the average prices at the quarter level were calculated. The data were processed in a statistical program “SPSS Statistics“.

The main goals in time series analysis are to describe, explain and predict - forecast the time series. In the process of forecasting, the authors had a time series for which data are known up to the moment h, X=(X1,....,Xh), and it is necessary to determine the future values for ℓ steps forward, i.e. Xh+ℓ where ℓ≥1. Value ℓ is called a forecast horizon (Ilić et al., 2014). For monthly time series it is good to forecast three steps ahead (next three months), for quarterly times series to forecast one (or two) quarters, and for daily time series to forecast for longer periods (days, even months), but it is questionable how precise these forecasts are. Beyond the aforementioned limitations, these would not be forecasts but projections (Dabetić, 2016). Projections require further considerations and some additional factors and research (Joksimović et al., 2020). Seasonal ARIMA models, like non-seasonal ones, take into consideration interdependence of consecutive observation of a time series, e.g. interdependence of observations of consecutive months (quarters) within a year. However, unlike non-seasonal time series, seasonal ones at the same time take into consideration interdependence of observations of the same months (quarters) in consecutive years (Mutavdžić et al., 2014).

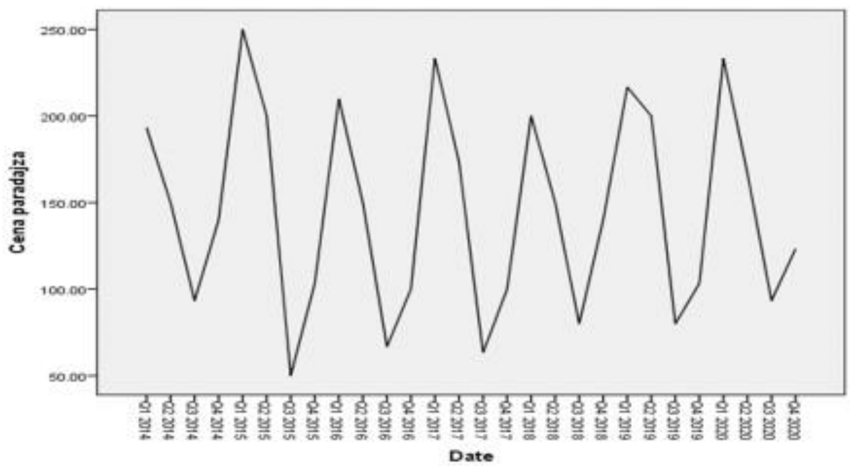
The seasonal ARIMA model for time series {Xt, tϵT} has the following form (Mladenović and Nojković, 2015):

$$\phi(B)(1-B)^d(1-B^s)^D X_t = \theta(B)\varepsilon_t ,$$

where the operators of seasonal and non-seasonal time differencing are applied d, i.e. D times, respectively. When making a seasonal ARIMA model, an interactive approach of Box and Jenkins model (Mladenović and Nojković, 2015) should be applied.

Results and discussion

Before analyzing the data on the tomato prices on markets in the Republic of Serbia, it was checked whether the data follow a normal distribution by applying two tests: the Kolmogorov-Smirnov and Shapiro-Wilk tests. Based on both tests, it was concluded that the data follow a normal distribution, which enables a further data analysis based on ARIMA modeling.



Source: Authors’ calculation in the“SPSS Statistics“, based on the STIPS data

Graph 1: Quarterly tomato prices at the markets in the Republic of Serbia in the 2014-2020 period

From the Graph 1, one can conclude that the data do not follow one trend (constant increase or constant decrease in prices), but there is a notable fluctuation in prices, based on which it can be concluded it is a stationary time series. During data modelling, based on the auto correlational and partial correlation function of a time series, several models were chosen to describe the price movements at the markets in the Republic of Serbia. The performances of the chosen model were tested and the authors came to conclusion that the most suitable ARIMA model (1,0,0)x(1,1,2) is the one with the parameters shown in Table 1.

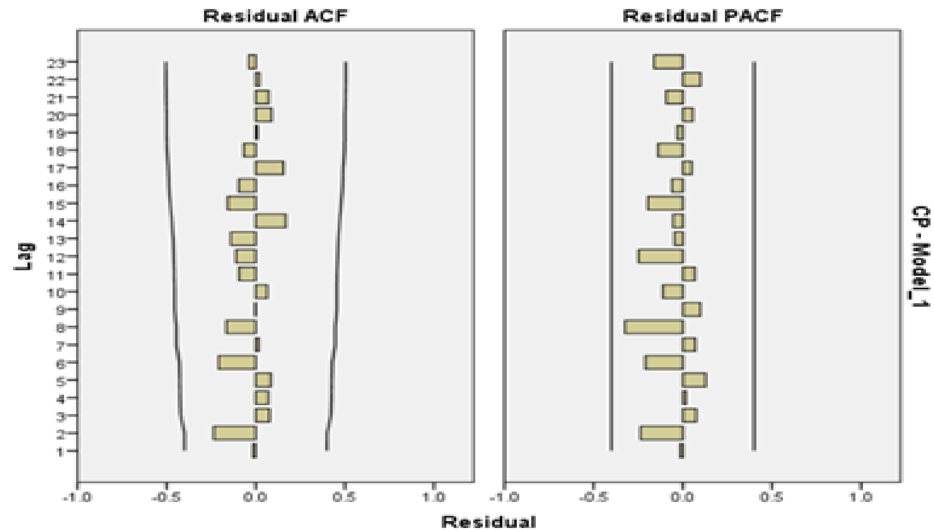
Table 1. Estimated parameters of the ARIMA (1,0,0)x(1,1,2) model

Variable		Estimates	Standard error	t	p-value
Constant		1.473	2.069	0.712	0.485
AR	First lag	0.286	0.226	1.265	0.221
AR, seasonal	First lag	-0.986	0.154	-6.416	0.000
MA, seasonal	First lag	0.136	94.623	0.001	0.999
	Second lag	0.860	82.364	0.010	0.992

Source: Authors’ calculation in the “SPSS Statistics“, based on the STIPS data

In general, if seasonal variations are assumed to change over time in a multiplicative manner, then a model in the following form is used (Mladenović and Nojković, 2015):

$$\left(1-\phi_1 L-\phi_2 L^2-\cdots-\phi_p L^p\right)\left(1-\Phi_1 L^s-\Phi_2 L^{2s}-\cdots-\Phi_P L^{Ps}\right)(1-L)^d\left(1-L^s\right)^D X_t =$$
$$\left(1-\theta_1 L-\theta_2 L^2-\cdots-\theta_q L^q\right)\left(1-\Theta_1 L^s-\Theta_2 L^{2s}-\cdots-\Theta_Q L^{Qs}\right)e_t$$



Source: Authors’ calculation in the “SPSS Statistics“, based on the STIPS data

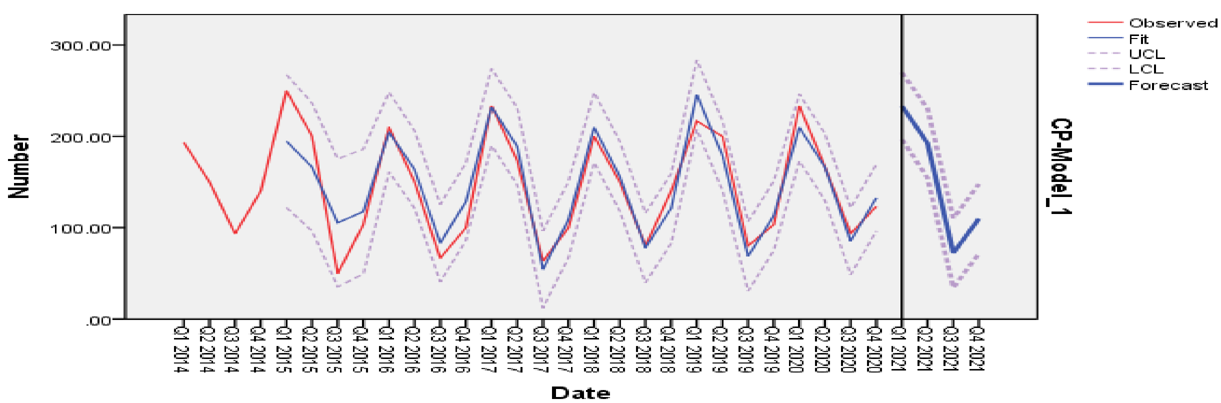
Graph 2: Correlogram results for the residuals of the estimated model

Based on the Graph 2, it can be concluded that the residuals do not contain any significant correlations on any of the lags, i.e. that the residuals follow the pattern of white noise.

Table 2: Estimated tomato prices at the markets of the Republic of Serbia based on the ARIMA model (1,0,0)x(1,1,2)

Quarters	Estimated prices	Confidence interval of 90% of the estimated prices		Real prices
		Lower bound	Higher bound	
I quarter of 2021	233.17	196.66	269.68	166.67
II quarter of 2021	192.16	154.19	230.13	183.33
III quarter of 2021	72.3	34.22	110.38	93.33
IV quarter of 2021	109.8	71.76	147.83	150.00

Source: Authors’ calculation in the “SPSS Statistics“, based on the STIPS data



Source: Authors’ calculation in the “SPSS Statistics“, based on the STIPS data

Graph 3: Quarterly tomato prices in the 2014-2020 period and the ARIMA-model estimated prices at markets in the Republic of Serbia

Based on Graph 3, it can be concluded that there is a deviation from the real prices and the ARIMA-model forecast prices for 2014 and 3rd quarter of 2015, while from the 4th quarter of 2015 onwards the forecast prices from the proposed model almost overlapped with real tomato prices at markets in the Republic of Serbia. The tomato price has a seasonal character and therefore constantly varies depending on the season, being lower in the summer than in the other quarters of the year. Moreover, the data obtained from the ARIMA model for the quarters of 2021 follow the same trend. Nevertheless, although the tomato price has been increasing worldwide (as forecast by the ARIMA model for the quarters of 2021), in the Republic of Serbia it has decreased for about 15% from the same period last year (STIPS data). The forecast price for the 1st quarter of 2021 was 233.17 RSD/kg, while the real price was considerably lower, amounting to 166.67 RSD/kg. Based on the STIPS data, however, in 2nd quarter of 2021 the tomato price at markets was 183.33 RSD/kg, while the forecast price was 192.16 RSD/kg. In the 3rd quarter the real price was about 20 RSD/kg higher than the price forecast by the ARIMA model. A considerably higher price than the forecast one can due to lower yields as a result of climate conditions during the summer production, that have happened in the Republic of Serbia mostly in an open field during that period of a year. Moreover, in 4th quarter of 2021 the real price was higher than the forecast one, almost completely overlapped with the higher bound of the forecast price. In that quarter it has happened a considerable increase in real tomato price at markets, and one of the reasons was dissatisfaction of tomato growers with the price in the previous quarter, which led to the lower production in the last quarter of 2021. At the same time, due to insufficient imports, the tomato price has been significantly increased in the last quarter. A great impact on price growth and such discrepancies from the forecast prices had the global crises caused by the COVID-19 pandemic, which has led to the growth of prices in general, including the price for inputs necessary for agricultural production and also the labor price, resulting consequently in the increase of prices for agricultural commodities.

Conclusion

Having analyzed the tendencies in price movement at markets in the Republic of Serbia, it can be concluded that a seasonal character prevails, i.e. greatly affects the tomato price. Apart from the seasonal character, tomato prices are greatly affected by the market, supply and demand, as well as the imports from the neighboring countries which are big producers of this vegetable crop and consequently offers tomatoes at lower prices (Greece, Albania, North Macedonia, etc.). Furthermore, in this case, given the analysis for 2021, the prices were greatly affected by the global crises caused by the COVID-19 pandemic. Studying the tendency of tomato price movements in the analyzed period (2014-2020), and prices forecast by using the ARIMA model, it can be concluded that due to numerous factors, the forecast prices do not differ from the real market prices. Therefore, the model can be considered reliable, since the real and forecast prices do not differ significantly, especially in 2nd quarter of 2021, whereas in 3rd and 4th quarter due to the dependence of the production on climate conditions and imports there was some discrepancies from the forecast prices, which implies the seasonal character of the production and its impact on the commodity prices.

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THE EFFECT OF DEMOGRAPHIC FACTORS AND CONSUMER BEHAVIOURS ON RED MEAT CONSUMPTION EXPENDITURES

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Abstract

The rearing of livestock is a vitally important issue for many rural communities in developing countries. It provides a regular contribution to livelihood and essential pathways out of poverty. Furthermore, a realisation that the consumption patterns among consumers in developing countries are shifting towards that of animal-based products, requires an overall policy to promote red meat supply and meet the increasing demand. According to data, provided by the Association of Red Meat Producers in Turkey, meat consumption is below that of those countries belonging to the Organization for Economic Development and Cooperation (OECD). While the annual per capita consumption of red meat was 12 kilos on average in 2019, it decreased to 7-8 kilos in 2020. Thus, policies to enable the increase of red meat consumption are of great importance. This research used a survey of 400 households from different provinces of Erzurum, Erzincan and Bayburt in the Eastern part of Turkey. It examined the effect of demographic factors and behaviours on the purchasing of red meat by consumers. The results of the analysis, revealing the effect of demographic characteristics and consumer behaviour on red meat consumption expenditures, are included. Solutions to the problems regarding red meat consumption are proposed. They have been determined by taking a holistic approach with respect to red meat consumption and the red meat supply chain.

Key words: *consumption, consumer behaviour, demographic factors, red meat.*

Introduction

Attributes of meat consumption are complicated. There have been significant changes in food consumption patterns in Turkey as in the world. Socio economic factors, incomes, urbanization, female labor participation, technological developments, ready-to-eat food consumption and health interests are key factors that impress not only the amount of the food consumption but also the kind of meat consumption. Meat demand is related to life standards, nutrition habits, livestock development and meat prices, as well as macroeconomic uncertainty and shocks to Gross Domestic Products (GDP). Marsh et al., (2004) emphasized the recalls of meat consumption contribute directly to industry costs have dramatically increased during the last two decades. Compared to other goods, meat is distinguished by great production costs and output prices and also meat consumption is associated with higher incomes and a switch - because of urbanisation - to food consumption changes that beneficence increased proteins from animal sources in diets. According to OECD (2021) data, meat consumption that is presented for beef, veal, and mutton in this study, with 36,853 kg /capita. Argentina is in the first place, while the second and the third place are occupied by United States and Brazil with 26,081 and 24,605 kg/capita respectively. The meat consumption in Turkey (9,818 kg/capita) remains relatively low in comparison to leading countries in the world. Red meat comes first among animal-based proteins and is frequently used as veal, beef and mutton. As pointed out by Cheah et al., (2022), people tend to incorporate more meat in their diet in an effort to boost their protein intake as it provides quality protein for human diet. A study conducted in UK has focused on identifying the optimal protein amount, timing and type of protein for sarcopenia prevention. Therefore, it is the suitable time for implementing healthy dietary changes, to delay the sarcopenia and also consumption of meat could be one of the keys to reducing malnutrition (Udomkun et al., 2018) as from the point of view of healthy diet, meat is a well origin of protein minerals and vitamins. While meat consumption habits change, especially inadequate meat consumption can cause various diseases. However, as argued by Randolph et al. (2007), the unfavourable publicity on animal breeding and animal source food is driven by health and food safety issues related to outbreaks of diseases and the link between the saturated fats and cholesterol found in animal source food and heart diseases, cancer, contributing to consumer nervousness about meat. For this reason, consumer behaviours affects their meat consumption, purchase and willingness to buy. Diamond (2002) emphasized that close contact with livestock improved human immunity to zoonotic diseases, in turn providing advantages to some groups. Thus livestock keeping development in developing countries are primarily intended to form income and meet the meat demand. According to FAO (2020), grain and grain products are in the first place among the most consumed food groups in Turkey. Red meat is the leading protein of animal origin. In this regard, red meat is necessary to increase the consumption of animal-derived protein, which is an important deficiency in a balanced diet. Low consumption in Turkey can be attributed to the decrease in supply of the red meat. Factors such as the decrease in the number of breeding animals, low meat prices, low milk prices, imbalance in input and output prices, illegal animal entry, which cause the decrease in red meat production, also significantly determine the consumption. When the meat prices are considered, it was understood that international meat prices fell down globally in 2020 because of the impact of COVID-19 pandemic. Logistical barriers and decreased food service and people spending temporarily did not improve import demand by Turkey. Pandemic related market inconvenience reduced incomes in meat importing, low income countries like Turkey significantly nibbling purchasing power and force households to substitute the intake of meat products with cheaper options. The fall in meat prices would have been greater if Turkey had not increased its import demand due to the pandemic, which continues to limit local production. Growth in livestock, combined with increased per animal productivity will promote the meat market. Increase in meat production is led mainly by growth meat consumption. To reveal the extent to which the changes in the consumption pattern affect the food consumption pattern of the households; it is economically important in the formation of national food policies, in the long-term plans of investors and in shaping the domestic market. The aim of this study is to empirically investigate the effects of demographic factors and consumer behaviour on red meat consumption expenditures and highlight the importance of policies to be implemented to increase red meat consumption. Previous studies on consumer behaviour linking red meat consumption have been limited, focusing on the impact of demographic factors or behaviours of households. The paper concludes with some discussions of the effect of demographic characteristics and consumer behaviour on red meat consumption. Primary data were collected through a household survey of red meat. The provinces in the eastern part of Turkey selected as the scope of the research are Erzurum, Erzincan and Bayburt.

Materials and Methods

The primary data of this research was obtained from face-to-face interviews with 400 households in Erzurum, Erzincan and Bayburt provinces in eastern part of Turkey. Purposive sampling method was used in this study to identify 400 households. Participants were selected from consumers who consume red meat. A semi-structured questionnaire was used and in the first part of the survey, the demographic characteristics of the interviewed consumers, in the second part, the purchasing behaviours of the households in consumption of red meat took place. Research findings has been explained with simple descriptive statistics. In addition, non-parametric tests were used in the statistical interpretation of the data.

Results and Discussion

Socio-Economic Characteristics of the Participants

The socio-economic characteristics of the participants are given in Table 1. According to the data, 85.25% of the interviewed participants are male. Looking at the distribution of the participants by age groups, it is seen that 61.81% of them are between the ages of 25 and 45. The participants who are over 45 years old is 32.41%. While 84% of the individuals participating in the interview are married, 15.50% are single. In terms of education level, it is seen that the participants are concentrated in three groups: primary school (26.32%), high school (29.32%) and undergraduate (21.30%). The total rate of these three groups is 76.94%. When the distribution of the participants according to their occupations is analysed, the most important share is composed of civil servants with 36.13%. This is followed by private sector employees (12.98%), self-employed (12.72%), farmers (9.41%) and retirees (7.38%). The household size of 68% of the participants is less than 4 people. The participants whose household size consists of 4 or more is 32%. It is possible to say that the participants are concentrated in the low and middle income groups. According to the monthly income distribution by considering the conditions of the period in which the survey was conducted, four income groups were taken as basis. The proportional distribution of the participants with an income of less than 458 US$, 458-1371 US$, 1372-2285 US$ and more than 2285 US$, respectively; 29.57%, 35.59%, 23.31% and 11.53%.

Table 1. Socioeconomic characteristics of the study participants

Characteristics	**%**	**Characteristics**	**%**
Gender		**Occupation**	
Female	14.75	Public servant	36.13
Male	85.25	Private sector employee	12.98
Age (years)		Self-employed	12.72
Less than 25	5.78	Farmer	9.41
25-45	61.81	Labor	7.89
More than 45	32.41	Retired	7.38
Marital status		Other	13.49
Married	84.00	**Household size**	
Single	15.50	Less than 4	68.00
Divorced	0.50	4 and more	32.00
Educational level		**Household monthly income**	
Illiterate	2.01	Less than US$ 458	29.57

Literate	3.01	US$458-1371	35.59
Primary school	26.32	US$1372-2285	23.31
High school	29.32	More than US$2285	11.53
Undergraduate	7.27		
Graduate	21.30		
Post-graduate	10.77		

Red Meat Purchase Behaviours of Participants

In this section, the red meat purchasing behaviors of the participants were examined. The red meat types preferred by the participants are shown in Figure 1. Accordingly, 62% of the participants prefer only beef. The rest of the participants do not limit their choice of red meat type to only beef. Thus; 29% of the participants prefer beef, lamb and goat meat, and 9% prefer beef and lamb.

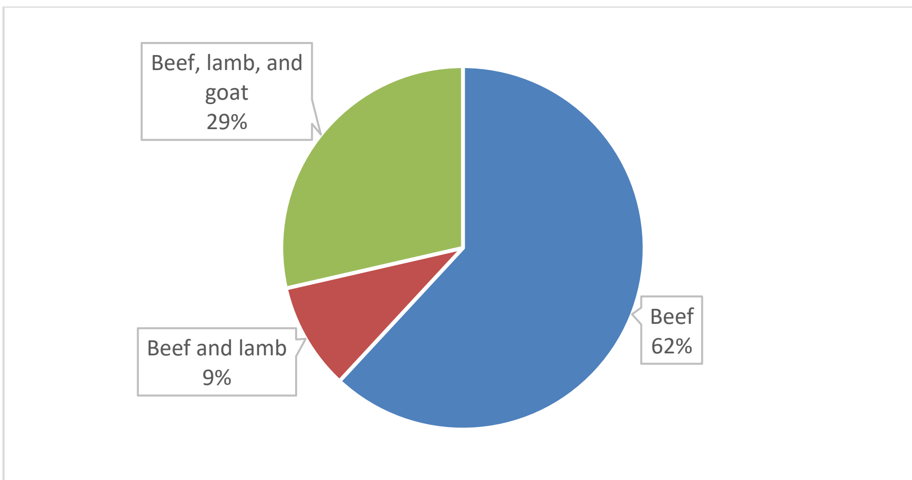

Figure 1. Types of red meat preferred by participants

The frequency of red meat consumption of the interviewed participants is given in Figure 2. As can be seen in Figure 2, the rate of those who consume red meat on a daily basis is quite low. (%9.52). The rate of the participants who consume the red meat 1-3 times a week is higher (%38.10). This is followed by those who consume the meat 4-5 times a week (%14.29). The ratio of those who consume the meat once a month or 2-4 times a month is 19.04% in total. The rate of the participants who consume the meat 1-3 times a year is 19.05%. This result reveals that approximately one fifth of the participants consume the red meat 1 or 3 times a year.

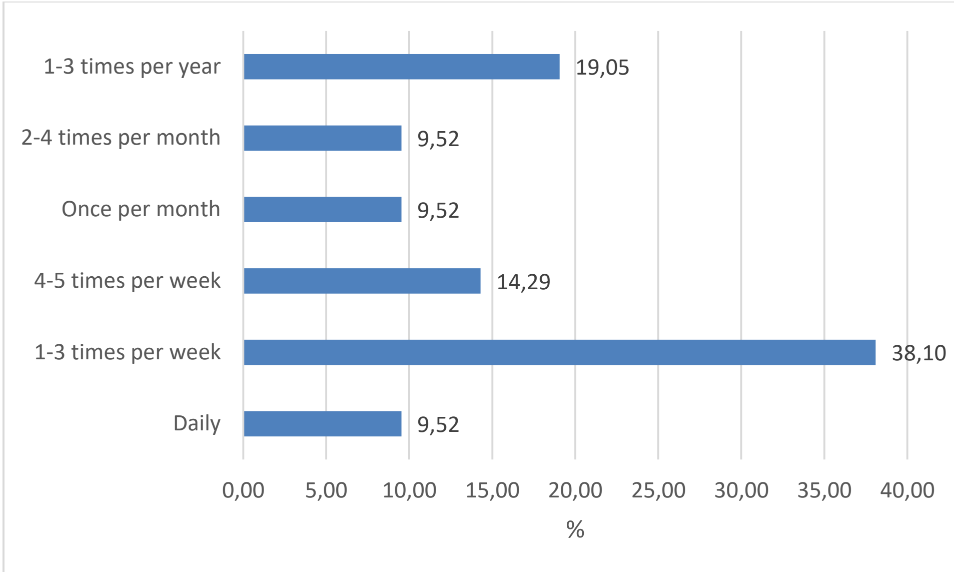

Figure 2. The frequency of red meat consumption of participants

The reasons why the interviewed participants prefer red meat are shown in Figure 3. The reason why most of the participants prefer red meat is based on a single criterion. Among these criteria, taste (29.42%) comes first, followed by the nutritive feature of red meat (20.59%). The rate of those who prefer the red meat only because of cheapness or habit is equal (5.88%). Among the participants, there are individuals whose reasons for choosing the red meat are based on more than one criterion. Among the reasons for choosing red meat of 26.47% of the participants are the criteria of taste, nutritive and habit. Some participants (5.88%) stated that taste and nutritive feature are among the reasons for choosing red meat.

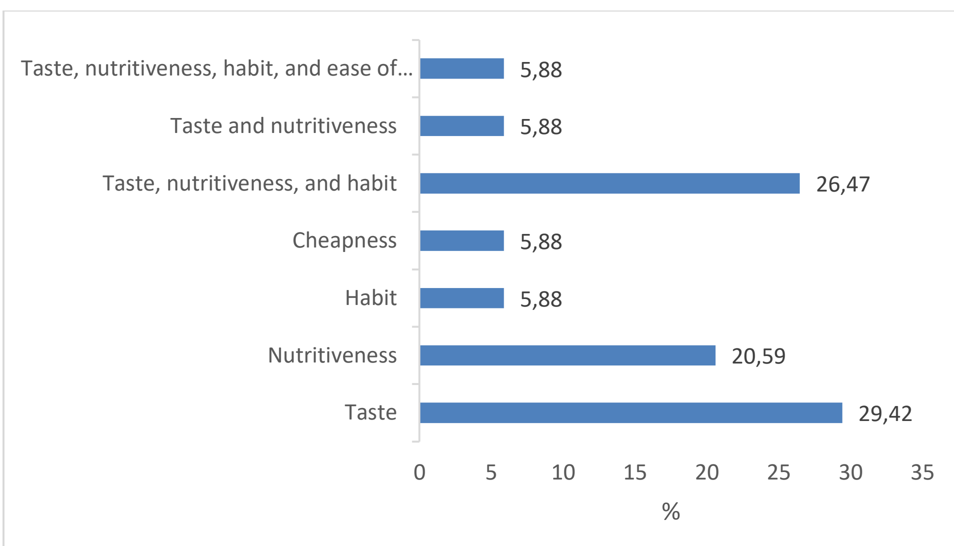

Figure 3. Participants' reasons for choosing red meat

In line with the information obtained from the participants, the monthly red meat consumption amounts per household are given in Figure 4. As seen in Figure 4, monthly red meat consumption per household is concentrated between 3.01-5 kg. As a matter of fact, 41.18% of the participants stated that their monthly consumption of red meat is between 3.01 and 5 kg. The rate of participants stating that the monthly consumption of red meat per household is between 1-2 kg is 29.41%. While the rate of monthly red meat consumption between 2.01-3 kg is 11.76%, the rate of those with more than 5 kg is 17.65%.

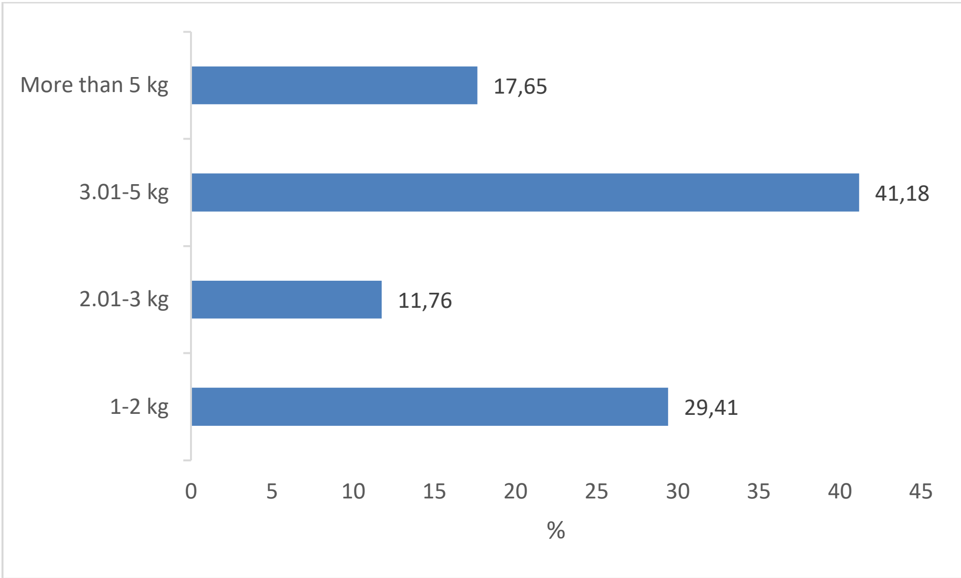

Figure 4. Monthly red meat consumption per household (kg)

According to the responds of the participants, monthly red meat consumption expenditure per household is 42.72 US$. This amount of expenditure varies between 4.57 and 457.06 US$. (Table 2).

Table 2. Monthly household expenditure on red meat (US$)

	Minimum	Maximum	Mean	Std. Deviation
Expenditure on red meat (US$)	4.57	457.06	42.72	42.82

The monthly expenditure for the red meat consumption varies according to the size of the household. As expected, as the size of the household increases, the amount of expenditure for the red meat also increases. As a matter of fact, this expenditure amount is US$ 40.17 for those with a household size of less than 4 people, and US$ 47.01 for those with a household size of 4 or more. However, there is no statistically significant difference between the expenditure amounts of the two household sizes (Table 3). The relationship between monthly household income groups and the amount of monthly expenditure for the red meat consumption was also examined. In the analysis carried out in this context, it was revealed that as the income level of the household increased, the monthly expenditure for the red meat increased statistically significantly. While the monthly expenditure of the household for the red meat is 27.79 US$ in the first income group (less than US$ 458US$), in the second (US$458-1371), third (US$1372-2285) and fourth (more than US$2285) income groups are respectively 34.84 US$, 44.75 US$ and 81.30'US$.

Table 3. Monthly household expenditure on red meat by household size and monthly household income

Characteristics	Expenditure on red meat (US$)	Mann-Whitney U	Sig.
Household size		10079,000	0.242
Less than 4	40.17		
4 and more	47.01		
		Kruskal-Wallis Chi-Square	
Household monthly income		41,408	0.000*
Less than US$ 458	27.79		
US$458-1371	34.84		
US$1372-2285	44.75		
More than US$2285	81.30		

* Statistically significant at the level of significance (p-value≤ 0.05)

Conclusions

In this study, the behaviour of households towards red meat consumption was analysed based on the survey data conducted in Erzurum, Erzincan and Bayburt provinces. The findings of this study indicate that red meat consumers in these three neighbouring provinces mostly prefer beef and consuming red meat 1-3 times a week is common. Taste and nutritive feature are the most prominent criteria for choosing red meat, and the monthly consumption of red meat per household is 3.01-5 kg and the rise in the income level of the household significantly increased the monthly expenditure for red meat. It is possible to come across many studies examining the red meat consumption profile of households both in Turkey and in other countries of the world. One of the common points encountered in many studies is that living standards are effective in red meat consumption. The findings in this study also supported this conclusion. This emphasis was also made in the OECD report on meat consumption. In this report, meat consumption is only associated with living standards. It is stated that meat consumption should be associated with diet, animal production, consumer prices, macroeconomic uncertainty and shocks in GDP. OECD statistics show that Turkey is below the OECD average in terms of meat consumption per capita. Among the main reasons for this are the decrease in animal production due to high production costs and the resulting high consumer prices. In countries such as Turkey, where meat consumption per capita remains low in terms of healthy nutrition, attempts can be made to increase meat consumption. However, it is emphasized that the increase in meat consumption in many countries goes far beyond its basic nutritional benefits. Previous studies have linked excessive consumption of red and processed meats to increased risk of heart disease, stroke, and certain types of cancer. In addition, compared to chicken, beef is stated to have a three to 10 times greater impact on land use, water and greenhouse gas emissions. In this context, red meat consumption should be evaluated in a broader dimension for both sustainable animal production and balanced consumption.

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MARKETING CHANNEL PREFERENCES OF DAIRY PRODUCERS AND THEIR PRICES: THE CASE OF GUMUSHANE PROVINCE IN TÜRKIYE

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Abstract

Dairy farming in Turkey is the main source of livelihood and an important source of income for most of the rural farmers. The dairy sector also provides economic benefits to all intermediary channels from production to consumption. Ensuring the sustainability of dairy farming requires a good analysis and development of the marketing channels used by the producers. Dairy producers may encounter different prices in the marketing channels they prefer in marketing their products. This can directly affect milk supply and consumption. In this study, milk marketing channels of traditional dairy cattle producers and their prices according to these channels were examined. The data of the study were obtained from face-to-face surveys conducted with 102 producers in Gumushane province. In the first part of the study, the socio-economic characteristics of the interviewed producers were discussed. In the second part, the marketing channels through which the producers sell their milk and the prices they obtain according to these channels are presented. The results revealed that dairy farmers use traditional marketing channels more than modern channels in raw milk marketing in Gumushane province. Among the various milk marketing channels most of the dairy farmers (%57) in Gumushane province prefer direct sale while %43 prefer the local traders. As a result, it can be said that the milk marketing channel preference of the dairy farmers varies depending on the type of the dairy farm, the development of the dairy industry and dairy farming systems in the region, milk prices and non-price factors.

Key words: *Milk production, Marketing channels, preferences, Milk prices, Turkey.*

Introduction

Agricultural activities, which depend on nature and economic factors, carry risks compared to industrial activities. The volume of agricultural activities is shrinking due to reasons such as price fluctuations, low profit margins, and high feed prices (ASUD 2021). Achieving these problems increases the economic value of the livestock in agricultural production and ensure that it becomes profitable for farmers. In milk production, which has an important place in agricultural production, some problems can be encountered both in Turkey and in the world, and these problems can directly affect agricultural economy of countries. The powerful economies of the world use agricultural activities in order to use their renewable resources effectively and to add value to economy. By increasing agricultural production and the demand, these countries can increase the volume of agricultural activities (Doğan and Kızıloğlu, 2015). The necessity of using more capital and keeping up with the market conditions and the development of technology complicate the management of agricultural production (Bozoğlu et al., 2001).
Milk production in the world is about 718 million tons per year and cow milk constitutes 83% of the world's milk production. The European Union (EU), USA, India and China are the leading

countries in milk production. According to FAO data (2022) on the cow milk production amount, with 154 million tons, EU countries are in the first place, while the second place is occupied by United States of America with 101 million tons and it is followed by India (87 million tons) and China (34 million tons). Turkey is in the 8th place with the amount of 20 million tons per year. Although Turkey is in the eight place in the production of cow milk in the world, the yield per cow remains relatively low in comparison to other countries. In terms of International Dairy Federation (2020) data, the yield is only 3.15 kg in Turkey. Although dairy farming can be practiced almost anywhere around the country (Şahin et al., 2021), the milk yield per cow is still low in Turkey. Therefore, among the important factors affecting the success of dairy farms such as efficiency, working with optimum capacity, being able to compete in the market, and making structural and economic analysis of the enterprises are important (Karagölge, 1996). It is clear that the current problems in milk production in Turkey affect directly the milk yield. Most of the dairy farms in Turkey are small size family enterprises and processing of milk into other products is not performed in modern farms like in developed countries (Küçük and Tapkı 2020). Despite the large number of small farms, the capacity utilization rate and efficiency are quite low. Several studies examining the economic aspects of dairy farming have been carried so far (Ermetin and Abacı 2022). Although there is a potential market for milk and milk products in Turkey, also in the province of Gumushane, the prices and the marketing channels affecting the profitability of dairy farms are not sufficiently known. In Turkey, in areas not suitable for industrial development but open to improvement of their agricultural economy, increasing the livestock is a good opportunity for the local economy. Agricultural activities are among the main sources of livelihood in the province of Gumushane, located in north-eastern Turkey. Although the time-honoured tradition of livestock offers potential, it does not currently reach the desired level due to recent problems experienced in the sector. The geographical and climatic conditions of the province also suffer various difficulties, especially logistical, in non-agricultural investments. In the face of these problems, the efficiency of the dairy farms in Gumushane is seen as an advantageous alternative for mobilizing the local economy. The aim of this study is to determine the socio-economic factors, the prices and the milk marketing channels of local dairy farmers. Furthermore, introducing the optimum type of dairy farms will be guide for decision-makers and dairy farmers who need such information.

Material and Methods

The main material of this study was provided from a face to face survey conducted with dairy farmers who produce raw milk in the research area. Research group obtained from the Breeding Dairy Cattle Association in the province of Gumushane. Purposive sampling method was used in the study. In this context, the three districts that produce the most milk in Gumushane were selected as the research area. In the study, quota sampling, which is one of the non-probabilistic sampling methods, was used while determining the sample size. Within the framework of this method, 102 dairy farms were included in the scope of the research.

A semi-structured questionnaire was used to collect data from dairy farms. There were questions about the demographic and structural characteristics of the dairy farms in the first part of the questionnaire, and in the second part, questions were about the marketing channels used by the dairy farmers. Findings are shown with simple descriptive statistics and graphs. In addition, in the study, national and international literatures were scanned in order to evaluate the status of raw milk marketing channels in Turkey compared to other countries.

Results and Discussion

Socio-Economic Characteristics of Dairy Farmers

The socio-economic characteristics of the interviewed dairy farmers are shown in Table 1. The age range of farmers varies between 26 and 73 years, with an average of 45.27. The average household size of farmers is 5.43 people. Household size varies between 2 and 10 people. A significant portion of the dairy farmers have primary school education with a rate of 70%. Only 26% of dairy farmers have received high school education. The ratio of dairy farmers who received pre degree and undergraduate education is equal (2%).
The interviewed farmers have approximately 17 years of experience in milk production. The experience of farmers in milk production ranges from 1 to 40 years. The agricultural land size owned by dairy farmers is 12.23 hectares. The smallest and largest land size, respectively 0.60 ha and 108 ha. The average herd size in the examined dairy farms is 31.97 heads, and the herd size varies between 7 and 300 heads.

Table 1. Socioeconomic characteristics of the dairy farmers

Characteristics	Mean	SD	Min	Max	Characteristics	%
Age (years)	45.27	8.78	26.00	73.00	Educational level	
Household size (person)	5.43	1.61	2.00	10.00	Primary school	70.00
Dairy farming experience (years)	16.94	10.15	1.00	40.00	High school	26.00
Farm size (hectares)	12.23	15.77	0.60	108.00	Pre degree	2.00
Heard size (number)	31.97	35.62	7.00	300.00	Undergraduate	2.00

Dairy Farmers’ milk marketing channels

In this part of the study, the raw milk marketing channels of the dairy farmers were discussed. In this context, first of all, information about raw milk marketing channels in Turkey was given. As seen in Figure 1 dairy farmers sell the raw milk through three different marketing channels. These are doorstep collection, cooperatives and direct selling places. It is stated that generally 20% of the milk is sold directly such as dairy farms, streets, local bazaars, restaurants and bakeries.
The main marketing channels of the farmers are dairy companies and milk cooperatives. The dairy companies operating in the dairy sector generally collect raw milk from the production area through their collectors, then process and market it to the domestic and foreign markets. The dairy cooperatives collect the milk of their partner producers from milk collection centres in the production area. These cooperatives process the milk and supply it to the market. It is possible to say that the dairy companies have a significant share in the raw milk sales of the dairy farmers. However, in some regions, agricultural cooperatives appear to be an important buyer of raw milk. Raw milk marketing channels of the interviewed dairy farmers differ slightly from the milk marketing structure in Turkey. As can be seen in next Figure, 57% of dairy farmers prefer direct sales, while 43% prefer dairy companies. It has been determined that direct selling farmers generally prefer bazaars. The fact that dairy farmers prefer the option of selling directly to consumers is based on some reasons. One of these reasons is the expectation of higher price compared to dairy companies. Another reason is that dairy farmers do not have analysis for quality control in raw milk sales. As a matter of fact, both dairy companies and dairy cooperatives perform quality control analyses for raw milk in milk collection centres. The small scale of the dairy farms in the research area is an important factor in the preference of direct sales

options by dairy producers. Actually, small-scale dairy farms generally cannot meet the quality criteria of dairy companies. Dairy companies prefer larger scaled dairy farms in order to avoid quality problems in milk purchase.

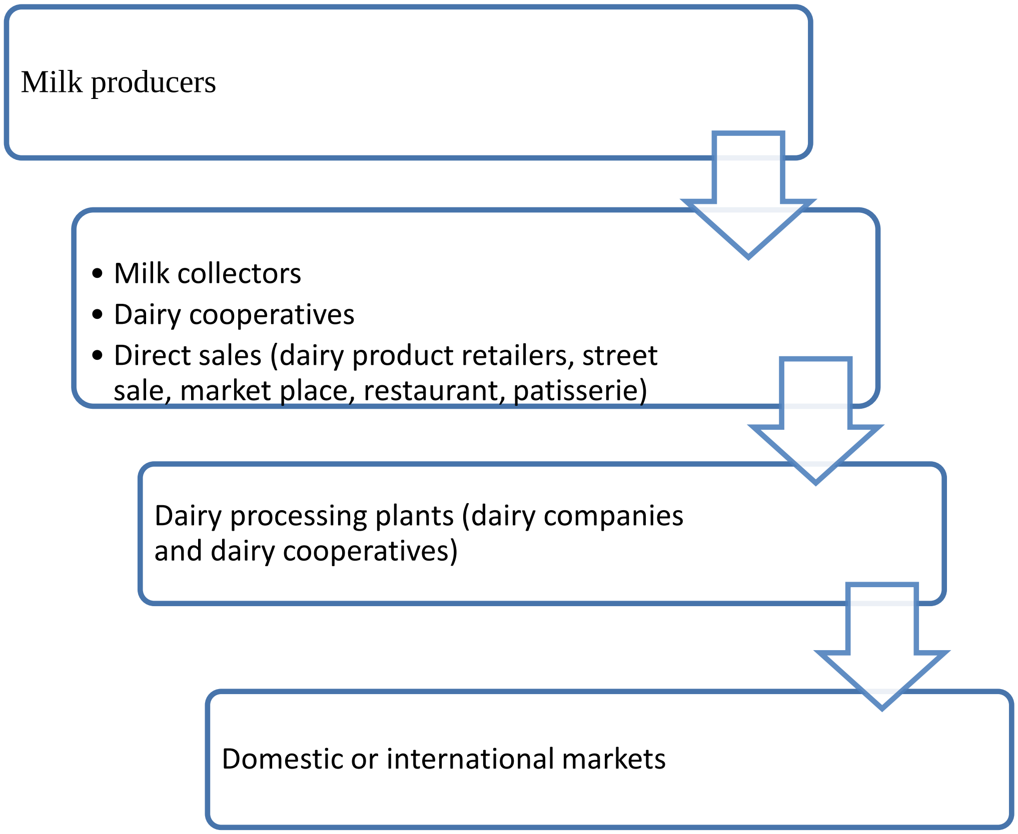

Figure 1. Raw milk-marketing channels in Türkiye
*Source: 1. Turkish National Dairy Council, 2018.; 2. TAGEM, 2018.

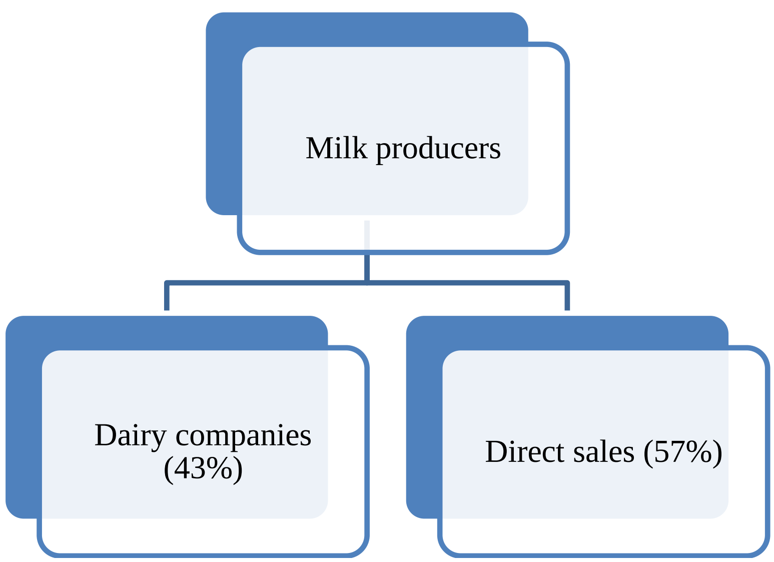

Figure 2. Marketing channels used by milk producers in research area

Table 2 is arranged in order to evaluate the status of raw milk marketing channels in Turkey compared to other countries. As seen in Table 2, the marketing channels used by dairy farmers in raw milk marketing vary according to countries. It is seen that the raw milk produced in the samples of the countries is marketed through two different marketing channels, which are described as traditional and modern (or formal). In the traditional marketing channel there are milk vendors, local traders, brokers, sweet shops, restaurants, etc. In addition, direct sales of milk to consumers should be added to traditional marketing channels. There are producer organizations and private dairy companies in modern or formal marketing channels.

It is seen that traditional marketing channels are used more widely in the marketing of raw milk in countries such as India and Rwanda. On the other hand, it has been determined that there is a greater tendency to use modern marketing channels in the marketing of raw milk in Uganda, Brazil, Indonesia and European Union (EU) countries. It can be said that producer organizations have an important place in the marketing of raw milk, especially in EU countries. According to 2016 data, it is stated that 74% of all marketed milk in Poland is processed by producer organizations. The processing rate of domestically marketed milk by producer organizations is 67% in Germany, 53% in France and 35% in Spain. It is noteworthy that not all farmers selling milk to cooperatives are members of cooperatives, especially in Poland. In Poland (Podlaskie) 56% of producers who are not members of PO (producer organizations) consigned milk mainly to POs, and in Spain (Galicia), France (Normandy) and Germany (Bayern) most of the milk produced by PO members was delivered to non-cooperative processing companies (Di Marcantonio et al., 2022). In a study carried out in Turkey, it was revealed that the delivery of milk by dairy farmers to agricultural cooperatives varies according to the types of dairy farms. In a study conducted by Yılmaz and Ata (2016), the rate of those who delivered their milk to producer organizations was 92% in small-scale traditional dairy farms, while it was 32% in large-scale farms. Delivery of milk to producer organizations may vary depending on reasons other than price and out of price. In this context, Wijnands et al. (2017) conducted a survey of dairy farmers' motivations and expectations to participate in a PO in some EU countries (Germany, France, Italy and Spain). In this study; better price (92% of farmers), a more stable price (67%), strengthening the position of producers in the value chain (63%) and guaranteeing the collection of whole milk (49%) were identified as the main motivations of dairy farmers.

Table 2. Marketing channels used by producers in milk marketing according to various countries

The study area	Number of households surveyed	Marketing channels used by milk producers	Authors
India	390	Traditional channels (milk vendor, sweet shop, or directly to consumer, contractor, etc: (70%) Modern channels (coops and private): (30%)	(Sharma, 2015)
Kiruhura District of Uganda	240	Formal channels: (Dairy cooperative union collection centres (processors) (38.1%), License raw milk traders’ collection centres) (34.1%) Informal channels: Milk vendors (bicycle and motor cycle) (24.9%),	(Nkwasibwe et al., 2015)

		Restaurants (3.1%)	
Burdur Province of Türkiye	100	Traditional Dairy Farms: cooperative (92%), dairy products factory (8%) Improved Dairy Farms: cooperative (32%), dairy products factory (68%)	(Yılmaz and Ata, 2016)
The state of Rio Grande do Sul of Brazil	199	Cooperative (49.2%), private companies (32.7%)	(Neutzling et al., 2017)
The Western Province of Rwanda	96	Brokers (49%), local vendors (36%), milk collection centres (15%)	(Innocent et al., 2018)
India	294	Local traders (37.4%), consumers (29.8%), cooperatives (26.1), commission agents (3.8%), processors (1%), others (1.9%)	(Kumar et al., 2018)
Bogor District of Indonesia	47	Cooperatives-milk processing industry (86.5% of the total milk production of dairy farmers), retailer (11.3%), consumer (2.1%)	(Hardini and Gandhy, 2022)
EU Member States (Germany (Bayern), France (Normandy), Poland (Podlaskie), and Spain (Galicia)	1148	-(Podlaskie): 56% of non-producer organization (PO) members mainly deliver milk to POs, -(Galicia, Normandy, and Bayern): a large share of the milk produced by PO members is delivered to non-cooperative processing companies	(Di Marcantonio et al., 2022)

Prices Obtained Dairy Farmers

The prices received by the interviewed dairy farmers according to their marketing channels are shown in Table 3. As seen in Table 3, when the dairy farmers sell their products to dairy companies, the price they receive was US$0.44 per liter of milk. On the other hand, the dairy farmers earn a price of 0.45 US$ per liter from their direct sales. As can be understood from this finding, there is not much difference between dairy companies and direct sales. The statistical analysis (Mann-Whitney U test) reveals that there is no significant difference in terms of milk prices between the two marketing channels.

Table 3. Milk prices received by dairy farmers by marketing channels (US$ per liter)

Marketing Channels	Mean	Std. Deviation	Minimum	Maximum	Mann-Whitney U	Sig.
Dairy companies	0.44	0.093	0.37	0.82	975.500	0.168
Direct Sales	0.45	0.096	0.27	0.82		
Total	0.45	0.095	0.27	0.82		

In terms of the sustainability of dairy farms, it is very important that the milk prices received by the producer cover the production cost. In this context, a question was asked to the dairy farmers about how much their milk prices covered the production costs. The answers given by the dairy farmers for this question are shown in Figure 3. As seen in Figure 3, 84% of the dairy farmers stated that the prices they obtained in milk sales did not cover their production costs. Only 16% answered “yes” to this question.

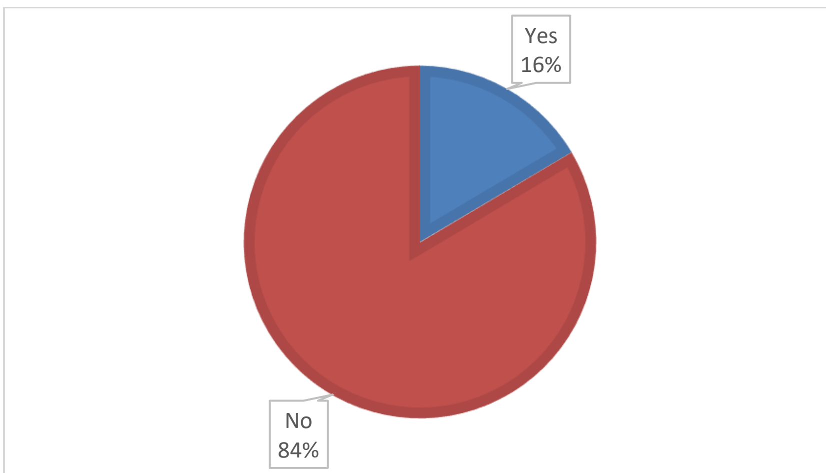

Figure 3. Do milk prices cover the production costs?

Conclusions

In this study, raw milk marketing channels of the dairy farmers were discussed based on the survey data. In addition, in this study, raw milk marketing channels in various countries were evaluated and comments were made in terms of Turkey.

The examined country samples show that raw milk is marketed through two different marketing channels as traditional and modern (or formal). According to the analysis, the marketing channels used by dairy farmers change according to the countries. It is understood that in some countries traditional marketing channels are dominant but in some countries modern (or formal) marketing channels. It can be also said that one of the traditional or modern marketing channels is more common in different regions of the same country. The findings indicate that the dairy producers use traditional marketing channels more than modern channels in raw milk marketing in Gumushane province. As a matter of fact, 57% of the interviewed dairy farmers prefer the direct sale option to market their raw milk, while 43% prefer dairy companies. Some studies conducted in other provinces of Turkey point out that modern marketing channels are used more widely than traditional channels. We can say that the marketing channel preference of the dairy producers varies depending on the type of business, the development of the dairy industry, producer organizations in the region, milk prices and non-price factors.

Milk prices keep its significance as one of the major criteria in the marketing channel preference of dairy farmers. In fact, dairy farmers with high production costs try to prefer buyers who offer higher prices for their milk. The findings at the time this research revealed that 84% of dairy farmers could not meet the production costs. In recent years, it is mentioned that a similar situation has been encountered in other countries. The gradual increase in production costs in dairy farming systems has a very negative effect on dairy cattle enterprises. In this context, sustainable dairy cattle farming can be an important option for dairy farmers, both to reduce production costs and to obtain higher prices from buyers. Sustainable livestock farming is seen as an opportunity to increase the durability and profitability of livestock farms. As sustainable products will be offered to the market with sustainable dairy farming system, dairy producers will gain competitive power in the market. At the same time, dairy producers will be able to contribute to reducing greenhouse gas emissions, ensuring animal welfare and protecting soil health through sustainable production.

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EXPLANATION OF THE REASONS FOR PRICE CHANGES IN THE SUPPLY CHAIN OF SELECTED AGRICULTURAL PRODUCTS

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Abstract

Marketing processes of agricultural products have a special importance due to their organic structure. Agricultural products are generally marketed through intermediaries due to the small scale of Turkish agricultural enterprises and the lack of organized structures in the sector. Since the actors in the supply chain include producers, brokers, traders, wholesalers, marketers, retailers and consumers, there is an increase in product prices in the process of delivering the product from the producer to the consumer. This increase in the supply chain is called the "market margin". The high market margin of agricultural products causes the perception that product prices are increased by the actors in the marketing channels and that unfair profits are obtained. Since changes in agricultural product prices have a significant share in consumer expenditures, prices are followed with interest by consumers and consumer behavior changes according to market conditions. In addition, due to its share in inflation, economic management and all segments of economic activity closely monitor the changes in the prices of agricultural products. Changes in the prices of agricultural products are closely followed throughout the country, and they are exposed to speculative approaches, discourses and accusations, especially by the media. This situation causes loss of motivation for those operating in the sector from production to consumption. For this reason, it is aimed to explain the reasons for price increases by examining the supply chains of selected agricultural products. For this purpose, producer and consumer prices of basic agricultural products were determined and market margins were calculated. The result of the study is thought to help producers, organizations and policy makers in the formation of policies related to the marketing of agricultural products and the development of marketing strategies.

Keywords: *Food, Market Margin, Marketing, Market, Agriculture.*

Introduction

Fresh fruit and vegetable industry (FFV); It is one of the most important sub-components of the agricultural sector. While there are producers, brokers, traders, cold storage stores and retailers operating with the vision from field to fork, products can be marketed through these intermediaries. Therefore, the entire process from the production of FFV products to the final consumer through these intermediaries is called the "supply chain". The supply chain is defined as examining all the relationships between production and consumption stages. These relationships are; While it consists of many integrated structures such as production, storage, packaging and distribution, different costs and pricing are made in each of these stages. Therefore, this increase in the supply chain is called the "marketing margin". The high marketing margin causes the perception that product prices are increased by the actors in the marketing channels and that unfair profits are obtained. FFV’S have a significant share in consumer expenditures, and changes in their prices are followed with interest by consumers and receive a high reaction. In addition, due to its share in inflation, the economy management and all segments of economic activity follow and closely monitor the changes in the prices of FFV products. The close monitoring of the changes in FFV prices throughout the country causes speculative approaches and discourses, especially the media, while at the same time it causes the motivation of the actors operating in the sector from production to consumption. Therefore, in many studies, the supply chain of agricultural products has been determined and the deficiencies in the supply chain have been discussed. However, although it was emphasized in the studies that the price changes in the supply chain structures of different agricultural products were high, the findings on the explanation of the reasons for the price changes were limited. (Akpinar et al., 2009; Nizam Bilgiç, 2011; Keskin and Demirbaş, 2014; Erol and Serin, 2015; Gökkür and Çelik, 2016; Güler and Saner, 2017; Tanyaş and Tümenbatur, 2018; Çakır, 2019; Yıldızbaşı and Üstünyer, 2019; Savaş, 2022; Tümenbatur et al., 2022). For this reason, within the scope of the study, the supply chain structures in developed countries were investigated, and the prices of the products in Turkey were determined by comparing the marketing margins and the reasons for the price increases were discussed.

Material and Methods

For this purpose, first of all, fresh vegetable and fruit products, which have a high weight on the inflation basket, were determined. In determining the products, their weights in inflation were taken into account as seen in Table 1, and the 14 main products with the highest weight were examined. In the study, tomato, cucumber, green pepper, eggplant, zucchini, onion, potato, banana, apple, lemon, orange, tangerine, pear and peach products were selected. The share of selected vegetables in food and non-alcoholic beverages was 7.76% and their share in total inflation was 1.98%. It has been determined that 63.80% of the effect of vegetables on inflation belongs to the products within the scope of the project. The share of fruit products in inflation was 1.12%, and the share in food and non-alcoholic beverages was 4.60%. Thus, the reasons for price changes in the supply chain were prepared by calculating the market margins for selected products according to the countries. 12 and its share in food and non-alcoholic beverages was determined as 4.60%. Thus, the reasons for price changes in the supply chain were prepared by calculating the market margins for selected products according to the countries.

Table 1. Inflation Rates of Selected Products (%)

Product	Inflation Rate	Product	Inflation Rate	Product	Inflation Rate
Tomato	0,81	Apple	0,24	Potato	0,22
Pepper	0,17	Banana	0,30	Onion	0,26
Cucumber	0,30	Lemon	0,11	Pear	0,06
Eggplant	0,17	Orange	0,17	Peach	0,07
Zucchini	0,05	Mandarin	0,17		

Results and Discussion

The marketing of agricultural products, especially fresh fruits and vegetables, is of particular importance due to their organic structure. For this reason, various marketing systems have been created in the fresh fruit and vegetable trade, and the products have been delivered to the consumers with the help of these systems. However, factors such as lack of yield due to climate, insufficient logistics infrastructure in small-scale agricultural enterprises and high costs cause the products to be marketed with the help of wholesale markets. In this context, fresh fruit and vegetable wholesale markets are important. These situations play an active role in delivering the product to the consumer in a short time and preventing economic losses by contributing to the stabilization of product prices, the prevention of unfair competition, and the elimination of unregistered production-sales.

Wholesaler markets enable small and medium-sized agricultural enterprises to access the market, leading to maximizing business opportunities and creating affordable prices for consumers (WUWM, 2022). In this direction, 200 million tons of products are marketed daily in fresh fruit-vegetable wholesale markets around the world, and 90% of the fresh fruit-vegetables sold in the markets are procured from these markets. In addition, 3 billion consumers daily buy these products from wholesale markets (WUWM, 2022). New Covent Garden in England-London, Rungius in France-Paris, Berlin, and Munich in Germany stand out among the wholesale markets that play a role throughout the world. Marketing of FFV products is generally carried out through these situations.

According to the data of 2020, there are 176 wholesalers in Turkey, 170 of which are in the status of municipality and 6 of them are in the status of private wholesalers (Çelik, 2020). The quantity and quality of the states in the marketing system have always been discussed, and it is thought that especially recently, wholesale markets reduce the effectiveness of marketing strategies. The increase in the difference between producer and consumer prices, which is also expressed as the marketing margin, is thought to be due to the wholesale markets, and in reality, this situation is a result of developed economies. As a matter of fact, improvements and rises in the entire supply chain, which includes processing, packaging, supply to the market and presentation to the consumer in developed economies, cause an increase in the marketing margin. As a matter of fact, as can be seen in Table 2, the marketing margins of the main products determined within the scope of the study are shown compared to the developed countries. Accordingly, when the United States (USA) and France, Australia and Japan countries are analyzed, the rate of change between producer prices and consumer prices varies between 200-322%, while this rate is between 150-180% in Turkey. This situation is also seen in basic food products (meat, sugar, rice, etc.). While the rate of change in selected developed economies was 221.98-299.29%, it was 148.37% on average in Turkey (Table 3). Therefore, it is necessary to examine all actors in the chain in order to determine the points required for improvement of the supply chain. When the Australian and Japanese countries are examined, the rate of change between producer prices and consumer prices varies between 200-322%, while this rate is between 150-180% in Turkey. This situation is also seen in basic food products (meat, sugar, rice, etc.). While the rate of change in selected developed economies was 221.98-299.29%, it was 148.37% on average in Turkey (Table 3). Therefore, it is necessary to examine all actors in the chain in order to determine the points required for improvement of the supply chain.

Table 0. Marketing Margin of the Products Inspected in the Study by Country (2021) – Dollars/Kg

Produce	USA				France				Australia				Japan				Turkey			
	Producer Price	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**
Tomato	1,26	4,30	3,04	241,54	0,89	2,36	1,47	165,77	1,11	3,98	2,87	258,56	2,66	5,07	2,42	90,96	0,34	0,52	0,18	52,20
Pepper	0,93	3,68	2,75	295,70	1,12	3,23	2,11	189,22	1,19	3,45	2,26	189,92	2,28	8,56	6,28	275,44	0,32	0,83	0,51	160,65
Cucumber	0,65	2,46	1,81	278,46	0,99	2,59	1,60	161,88	1,42	4,23	2,81	197,89	2,64	7,99	5,35	203,00	0,40	1,03	0,63	157,61
Zucchini	0,32	1,56	1,25	395,24	0,78	2,88	2,10	269,23	0,65	3,11	2,46	376,26	1,68	6,56	4,88	290,71	0,27	0,61	0,34	124,00
Eggplant	0,68	2,22	1,54	226,47	0,95	2,59	1,64	172,63	1,25	3,65	2,40	192,94	3,22	11,23	8,01	248,76	0,43	1,24	0,81	189,84
Potato	0,21	1,94	1,73	832,69	0,38	1,22	0,84	224,47	0,42	2,63	2,21	530,70	1,21	2,34	1,13	93,23	0,11	0,27	0,16	148,43
Onion	0,32	1,50	1,18	368,75	0,32	1,09	0,77	240,63	0,43	2,25	1,82	420,83	0,95	3,46	2,51	264,21	0,04	0,17	0,13	298,41
Banana	0,65	2,24	1,59	244,62	0,45	1,45	1,00	222,22	1,19	1,88	0,69	57,98	1,12	2,79	1,67	149,11	0,32	0,97	0,65	200,00
Apple	0,85	3,34	2,49	293,40	0,73	1,06	0,33	45,80	1,42	3,38	1,96	138,03	2,82	5,78	2,96	104,96	0,16	0,43	0,27	167,23
Lemon	0,67	2,22	1,55	230,60	0,69	1,84	1,15	166,67	0,98	2,22	1,24	126,53	1,00	4,29	3,29	329,00	0,15	0,44	0,29	196,36
Pear	0,61	1,98	1,37	226,73	0,65	1,74	1,09	167,69	0,56	2,56	2,00	357,96	4,82	12,33	7,51	155,65	0,22	0,63	0,40	180,00
Peach	1,58	3,46	1,88	119,54	1,70	2,99	1,29	75,68	1,67	4,23	2,57	154,05	6,31	18,65	12,35	195,80	0,24	0,70	0,46	190,14
Orange	0,76	4,00	3,24	426,32	0,72	3,12	2,40	333,33	0,93	2,82	1,89	203,23	1,14	4,98	3,84	335,31	0,13	0,37	0,24	189,89
Mandarin	0,96	4,22	3,26	338,21	0,98	3,31	2,33	237,76	1,47	4,56	3,09	209,57	2,31	5,85	3,54	153,69	0,12	0,52	0,39	321,67

Resource: FAO (2021)

Table 3. Market Margin for Basic Food Products by Country (2021)

Produce	USA				France				Australia				Japan				Turkey			
	Producer Price	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**	**Producer Price**	**Consumer Price**	**Absolute Marketing Margin**	**Change Rate**
Red Meat	9,56	26,38	252,19	175,94	2,69	10,30	27,71	282,90	4,57	17,73	80,96	288,30	4,21	18,55	78,10	340,62	3,33	8,70	29,00	161,00
Milk	0,55	1,93	1,06	250,91	0,23	0,90	0,20	300,00	0,54	1,61	0,87	198,15	0,54	1,84	0,99	240,74	0,40	0,89	0,36	122,50
Egg	0,84	3,32	2,79	295,24	0,76	2,99	2,26	296,03	1,64	4,81	7,89	193,29	0,65	1,99	1,29	206,15	0,97	1,95	1,89	101,72
Rice	0,98	3,62	3,55	269,39	0,45	1,53	0,69	240,00	0,41	1,50	0,62	265,85	1,37	4,75	6,48	247,99	0,33	0,96	0,32	188,00
Sugar	0,21	1,14	0,24	442,86	0,22	0,89	0,20	299,10	0,53	1,43	0,76	169,81	0,53	1,54	0,82	190,57	0,23	0,65	0,15	178,57
Flour	0,26	1,31	0,34	403,85	0,32	0,99	0,32	210,34	0,18	0,94	0,17	422,22	0,79	1,61	1,26	105,04	0,33	0,92	0,31	176,00
Oil	0,88	3,14	2,76	256,82	0,95	2,50	2,39	162,05	1,29	3,98	5,13	208,53	1,10	3,55	3,91	222,73	1,20	2,53	3,04	110,83

Resource: FAO (2021)

When the Australian and Japanese countries are examined, the rate of change between producer prices and consumer prices varies between 200-322%, while this rate is between 150-180% in Turkey. This situation is also seen in basic food products (meat, sugar, rice, etc.). While the rate of change in selected developed economies was 221.98-299.29%, it was 148.37% on average in Turkey (Table 3). Therefore, it is necessary to examine all actors in the chain in order to determine the points required for improvement of the supply chain. 37 occurred (Table 3). Therefore, it is necessary to examine all actors in the chain in order to determine the points required for improvement of the supply chain. 37 occurred (Table 3). Therefore, it is necessary to examine all actors in the chain in order to determine the points required for improvement of the supply chain.

Conclusions

In addition to the competition and protective policies in the global economy, the Covid 19 epidemic and regional political crises have led to an increasing trend in food prices. The demand for food products has increased with the increasing welfare, especially in economies that are crowded and growing in population, while yield losses due to climate change, increase in input costs and the formation of monopolistic structures have caused product prices to rise rapidly. A similar situation is valid in the Turkish economy, and as of 2003, thanks to the structural and economic reforms, the demands and tastes of the consumers have changed, the production technology has been renewed, and therefore the consumption habits of the households have also changed. As a result of this, the demand for products increased with the increasing welfare, and product prices increased, especially as the demand increase in FFV products exceeded the increase in supply. In addition to these, continuous improvements have been made due to the deteriorated supply chain structure in recent years, and the supply chain has increased with new technologies and opportunities. As a result of these, both the lack of supply, production costs and the improvements in the supply chain have increased the product prices. Therefore, it can be said that there is no unfair price formation in all stages of the supply chain from production to consumption in FFV products, and price increases are experienced as a natural process. For this reason, suggestions that will take into account producer-consumer welfare in price increases are listed below:

Giving incentives for the use of substitute domestic inputs instead of imported inputs

1. Observing the supply-demand balance by making production and consumption plans
2. Developing marketing organizations where manufacturers can be competitive within the system
3. Increasing awareness for the public
4. Reorganization and inspections of wholesale markets under public supervision
5. Limitation of non-market FFV trade
6. Development of alternative transportation models
7. The price increases are weighted according to the seasons.

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COMPARISON OF FOOD SAFETY BY COUNTRY

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Abstract

As a result of the increase in food demand due to population growth and the fact that food safety is among the priority issues for many countries and international organizations, the number of scientific studies carried out to measure and understand food safety has increased. The heterogeneity of available data makes cross-country comparison difficult; encouraged international organizations to create unified and comprehensive indexes. Indices using different variables have been developed by institutions such as FAO, IFPRI, EIU, WFP, UN in order to reveal the status and developments of food security in a particular country or region, to estimate the reasons, severity and duration of food safety, and to offer solutions by determining the necessary measures. The most widely used among these indices are the Global Food Security Index (GFSI) and FAO food safety indices, and it is aimed to compare the food safety indices by country within the scope of this study. In addition, since the indices used in the study are calculated with the combination and weighting of different indicators, there are inadequacies in terms of revealing the severity of food safety and its main causes, determining risk factors and policy preferences. In this study, besides the FAO and GFSI indices, production, and marketing losses, which are accepted as important indicators of food safety, were compared according to the countries in terms of product groups. Thus, the main reasons preventing food safety were analyzed in the sense of accessibility, availability, quality, and safety dimensions. Policies were reviewed according to the determined product groups and suggestions were made for the realization of new investments in the sector.

Keywords: *Food Safety, Marketing Losses, Welfare, Agriculture, Production Losses.*

Introduction

Meeting the food needs of the increasing population quantitatively and qualitatively allows them to have a balanced and adequate diet. These nutritional conditions are important not only in terms of vital activities, but also in the development of society. Adequate and balanced nutrition of societies contributes to the development of social and economic welfare by increasing their business potential. However, while 22.6% of the world's population faced moderate and severe food insecurity in 2014, this rate increased to 30.4% in 2020. In terms of continents, it is 59.6% in Africa, 40.9% in Latin America and the Caribbean, and 25.8% in Asia (FAO, 2021). Therefore, food security cannot be met equally in all societies. The reasons for this are; geographical locations of countries, climate characteristics, agricultural areas, irrigation possibilities, production patterns, production potentials, their use of technology, agricultural policies, financial strength and relations with other countries. In addition to these, different measurement criteria and methods have been developed in the current conditions related to food

safety, which is important on a global scale. The excess of these parameters makes it difficult to compare heterogeneous data and countries. This situation encouraged international organizations to create unified and comprehensive indexes.
FAO, IFPRI, EIU, WFP, UN etc. Indices using different variables have been developed by institutions in order to reveal the food security situation and developments in certain countries or regions, to predict the reasons, severity and duration of food safety, to determine the necessary measures and to offer solutions (Yürekli et al., 2014; Dağdur and Olhan, 2015). The most widely used among these indices are the Global Food Safety Index (GFSI) and the FAO food safety index, which are put forward by the Economist Intelligence Unit. Within the scope of the study, it is aimed to compare countries according to GSFI. For this purpose, the status of countries in the foreground in terms of indices has been examined.

Material and Methods

In the study, the food safety index values of the countries according to GFSI were compared with the help of secondary data. Due to the global importance of the subject, other scientific studies, reports, and statistics on food safety have been used. In addition, in this study, food loss rates of strategic products (beans, eggs, corn and their products, meat, milk (except butter), rice, sugar, sunflower seeds, tomatoes and their products, wheat and their products) were determined due to their importance in terms of food safety. (FAO, 2022). The following formula was used to calculate this ratio.

$$Food\ Loss\ Rate = \frac{Amount\ of\ product\ lost}{Production\ amount\ of\ the\ product} * 100$$

Results and Discussion

In food production; soil, water, worker, seed/seedling/sapling, agricultural mechanization, temperature, precipitation, fertilizer, pesticide etc. factors are important. The quantities and qualities of these factors are limited and optimal use of these resources must be ensured to ensure food safety. However, it is not possible to achieve this balance in every country/region, depending on the distribution of resources on earth. Therefore, production planning can help ensure food safety. Food safety; It is the provision of quality supply of agricultural products, which are the raw materials of food necessary for the healthy sustainability of human life, and the hygienic accessibility of the processing and marketing organization for all income groups (Karakayacı et al., 2022). Therefore, food security lays the groundwork for social and economic security. In the UN "Sustainable Development Goals", the issue of food safety is given importance and priority is given to solving the problem of poverty and hunger, raising healthy individuals, reducing inequalities, providing access to clean water and protecting public health. (UN, 2022). Therefore, food safety must be ensured in order to maintain a healthy human life. In order to ensure food safety and access to sustainable food, the existing structure should be measured and policies should be developed accordingly. There are various organizations around the world that determine food safety with different parameters. The most common of these are the GFSI and FAO food safety index put forward by the Economist Intelligence Unit. In GFSI, it determines the criteria of accessibility, availability, quality-safety and natural resources-durability in terms of food safety by countries. Within the scope of the study, countries were compared in terms of these four sub-measurement criteria (Anonymous,2022).

Table 1 shows the values of leading countries in terms of food safety according to GFSI criteria. In the world, the average of accessibility is 66.8%, availability is 56.7%, quality-safety is 68.0%, and natural resources-resilience is 50.8%, with a general average of 60.9%. Turkey ranks 48th among countries with 65.1% in general average and has values above the world average.

Table 2. Global Food Security Index by Country (%)

No	Overall Food Security Environment		1) Affordability		2) Availability		3) Quality And Safety		4) Natural Resources & Resilience	
	Country	Skor	Country	Skor	Country	Skor	Country	Skor	Country	Skor
1	Ireland	84,00	Denmark	93,10	Singapore	82,90	Canada	94,50	Norway	76,10
2	Austria	81,30	Ireland	92,90	China	78,40	United States	94,30	Finland	75,10
3	United Kingdom	81,00	Finland	91,70	Canada	77,70	Ireland	94,00	Ireland	74,10
4	Finland	80,90	United Kingdom	91,10	Switzerland	76,90	Finland	93,80	Czech Republic	70,90
5	Switzerland	80,40	Sweden	91,00	Japan	75,70	Denmark	93,50	New Zealand	70,80
6	Netherlands	79,90	New Zealand	90,90	Austria	75,20	Sweden	92,30	United Kingdom	69,00
7	Canada	79,80	Israel	90,60	Israel	75,20	Netherlands	92,20	Uruguay	68,50
8	Japan	79,30	Austria	90,50	Ireland	75,10	France	92,10	France	67,50
9	France	79,10	France	90,30	Qatar	74,40	Austria	91,20	Sweden	67,30
10	United States	79,10	Germany	90,10	Netherlands	73,70	Israel	90,70	Costa Rica	67,00
	Turkey (48th)	65,10	Turkey (67th)	67,60	Turkey (42nd)	61,60	Turkey (47th)	75,80	Turkey (27th)	56,40
	Average	60,90		66,80		56,70		68,00		50,80

Resource: Anonymous (2022)

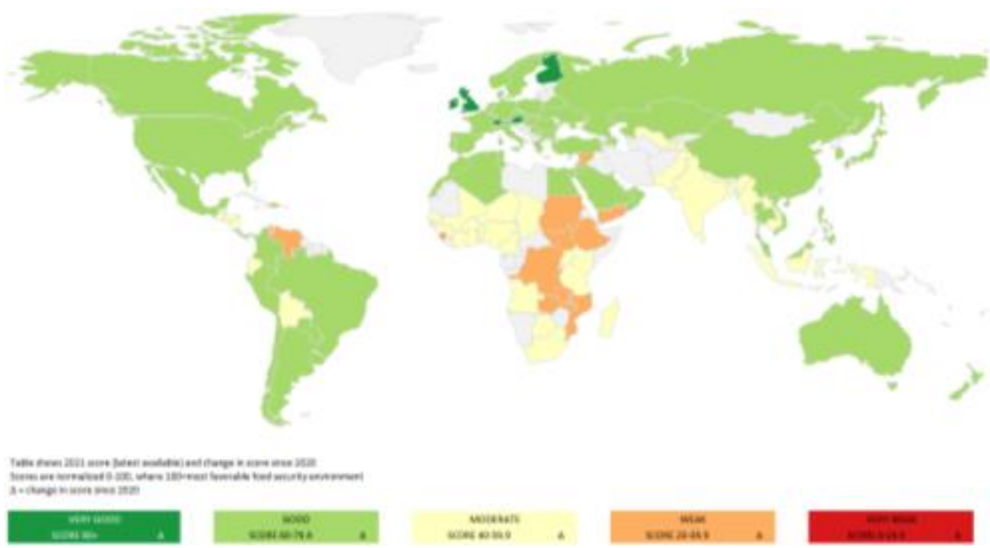

Figure 1. Mapping the Global Food Security Index by Country (2020)

Resource: Anonymous (2022)

In Figure 1, countries are mapped according to their overall averages in the global food security index. In the figure, countries with an index value between 0-19.9% are categorized as very weak, between 20.0-39.9% as weak, between 40.0-59.9% as normal, between 60.0-79.9% as good, and above 80.0% as very good. As seen in Figure 1 and Table 1, Ireland, Austria, United Kingdom, Finland, and Switzerland are very good countries according to the general food security index. In terms of accessibility, the top 10 countries (Denmark, Ireland, Finland, United Kingdom, Sweden, New Zealand, Israel, Austria, France, and Germany) are over 90.0%, while Turkey ranks 67th with 67.6%. In the availability comparison, only Singapore is the very good

country with 82.9%, while China, Canada, Switzerland, Japan, Austria, Israel, Ireland, Qatar and Netherlands are among the good countries. Turkey ranks 42nd in this category with 61.6%. Canada, United States, Ireland, Finland, Denmark, Sweden, Netherlands, France, Austria and Israel have a value of over 90.0% in quality and safety, which is another indicator, and Turkey ranks 47th with 75.8% and is among the good countries. Natural resources and resilience are the last index, and the top 10 countries are in the good countries category with a value between 67-76.1%. Turkey, on the other hand, ranks 27th with a normal rating of 56.4% (Table 1).
Turkey's global food security index for 2021 is given in Table 2 in detail. As seen in the table, an increase of 11.0% was observed in the accessibility value in the country compared to the previous year, and in 2021 it had a value close to the world average. In the sub-criteria, the highest increase compared to the previous year was found in the change in average food costs (31.0%) and food safety programs (25.0%). Due to the lack of change in the sub-criteria in availability, there was an increase of 0.9% compared to the previous year, and supply adequacy decreased by 1.1%. Dietary diversity, nutritional standards, micronutrient availability, protein quality and food safety parameters are included in the quality and safety index, and food safety has increased by 6.5% compared to the previous year. The last measurement criterion, natural resources and durability, decreased by 0.6%. The main reason for this is the decrease in the sensitivity of resources to climate change. As a matter of fact, commitment to nature is high in the agricultural sector, which is the basic building block of food production. Especially the current climatic changes have increased the need for natural resources, and this has led to a decrease in sensitivity to food security.

Table 3. Turkey's 2021 Global Food Security Index

Series	Score	Change	Rank	Average Score
OVERALL FOOD SECURITY ENVIRONMENT	**65,10**	**+3,90**	**48**	**60,90**
1) Affordability	67,60	+11,00	67	66,800
2) Availability	61,60	+0,90	42	56,70
3) Quality And Safety	75,80	+1,10	47	68,00
4) Natural Resources & Resilience	56,40	-0,60	27	50,80
1) AFFORDABILITY	**67,60**	**+11,00**	**67**	**66,80**
1.1) Change in average food costs	31,00	+31,00	=99	70,40
1.2) Proportion of population under global poverty line	97,60	-0,90	=45	73,90
1.3) Inequality-adjusted income index	64,50	-1,30	36	54,50
1.4) Agricultural import tariffs	0,00	0,00	=110	63,30
1.5) Food safety net programs	100,00	+25,0	=1	72,10
1.6) Market access and agricultural financial services	87,00	-0,10	35	63,80
2) AVAILABILITY	**61,60**	**+0,90**	**42**	**56,70**
2.1) Sufficiency of supply	72,20	-1,10	=38	58,70
2.2) Agricultural research and development	48,50	+6,20	31	42,10
2.3) Agricultural infrastructure	58,70	0,00	=37	47,50
2.4) Volatility of agricultural production	78,90	+4,40	=40	61,00
2.5) Political and social barriers to access	41,60	0,00	90	58,70
2.6) Food loss	59,50	0,00	92	73,70
2.7) Food security and access policy commitments	50,00	0,00	=24	43,80
3) QUALITY AND SAFETY	**75,80**	**+1,10**	**47**	**68,00**
3.1) Dietary diversity	56,70	0,00	=39	48,30
3.2) Nutritional standards	47,10	0,00	=93	62,10
3.3) Micronutrient availability	95,60	0,00	16	78,30
3.4) Protein quality	75,20	0,00	49	68,40
3.5) Food safety	93,10	+6,50	45	80,10
4) NATURAL RESOURCES & RESILIENCE	**56,40**	**-0,60**	**27**	**50,80**
4.1) Exposure	69,50	0,00	=42	65,00
4.2) Water	10,00	0,00	=52	19,70
4.3) Land	85,40	+0,10	=27	70,30
4.4) Oceans, rivers and lakes	4,00	0,00	105	27,40
4.5) Sensitivity	85,80	-7,30	36	69,60
4.6) Political commitment to adaptation	63,10	0,00	=28	45,30
4.7) Demographic stress	79,30	+2,50	32	59,90

Great	Good	Normal	Weak	Poor

***Source:** Anonymous (2022)

One of the risks in meeting the needs of the society in food safety is food losses. Food losses occur in the production, supply and consumption stages, and in the production stage; Factors such as wrong agricultural practices, diseases and pests, not using quality inputs, climatic events, worker practices cause food losses (Songür and Çakıroğlu, 2016; Dölekoğlu, 2017; Tahmaz and Aksoy, 2022). Food losses during the supply phase; It covers the losses that occur in the process from the product to the delivery of the product from the producer to the consumer. Food losses occurring in consumers, on the other hand, consist of losses arising from waiting for the product and not being eaten and thrown away. In Table 3, the food loss rates of selected products by country are given as of 1990 and 2019. Food loss rates are obtained by dividing the amount of production produced in the country by the amount of loss in that product. In this respect, the highest production loss in beans was experienced in Germany with 16.67% in 1990, and it is between 3.11-3.33% in Turkey. The reason for the high loss rate in Germany is the low amount of production and the proportional excess of the loss amount. While Brazil (2.5 million tons) and China (1.5 million tons) are in the top ranks in bean production, the loss rate is between 3.09-5.27%. Worldwide, there was a 4.52% loss in bean production in 1990 and a 5.80% loss in 2019. China and the USA are in the top ranks in egg production, and the rate of egg loss increased from 3.95% to 5.32% between 1990 and 2019 worldwide. In Turkey, there is a decrease, from 5.97% in 1990 to 4.98% in 2019. The USA ranks first in corn production by a large margin. On the other hand, the loss rate in the country was 4.55% in 2019, which is below the world average (5.34%). Other products selected in the comparison of food losses are meat and milk, and these products have alternative uses, The loss rate is low because it is consumed in different ways and because it is a food product, it is carefully presented to consumption. 30-35% of rice production is produced in China and the loss rate is around 4.9%. The loss rate of sunflower seeds increased in selected countries and increased from 1.55% in 1990 to 3.35% in 2019. Tomato is in perishable form and has the highest loss rate (9-10%) among the selected products. While the loss rate in wheat and its products was 5.17%, it decreased to 3.64% in 2019.

Table 4. Production Losses by Country (1990-2019) (%)

Countries	Years	Beans	Egg	Maize and Maize Products	Meat	Milk - Excluding Butter	Rice (Ground Equivalent)	Sunflower Seeds	Tomatoes and Tomato Products	Wheat and Wheat Products
Brazil	1990	3,09	3,03	10,70	0,00	5,00	12,61	0,00	10,00	11,12
	2019	4,71	3,11	10,14	0,00	9,25	10,09	7,52	8,37	4,55
Canada	1990	1,90	1,89	3,28	3,22	0,00	0,00	0,00	4,45	0,03
	2019	2,52	1,88	4,55	3,39	0,00	0,00	7,94	8,16	0,06
China	1990	3,25	5,19	8,04	0,00	5,12	5,76	2,99	7,15	6,51
	2019	5,27	5,32	4,59	0,00	5,00	3,95	3,80	10,96	2,20
France	1990	0,00	0,79	0,55	0,00	0,21	1,23	3,05	12,53	0,93
	2019	0,00	0,89	0,90	0,00	0,21	3,61	3,93	17,49	0,90
Germany	1990	16,67	1,02	3,61	0,14	0,12	0,00	0,00	160,78	2,39
	2019	0,00	1,31	2,81	0,06	0,11	0,00	2,17	114,95	2,68
Sweden	1990	0,00	1,72	0,00	0,00	2,00	0,00	0,00	25,00	2,63
	2019	0,00	2,10	0,00	0,00	0,00	0,00	0,00	47,06	3,22
Turkey	1990	3,33	5,97	4,00	0,00	5,93	3,92	3,02	15,00	16,73
	2019	3,11	4,98	3,53	0,00	4,69	3,20	3,48	15,00	9,83
United Kingdom of Great Britain and Northern Ireland	1990	0,00	1,43	0,00	0,00	0,00	0,00	0,00	3,60	1,95
	2019	0,00	1,23	0,00	0,00	0,00	0,00	0,00	3,28	0,50
USA	1990	0,00	2,31	0,00	0,00	0,00	11,14	0,00	5,16	0,00
	2019	0,00	2,03	4,55	0,00	0,00	3,90	6,24	8,37	4,54
World	1990	4,52	3,95	4,82	0,71	2,59	5,42	1,55	9,17	5,17
	2019	5,80	5,32	5,34	0,53	2,95	4,18	3,35	9,51	3,64

*Source: FAO, 2022.

Conclusions

Despite the population's tendency to increase, the limited production factors create the problem of hunger and the number of people struggling with hunger is increasing day by day. Hunger and malnutrition, on the other hand, lead to the proliferation of chronic diseases and reduce the quality of the workforce. In addition, the high dependence on nature in agriculture and the current climate change cause migration to the city, which increases the pressure on cities and leads to unplanned urbanization. The increase in urbanization creates the basis for the loss of quality of agricultural lands and the reduction of agricultural lands. As a result of these, the level of meeting the food needs of the society decreases and the risk of food safety occurs. Another factor that is effective in increasing this problem is food waste and losses. Food waste and losses; It takes place at the stages of the value chain, including agricultural enterprises, and at the consumer table. With the effect of food losses and climate change, there is excessive use of resources (pharmaceuticals, fertilizers, etc.) in agricultural production. This situation causes water and soil pollution and prevents the production of high quality and efficient products. The low quantity and quality of the products is insufficient to meet the demand. High demand and product production can be affected by climate change, non-purpose land use, food loss, etc. The fluctuations of food products over the years, depending on the reasons, cause volatility in the prices of food products. This situation puts pressure on the purchasing behavior of consumers. The following suggestions have been developed to solve these problems. These are;

- Population planning should be done,
- Production planning should be done in order to increase the quality and quantity in production,
- Food production with high nutritional value should be ensured,
- The use of early warning systems in the fight against climate change should be expanded,
- Alternative job opportunities to the agricultural sector in rural areas should be established
- Misuse of agricultural lands should be limited,
- Policies to increase the competence levels of countries should be developed,
- Action plans with universal validity should be established,
- International food markets should be improved,
- Purchasing power of consumers should be increased,
- Effective management of natural resources should be ensured,
- Community awareness should be raised to reduce food waste and losses.

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THE EFFECT OF AGRICULTURAL INPUT PRICES ON FARMERS' PURCHASE POWER

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Abstract

The sustainability of agricultural production depends on the costs of the farmers in the production processes and the income they earn after production. The increase in the price of the inputs used in agricultural production causes a decrease in profit margins and adversely affects production. In general, the course of price changes in the markets is followed by price index values. Price indices are important for understanding the purchasing power of farmers and monitoring the current conditions in the sector. In this study, the main purpose is to examine the changes in the purchasing power of farmers according to products and years and to determine the effect of price changes on agricultural production. For this purpose, secondary data were used in the study; in the selection of the data, 45 products, which have a significant weight in the inflation basket, were selected. Producer prices and input price indices of selected products were compiled from TURKSTAT. The changes in the purchasing power of the farmers according to the products and years were calculated. Changes in the purchasing power of farmers contain important clues in terms of determining social welfare in rural areas and sustainability of agricultural production. By evaluating the results obtained in these aspects, discussions were made on the current situation, and policy recommendations were developed for agricultural input markets and the purchasing power of farmers.

Keywords: *Farmer, Price, Welfare, Purchasing Power, Agriculture.*

Introduction

Agricultural production activities; It makes rural life sustainable by increasing social welfare and farmer incomes by meeting food needs. With the effect of globalization, micro components are needed to be effective in order to be sustainable in macro terms. Profit margins of businesses that are micro components of economies; can provide competitive advantage by reducing and controlling costs (Özçelik and Avcı Öztürk, 2019). The continuation of the activities of agricultural enterprises is shaped by the changes in input costs. Reducing input costs and increasing profit margins in agricultural enterprises will increase the willingness of farmers towards production activities.

At the same time, the cost of agricultural production; It is also important to determine the level of use of physical inputs (fuel oil, pesticides, fertilizers, etc.) used in enterprises, to plan the workforce and mechanization, to make financial programs and to maintain the budget balance (Demircan et al. 2005). Stability in input prices is important for the sustainability of production, and prices vary depending on supply and demand. In addition to the effect of changes in prices on the use of resources on the supply side, negative effects of the market such as inflation may also be effective in determining product prices (Yavuz, 2021). Within the scope of this study, the index values of producer prices and input price index values calculated by the Turkish Statistical

Institute (TURKSTAT) were used. These values have a direct impact on the welfare of the producers and provide information in terms of the sustainability of agricultural enterprises.

Material and Methods

The main purpose of this study is to examine the changes in the purchasing power of farmers by years and selected products. For the determined purpose, all kinds of printed materials, reports, statistics and publications related to the subject were examined. Within the scope of the study, 45 products, which have a significant weight in the inflation basket, were selected. Determined products; Producer prices and input price indices were compiled from the Turkish Statistical Institute (TURKSTAT), and the changes in the purchasing power of the farmers according to the products and years were calculated. The change in the purchasing power (PPOF) of the farmers was calculated with the help of the following formula.

$$PPOF = \frac{Price\ Index\ of\ Goods\ Sold\ by\ Farmer}{Price\ Index\ of\ Goods\ Purchased\ by\ Farmer}$$

Reference: Güneş, 1996; Oğuz and Bayramoğlu, 2018.

The calculated rate also expresses the effect of price fluctuations on farmers. If the value obtained with this formula is greater than 1, it can be said that the purchasing power of the farmer increases for the relevant product, and if it is less than 1, the purchasing power of the farmer for the relevant product weakens. Therefore, it can be said that as a result of the calculation of the purchasing power of the farmers, important indicators for rural development and social welfare are obtained.

Results and Discussion

Producer Price Index (PPI); It refers to the price changes that occur in the input prices of products that are subject to domestic trade, produced for the economy of a country in a certain period (Terzi and Tütüncü, 2017). While calculating the index values of the data used in the study, the year 2005 was taken as the basis and the index value was accepted as 100 (Table 1). The index value for wheat, which was determined as 106.25 in 2016, was calculated as 261.46 in 2021. Producer prices of wheat increased by 59.36% as of the years examined. As of April 2022, the increase in producer price increased to 80.60% in wheat. When the course of the wheat price increase and the periodic changes in the last year are examined, it is possible to say that the Covid-19 epidemic period has an impact on food prices. With the effect of the Covid-19 global epidemic; Concerns about access to food have arisen due to the negativities that will occur for the agricultural food chain, the decrease in the wages of the workforce and the loss of income in small businesses, the rapid price increases caused by the changes in food demand and supply (Torun Kayabaşı, 2020). In addition, the increase in food demand in the same period caused prices to rise, therefore, concerns about world food supply and security increased (Abbott et al., 2008; Esmaeili and Shokoohi, 2011; Güngör and Erer, 2022). While these developments and sudden changes in the market increased the importance of food, they also caused an increase in producer prices. As of the years examined, there has not been a product with a decreasing producer price index. In the fresh fruit and vegetables category, the value of producer prices showed a higher change. When these products are compared with other agricultural products such as grains; As a result of their faster deterioration, shorter storage times and higher investment costs in the process of converting them into value-added products, price changes are higher.

Table 5. Agricultural Products Producer Price Index for Selected Items by Years (2005=100)

Products	2016	2017	2018	2019	2020	2021	2022 January	2022 February	2022 March	2022 April
Wheat	106,25	116,76	123,45	155,56	192,38	261,46	386,31	456,12	482,46	547,74
Maize	104,02	115,79	131,31	159,20	188,38	263,86	356,32	453,36	545,03	611,10
Barley	105,07	121,66	131,43	163,81	194,60	285,79	438,43	455,16	519,11	588,34
Haricot bean	99,92	111,26	125,80	161,76	209,40	231,37	277,88	285,06	283,45	338,92
Sunflower	107,99	123,26	136,86	159,96	235,99	322,01	398,54	427,99	507,44	581,59
Rice	89,02	112,49	139,04	179,02	226,38	251,19	322,50	344,58	364,34	449,91
Green beans	106,62	118,32	110,19	128,89	125,56	194,27	343,82	526,50	589,45	840,45
Cauliflower	98,37	90,81	88,30	132,83	137,25	169,47	170,32	268,78	321,98	-
Cabbage	105,06	102,63	99,48	147,59	139,35	173,77	191,81	233,95	265,51	-
Lettuce	104,07	96,54	106,62	157,04	135,94	170,67	182,53	250,01	310,83	542,81
Watermelon	122,27	114,75	153,31	182,23	241,16	258,26	-	-	-	-
Melon	115,29	108,81	133,41	157,99	210,89	225,99	-	-	-	-
Tomato	88,75	102,19	114,61	140,02	128,21	129,15	185,20	274,54	292,52	656,24
Cucumber	105,92	129,87	123,87	146,81	140,71	180,20	397,70	562,96	560,12	609,22
Pepper	97,40	113,10	96,53	156,03	154,09	154,74	458,18	584,80	717,32	876,91
Eggplant	85,75	108,80	99,64	149,50	129,96	159,77	383,04	548,75	727,50	765,96
Onion	113,57	79,97	137,98	211,01	158,21	126,51	116,83	121,94	169,41	230,38
Carrot	83,72	86,53	99,07	136,94	126,57	185,16	211,57	264,17	383,88	370,34
Potato	54,30	61,38	85,91	141,98	102,30	99,93	221,76	235,92	278,03	358,86
Sugar beet	115,85	121,39	125,66	141,33	182,32	217,04	226,01	226,01	226,01	226,01
Raw tobacco	111,04	131,77	151,13	158,48	184,31	205,20	215,68	215,68	215,68	215,68
Cotton unseed	126,70	158,11	179,56	203,23	228,72	361,39	727,99	880,62	938,01	1192,69
Grapes	98,38	101,90	104,03	154,66	193,99	203,12	-	-	-	-
Fig	96,64	115,64	139,22	168,71	192,15	284,90	-	-	-	-
Banana (Local)	94,12	115,82	139,79	192,37	234,06	248,33	-	-	-	-
Orange	96,05	113,35	127,00	167,85	237,06	302,61	297,54	324,76	373,41	391,90
Mandarin	95,40	107,48	118,60	137,09	187,79	231,51	322,07	405,34	-	-
Lemon	130,69	146,38	133,15	159,23	197,24	143,63	151,78	193,04	-	-
Apple	90,34	105,65	119,43	154,12	194,29	218,29	-	-	-	-
Pear	95,42	97,19	93,00	129,11	163,04	210,56	-	-	-	-
Peach	96,89	98,03	89,75	129,29	175,19	245,94	-	-	-	-
Plum	97,54	112,44	90,58	153,85	160,70	208,25	-	-	-	-
Apricot	96,29	88,68	109,98	139,74	160,49	251,41	-	-	-	-
Cherry	108,51	129,16	94,66	153,55	175,38	192,06	-	-	-	-
Strawberry	92,50	107,05	123,68	165,50	168,02	212,24	-	-	-	518,31
Hazelnut	68,68	63,59	68,74	104,86	138,45	144,60	186,35	209,90	219,14	217,88
Walnut	106,61	107,31	113,88	133,16	142,72	166,80	188,78	196,70	205,39	221,40
Pistachio	85,13	111,20	166,27	202,14	234,78	235,98	341,71	344,75	349,46	379,27
Pomegranate	89,43	103,17	119,12	182,70	208,02	255,88	274,05	293,63	-	-
Oil	114,64	118,85	134,32	149,24	162,10	187,57	225,51	227,24	242,79	303,16
Tea leaf	114,03	127,23	137,19	180,85	208,18	245,21	258,57	258,57	258,57	258,57
Cows’ milk	97,60	104,98	127,11	152,99	189,47	238,61	357,96	375,25	390,97	420,17
Ewe’s milk	105,20	113,08	128,96	148,68	175,44	233,62	250,53	261,08	267,03	317,79
Goats’ milk	104,39	110,74	123,26	152,62	189,73	242,55	294,25	302,65	312,25	346,22
Egg	106,27	113,84	130,21	136,05	141,12	187,32	265,13	289,37	321,07	320,73

Reference: TURKSTAT (2022)

Changes in agricultural input prices are analyzed in Table 2. Under the heading of energy and oils; diesel, electricity, coal, wood, oil and LPG. By the years examined, the largest proportional price change occurred in diesel. This had an impact on the energy and fats heading in general. Under the heading of fertilizers and soil improvers, plain and compound fertilizers; Fungicides, herbicides, insecticides and acaricide are included under the title of agricultural pesticides. In animal feeds, there are coarse and concentrated feeds. There has been an increase in the prices of all inputs over the years examined. The highest increase was in the fertilizer and soil improvers category. While the average index value of fertilizers and soil developers was 91.68 in 2016, it became 767.50 as of April 2022, with an average of 8 times increase. Fertilizers in plant production and feeds in animal production are important cost items for producers. It is estimated that 17% of the input costs in crop production consist of fertilizers on average (Çobanoğlu et al., 2021). Therefore, increased input costs are directly reflected in the price of agricultural products. If the prices of agricultural inputs and agricultural products cannot increase linearly, losses occur for the welfare of the producers.

Table 6. Agricultural Input Price Index by Years (2015=100)

	2016	2017	2018	2019	2020	2021	**2022 January**	**2022 February**	**2022 March**	**2022 April**
Agriculture-IPI (Input 1 + Input 2)	103,52	113,09	136,04	156,99	172,45	220,54	306,24	357,36	411,54	442,21
Goods and services used in agriculture (Input 1)	102,78	112,02	134,29	154,80	169,39	217,41	307,10	361,43	419,69	451,32
Seed and planting material	104,66	113,24	125,55	149,92	159,59	174,68	178,88	214,06	226,36	238,63
Energy and fats	102,72	119,71	148,88	175,54	178,00	218,22	395,88	437,11	539,05	560,86
Fertilizer and soil improvers	91,68	92,55	121,85	154,72	160,68	272,39	484,19	482,18	697,49	767,50
Pesticide	109,89	114,17	130,51	144,90	144,83	164,71	213,19	283,02	295,92	306,98
Veterinary expenses	102,52	107,65	114,12	120,49	118,46	136,30	161,05	160,70	174,34	171,11
Animal feed	103,57	111,12	133,04	149,93	170,66	220,20	283,46	371,76	417,34	454,23
Machine maintenance costs	106,59	124,79	158,98	178,42	212,54	278,37	428,71	444,22	456,35	471,20
Building maintenance costs	101,89	112,65	142,75	165,24	178,19	250,22	393,14	425,75	477,36	529,72
Other goods and services	103,74	114,10	129,92	150,50	167,05	200,09	242,14	255,46	263,91	286,24
Goods and services that contribute to agricultural investment (Input 2)	108,68	120,52	148,21	172,15	193,66	242,26	300,26	329,08	354,90	378,88
Materials	107,68	117,33	142,64	165,28	185,76	218,77	240,05	269,14	287,71	307,16
Farm buildings (non-residential)	111,63	129,91	164,62	192,39	216,96	311,47	477,67	505,70	552,86	590,19

Reference: TURKSTAT (2022)

In Table 3, the changes in the purchasing power of the farmer obtained as a result of the ratio of the producer price index and the input price index are examined. A value greater than 1 was obtained for 17 products out of 45 selected products. Farmer purchasing power has increased for these 17 products. The product with the highest increase in purchasing power over the average values was sunflower. Despite the increase in input prices, the ability of the product to be converted into high value-added products and the fluctuations in oil prices contributed to the increase in farmer incomes. The most disadvantaged product in terms of weakening purchasing power is potato with 0.65. The product has disadvantages due to the fact that the price of the potato is kept constant within the speculative discourses, and the appropriate market conditions and infrastructure cannot be established despite its storage feature. In a study conducted by Erdal and Gürler (2003) the effect of inflation on agricultural products in Turkey was examined on the basis of inputs. In the study, it was determined that the plant product that suffered the most from inflation was sugar beet and the animal product was egg. While the value of purchasing power in sugar beet was 1.12 in 2016, it decreased to 0.51 in April 2022 and took an average value of 0.85. The purchasing power of eggs, which was 1.03 in 2016, increased to 0.73 in the last month and 0.87 on average for 2022. For sugar beet and egg producers, the decline in purchasing power generally continued and producer welfare was lost.

Table 7. Changes in Farmer Purchasing Power by Years and Products

Products	2016	2017	2018	2019	2020	2021	2022 January	2022 February	2022 March	2022 April	Average
Wheat	1,03	1,03	0,91	0,99	1,12	1,19	1,26	1,28	1,17	1,24	1,12
Maize	1,00	1,02	0,97	1,01	1,09	1,20	1,16	1,27	1,32	1,38	1,14
Barley	1,01	1,08	0,97	1,04	1,13	1,30	1,43	1,27	1,26	1,33	1,18
Haricot bean	0,97	0,98	0,92	1,03	1,21	1,05	0,91	0,80	0,69	0,77	0,93
Sunflower	1,04	1,09	1,01	1,02	1,37	1,46	1,30	1,20	1,23	1,32	1,20
Rice	0,86	0,99	1,02	1,14	1,31	1,14	1,05	0,96	0,89	1,02	1,04
Green beans	1,03	1,05	0,81	0,82	0,73	0,88	1,12	1,47	1,43	1,90	1,12
Cauliflower	0,95	0,80	0,65	0,85	0,80	0,77	0,56	0,75	0,78	-	0,77
Cabbage	1,01	0,91	0,73	0,94	0,81	0,79	0,63	0,65	0,65	-	0,79
Lettuce	1,01	0,85	0,78	1,00	0,79	0,77	0,60	0,70	0,76	1,23	0,85
Watermelon	1,18	1,01	1,13	1,16	1,40	1,17	-	-	-	-	1,18
Melon	1,11	0,96	0,98	1,01	1,22	1,02	-	-	-	-	1,05
Tomato	0,86	0,90	0,84	0,89	0,74	0,59	0,60	0,77	0,71	1,48	0,84
Cucumber	1,02	1,15	0,91	0,94	0,82	0,82	1,30	1,58	1,36	1,38	1,13
Pepper	0,94	1,00	0,71	0,99	0,89	0,70	1,50	1,64	1,74	1,98	1,21
Eggplant	0,83	0,96	0,73	0,95	0,75	0,72	1,25	1,54	1,77	1,73	1,12
Onion	1,10	0,71	1,01	1,34	0,92	0,57	0,38	0,34	0,41	0,52	0,73
Carrot	0,81	0,77	0,73	0,87	0,73	0,84	0,69	0,74	0,93	0,84	0,79
Potato	0,52	0,54	0,63	0,90	0,59	0,45	0,72	0,66	0,68	0,81	0,65
Sugar beet	1,12	1,07	0,92	0,90	1,06	0,98	0,74	0,63	0,55	0,51	0,85
Raw tobacco	1,07	1,17	1,11	1,01	1,07	0,93	0,70	0,60	0,52	0,49	0,87
Cotton unseed	1,22	1,40	1,32	1,29	1,33	1,64	2,38	2,46	2,28	2,70	1,80
Grapes	0,95	0,90	0,76	0,99	1,12	0,92	-	-	-	-	0,94
Fig	0,93	1,02	1,02	1,07	1,11	1,29	-	-	-	-	1,08
Banana (Local)	0,91	1,02	1,03	1,23	1,36	1,13	-	-	-	-	1,11
Orange	0,93	1,00	0,93	1,07	1,37	1,37	0,97	0,91	0,91	0,89	1,04
Mandarin	0,92	0,95	0,87	0,87	1,09	1,05	1,05	1,13	-	-	0,99
Lemon	1,26	1,29	0,98	1,01	1,14	0,65	0,50	0,54	-	-	0,92
Apple	0,87	0,93	0,88	0,98	1,13	0,99	-	-	-	-	0,96
Pear	0,92	0,86	0,68	0,82	0,95	0,95	-	-	-	-	0,86
Peach	0,94	0,87	0,66	0,82	1,02	1,12	-	-	-	-	0,90
Plum	0,94	0,99	0,67	0,98	0,93	0,94	-	-	-	-	0,91
Apricot	0,93	0,78	0,81	0,89	0,93	1,14	-	-	-	-	0,91
Cherry	1,05	1,14	0,70	0,98	1,02	0,87	-	-	-	-	0,96
Strawberry	0,89	0,95	0,91	1,05	0,97	0,96	-	-	-	1,17	0,99
Hazelnut	0,66	0,56	0,51	0,67	0,80	0,66	0,61	0,59	0,53	0,49	0,61
Walnut	1,03	0,95	0,84	0,85	0,83	0,76	0,62	0,55	0,50	0,50	0,74
Pistachio	0,82	0,98	1,22	1,29	1,36	1,07	1,12	0,96	0,85	0,86	1,05
Pomegranate	0,86	0,91	0,88	1,16	1,21	1,16	0,89	0,82	-	-	0,99
Oil	1,11	1,05	0,99	0,95	0,94	0,85	0,74	0,64	0,59	0,69	0,85
Tea leaf	1,10	1,13	1,01	1,15	1,21	1,11	0,84	0,72	0,63	0,58	0,95
Cows' milk	0,94	0,93	0,93	0,97	1,10	1,08	1,17	1,05	0,95	0,95	1,01
Ewe's milk	1,02	1,00	0,95	0,95	1,02	1,06	0,82	0,73	0,65	0,72	0,89
Goats' milk	1,01	0,98	0,91	0,97	1,10	1,10	0,96	0,85	0,76	0,78	0,94
Egg	1,03	1,01	0,96	0,87	0,82	0,85	0,87	0,81	0,78	0,73	0,87

Reference: Calculated by the authors.

Conclusions

For the sustainability of agricultural production, first of all, it is necessary to protect farmer incomes and create social welfare for rural areas. Within the scope of this study, what needs to be done in terms of determining the purchasing power of farmers and sustainability of production has been revealed. Sunflower (1,20) and corn (1,18) are products that have contributed to the purchasing power of the farmer despite the increasing input costs due to their industrial usage areas. It is thought that the production of these plants will continue due to their contribution to purchasing power compared to other selected products. However, a production planning should be made for these products, taking into account the environmental effects such as the high irrigation needs in the production processes.

Hazelnut is one of the products with the lowest average value in terms of purchasing power. This situation creates a disadvantage for Turkey, which ranks first in the world in hazelnut (0.61) production. The decrease in purchasing power will cause the welfare to be adversely affected, and the producers to turn to alternative income sources and to break away from agriculture over time. For this reason, it is necessary to take early measures and to encourage investments in this field by evaluating the advantages of Turkey, especially in terms of value-added production.

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RAINFED CROP PROJECTIONS IN THE SEMI-ARID UNDER SCENARIOS OF PLUVIOMETRIC INSTABILITY IN CEARÁ STATE IN BRAZIL

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Abstract

This paper aimed to analyze the projections of rainfed crops (rice, beans, cassava and corn) in the semi-arid region under scenarios of rainfall instability in Ceará, in the period from 1945 to2020. The data were collected from the Meteorology and Water Resources Foundation of Ceará (FUNCEME) and the Brazilian Institute of Geography and Statistics (IBGE), through the IBGE System of Automatic Recovery - SIDRA, which provides data on Municipal Agricultural Production (PAM - 2020). The rainfall periods were organized in: drought; normal and rainy. This was done using the historical average and standard deviation of the rainfall from 1945 to 2020. The projections of production variables were made through the Autoregressive Integrated Moving Average (ARIMA) methodology, developed by Box and Jenkins (1976). The results show that the rainfall distribution in Ceará State, between 1945 and 2020, was quite unstable, with the Coefficient of Variation (CV) of the rainfall periods ranging between 33% in normal period to 54% in the drought period. Based on the proposed study we can confirm the general hypothesis, concluding that rainfall has an important impact on rainfed agricultural productionin Ceará State, especially in the variables: harvested area, yield and prices of rice, beans and corn crops, interfering in their average and thus influencing the income generation of farmers in the State. In the particular case of cassava, it was possible to conclude that due to the characteristics of its production, where it adapts well to climatic adversities, the interference ofrainfall.

Keywords: *Dryland Agriculture, Semi-arid, Forecast, Rainfall Instability, Drought.*

Introduction

The aggressive conditions of the semi-arid climate, with rainfall concentrated, ingeneral, in the first three to four months of the year, with high temperatures, and with predominantly low relative humidity, make agricultural practices difficult, especially when performed without the use of appropriate technologies. Another characteristic of this climatic regime is its instability in rainfall precipitation both spatially and temporally (Carvalho, 2014; Lemos, 2020; Melo, et al., 2019; Mohammed; Scholz, 2019; Salviano, 2021).

In general, dry land agricultural activities that depend exclusively on rainfall prevail in the Brazilian semi-arid region. Soil and water management in areas where rainfed agriculture prevails are the main constraints in maintaining sustainable production and productivity of these crops (Rockstrom et al., 2010; Mohinder et al., 2017; Wani, et al., 2009).

The dry land agriculture practiced by Ceará farmers, mostly family farmers (Lemos et al., 2020), who do not use irrigation, genetically modified seeds adapted to the hostile conditions of the semi-arid environment, make this type of activity in this state to have difficulties in its conduct and be an economically profitable activity. Therefore, the rainfall instability is a phenomenon

that is directly related to the difficulties in the production of these dry land crops, as are, in large scale, the productions of rice, beans, cassava, and corn (Costa Filho, 2019; Fischer et al., 2002; Lemos et al., 2020; Mendelsohn, 2009; Pereira, 2018;
Rosenzweig; Hillel, 2005; Salviano, 2021; Thornton et al., 2008; Wani et al., 2009).
According to the last definition of the Semi-arid occurred in December 2021, the Semi- arid region now has 1,427 municipalities. Of the 184 municipalities in the state of Ceará, 171 are currently recognized by the Federal Government as being inserted in this climatic regime. Ceará is the Brazilian state with the largest relative area and population within this climatic regime (BRASIL, 2021).
Given the contextualization of the problem addressed in this paper, it is necessary to raise the following question: i) can forecasts of production defining variables (harvested area, land productivity and price) of rice, beans, cassava and corn in the semi-arid region of Ceará State, capture the exogenous effect of the instability of rainfall?
Given these considerations, this study aims to: i) assess the descriptive statistics of the variables involved in the production of rice, beans, cassava and corn in Ceará State between 1945 and 2020; ii) estimate how the producers of Ceará State prepared their projections about the harvested area, productivity and average price of these crops in the period between 1945 and 2020; and iii) measure the impact of rainfall on the forecast models estimated for the production of agricultural crops under analysis.

Material and Methods

The data related to rainfall (C_t) was collected from the Meteorological and Hydrological Resources Foundation of Ceará State (FUNCEME). The ones regarding harvested areas (A_t), productivity (R_t) and prices (P_t) of the crops were collected from the Brazilian Institute of Geography and Statistics (IBGE). The period of study covers the interval between 1945 and 2020. Prices in Brazilian currency were corrected to 2020 values and converted to American Dollar of 2020.

Measurement of instabilities associated with the studied variables

The stability/instability of the selected variables was measured by the Coefficient of Variation (CV). The greater the magnitude of the CV, the more unstable or more heterogeneous will be the distribution of observed values of a random variable around its mean (Garcia, 1989;Gomes, 1985; Santos; Dias, 2021; Sorensen, 2000).
It is worth noting that in order to use the CV as a measure of homogeneity orheterogeneity of a distribution, it is necessary to establish the parameters of its critical values. In this conception, Gomes (1985) set limits for the classification of the calculated CVs, analysis performed for the standard of agricultural experimentation, as described in Table 1.

Table 1. Classification of CV levels according to their ranges

Classification of CV	Ranges of CV
Low	CV < 10%
Medium	10% ≤ CV < 20%
High	20% ≤ CV < 30%
Very high	CV ≥ 30%

Source: Gomes (1985).

Used models to make the projections of the decision variables

A time series is a group of observations ordered in time, and that exhibit serial dependence. In time series analysis, some concepts are relevant for the preparation of forecasting models. In this conception, it is worth emphasizing that a stochastic process is configured as a collection of random variables ordered in time (Gujarati and Porter, 2011).
Thus, considering the time series represented by the random variable Y_t. Its expected value $E(Y_t)$ will be different from the observed value due to the occurrence of random factors (ξ_t) along its path. This information can be summarized by Equation (1).

$$Y_t = E(Y_t) + \xi_t \tag{1}$$

This study aims to assess the impact of annual rainfall to predict the variables: harvestedareas, yields and prices of the rainfed crops studied. The hypothesis to be tested is that these impacts will occur on the noises (ξ_t) generated in the forecast model Equation (2):

$$\xi_t = f(C_t) \tag{2}$$

Substituting this value of ξ_t into Equation (1) yields the result that will be tested in this research:

$$Y_t = E(Y_t) + f(C_t) \tag{3}$$

The values of $E(Y_t)$ are estimated in this research using the Autoregressive Integrated Moving Average (ARIMA) process developed by Box Jenkins (1976). Following is a brief explanation of the ARIMA method as it applies to this study.

Brief summary of the ARIMA model

This model is suitable for time series that are stationary, or variables whose means, variances, and autocovariances are constant over time (Gujarati: Porter, 2011; Wooldrige, 2013). Admitting that the time series Y_t can be represented by Equation (4):

$$Y_t = \mu + \Sigma\psi_k u_{(t-k)} = \mu + \psi(B)u_t \tag{4}$$

Where the definition of the linear filter (ψ) is represented as follows:

$$\psi(B) = \theta(B)/\phi(B) \tag{5}$$

Equation (5) can be evidenced from the definition of the polynomials described below:

$$\theta(B)=1-\theta1B-\theta2B^2-....-\theta qB^q$$

and

$$\phi(B)= 1-\phi1B-\phi2B2-...-\phi pB^p$$

For this condition, Box et al. (2016) state that: 1) $\phi(B)$will be called an autoregressive operator; it is considered stationary, the roots of $\phi(B)= 0$ are outside the unit circle; 2) $\psi(B)$ will be called a generalized autoregressive operator, i.e., a non-stationary operator with "d" roots of $(B) = 0$ equal to unity, i.e., "d" unit roots; and 3) (B) will be called the moving average operator; it is assumed to be invertible and that the roots of $(B) = 0$ are outside the unit circle.Given the previous demonstrations, we define $\tilde{Y}_t = Y_{t-\mu}$, so it will be possible to obtain its transformation, as shown in Equation (6):

$$\phi(B)\tilde{Y}_t = \theta(B)u_t \tag{6}$$

For the definition of Equation (6), the random term "u_t" must present the following characteristics to be considered "white noise" (Cochrane, 1997):

i) $E(u_t)=0$; ii) $E(u_t^2)=\sigma_u^2 < \infty$; e iii) $E(u_t,u_{t+k})= 0$, when $k=\pm1,\pm2, ...$

Based on this set of information Equation (6) can be rewritten and is known as the p-order autoregressive and q-order moving average or ARMA (p,q) as shown in Equation (7):

$$\tilde{Y}_t = \theta(B)\phi^{-1}(B)u_t \tag{7}$$

When the series is not stationary, it is necessary to provide this characteristic by performing differentiations on the time series. In general, with up to three differentiations it is possible to transform into stationary a series that was shown to be no stationary (Box, Jenkins, 1976; Gujarati; Porter, 2011: Greene, 2012; Gujarati; Porter, 2011; Wooldrige, 2013).
The steps to predict the values of a time series thru the Box-Jenkins model it is necessary to examine if the series is stationary. To better fit the models, the present research used some criteria to achieve the proposed objectives. One of them is that the smaller the number of parameters, the better is the model adjustment. Besides this, other parameters were used: unit root test - Augmented Dickey-Fuller (ADF); coefficient of determination (R^2); percentage of the absolute average error (MAPE); Ljung-Box Q statistic; and Pearson's correlation coefficient between the observed series and the series projected by the estimated models (Box; Jenkins, 1976; Box et al., 2016; Greene, 2012; Wooldridge, 2013).

Relationship between the forecast models and rainfall in the state of Ceará

This research admits that, in the forecast scenario the shocks ξ_t that appear in Equation (2) were caused by exogenous variable: rainfall (C_t) as shown in Equation (8)

$$\xi_t = \lambda_0 + \lambda_1 C_t + \upsilon_t \tag{8}$$

In Equation (8) the coefficient λ_0 represents the linear parameter; λ_1 is the angular coefficient which, being statistically different from zero, gauges the sensitivity of the error termof the forecast model (ξ_t) to the oscillations of the annual rainfall (C_t). The random term υ_t, by hypothesis, is also endogenously "white noise" in Equation (8).

Results and Discussion

Initially, the descriptive statistics associated with the annual rainfall occurring in Ceará between 1945 and 2020 were estimated, as well as those referring to the harvested areas, yields, and prices of rice, beans, cassava, and corn. These results are shown in Table 2.

Table 2. Descriptive statistics associated to the research variables

Variables	**Mínimum**	**Máximum**	**Average**	**CV (%)**
Annual rainfall (mm)	286,90	1773,40	777,76	33,56
Harvested area with rice (ha)	5250,00	79993,00	40532,01	45,34
Yield per hectare of rice ($kg.ha^{-1}$)	409,68	3130,82	1939,54	33,87
Average price of rice (U$S.$kg^{-1}$)	0,17	1,14	0,52	43,94
Harvested area with bean (ha)	74775,00	765654,00	381713,85	42,14
Yield per hectare of bean ($kg.ha^{-1}$)	116,85	608,22	340,80	40,44
Average price of bean (U$S.$kg^{-1}$)	0,41	2,91	0,93	54,43
Harvested area with cassava (ha)	32283,00	176000,00	89810,80	39,75
Yield per hectare of cassava ($kg.ha^{-1}$)	3356,92	16905,08	10178,85	32,25
Average price of cassava (U$S.$kg^{-1}$)	0,00	0,23	0,08	51,15
Harvested area with corn (ha)	78460,00	726777,00	433491,71	39,21
Yield per hectare corn ($kg.ha^{-1}$)	120,00	1254,14	646,66	42,3
Average price of corn (U$S.$kg^{-1}$)	0,10	0,67	0,32	41,53

Source: Prepared by the authors based on data from FUNCEME (2022) and (IBGE, 2020). Note: Prices were deflated having 2020 as base year.

From the evidence shown in Table 2, one can infer the high instability observed in the annual precipitation observed for Ceará between 1945 to 2020, captured by the CV of the orderof 33.6% classified as "very high" in the scale designed by Gomes (1985). It is observed that this rainfall instability was transmitted in synergy to all variables studied, all classified as "very high" according to that reference, with CVs ranging from 32.2% for the annual productivity ofcassava to 54.5% for the average price of beans (Table 2).

Results found in the estimation of ARIMA models to perform the forecasts

The results found in the estimations of the parameters of the forecast models are shownin Table 3. The results presented in Table 3 suggest that only the cassava harvested area series was originally stationary. The other series were not stationary and needed to be process to be stationary. In all cases, required only one difference (d = 1). Overall, the estimated models are parsimonious with respect to the number of estimated parameters.
It is observed that all estimates show statistically non-significant Ljung Box statistics, at least with 10% error. This ensures that the noise generated in all estimates is random. In all the estimated models it was observed that the linear parameters were not statistically different from zero. From the evidences shown in Table 3, it also appears that the percentages of the mean absolute errors (MAPE), also presented relatively low magnitudes, with the smallest estimated value for the rice price series (15.476). However, it was observed that in the best adjustment for the cassava price forecast the estimated MAPE was 101.726, quite high. This suggests caution in using these estimates to forecast cassava prices, although they were the bestestimates found in the survey. The research also estimated the correlation coefficients betweenthe observed values and the predicted ones. All these correlations were statistically different from zero with an error of at least 1%.

Table 3. Models fitted to the forecasts of harvested areas, productivity and prices of rice, beans, cassava and corn in Ceará State between 1945 and 2020.

	Variables		Area	Productivity	Price
	Estimated Models		ARIMA (0,1,1)	ARIMA (0,1,1)	ARIMA (2,1,2)
Rice	Constant		0,000	0,000	0,000
	AR	Lag1	0,000	0,000	0,598 *
		Lag2			-0,622*
	MA	Lag1	0,455*	0,643*	0,997*
		Lag2			-0,578*
	R^2		0,638	0,579	0,771
	Ljung Box		14,857 NS	10,765NS	20,049NS
	MAPE		27,059	24,334	15,476
	R Pearson		0,803*	0,764*	0,880*
	Variables		**Area**	**Productivity**	**Price**
	Estimated Models		**ARIMA (0,1,1)**	**ARIMA (0,1,1)**	**ARIMA (2,1,2)**
Bean	AR	Lag1	0,000	-0,261**	0,670*
		Lag2			-0,433*
	MA	Lag1	0,725	0,678*	1,295
		Lag2			-0,679
	R^2		0,481	0,342	0,424
	Ljung-Box		8,149NS	16,278NS	13,836NS
	MAPE		32,943	35.309	32,304
	R Pearson		0,701*	0,597*	0,671*
	Variables		**Area**	**Productivity**	**Price**
	Estimated Models		**ARIMA (0,1,1)**	**ARIMA (0,1,1)**	**ARIMA (2,1,2)**
Cassava	Constant		88232,191*	0,000	0,000
	AR	Lag1	0,847*	0,000	0,347**
	Lag2				
	MA	Lag1	0,000	0,439*	0,741
	Lag2				
	R^2		0,728	0,700	0,433
	Ljung Box		17,298NS	8,323NS	24,103NS
	MAPE		16,040	15,785	101,726
	R Pearson		0,853*	0,842*	0,601*
	Variables		**Area**	**Productivity**	**Price**
	Estimated Models		**ARIMA (0,1,1)**	**ARIMA (2,1,0)**	**ARIMA (0,1,1)**
Corn	Constant		0,000	0,000	0,000
	AR	Lag1	0,000	-0,789*	0,000
		Lag2		-0,438*	
	MA	Lag1	0,661*	0,000	0,517*
		Lag2			
	R^2		0,475	0,074	0,620
	Ljung Box		10,817NS	22,067NS	0,662NS
	MAPE		33,342	48,769	17,040
	R Pearson		0,698*	0,388*	0,792*

Sources: Estimated values from IBGE data (2020). Note: *Sign. at 1%; **Sign.at 10%: NS = not significant atleast 15% error.

Relation between the residues generated in the models and rainfall

Table 4 shows the results found in the estimation of the relationship between rainfall and the estimated residuals in each of the created models. In general it is found that: the rainfallaffects the residuals that make the predicted values differ from the observed values.
It is observed that only in the cases of forecasts for prices of rice, cassava and for the model related to the area harvested with cassava, the regression coefficients estimated was not statistically different from zero at least on 10% of error. These results may reflect the decisionsof farmers always seeking the cultivation of cassava in their areas, regardless of the history of rainfall. This reinforces the relevance of this activity as an important source of food security, inthe feeding of domestic animals and income enhancement (Table 4).

Table 4. Results of the relationship between the residuals of the models adjusted for forecast and the annual rainfall observed between 1945 and 2020 in Ceará.

Crops	**Variables**	**Constant**		**Regressor**		**R^2 Ajusted**
		Coef.	**Sig.**	**Coef.**	**Sig.**	
Rice	Harvested Area	-12205,024	0,002	15,669	0,001	0,123
	Productivity	-482,001	0,001	0,672	0,000	0,156
	Average price	0,022	0,917	-7,577E-005	0,768	0,001
Bean	Harvested Area	-140620,973	0,001	183,044	0,000	0,155
	Productivity	-96,334	0,018	0,119	0,017	0,063
	Average price	2,056	0,004	-0,003	0,002	0,124
Cassava	Harvested Area	-3282,076	0,630	4,105	0,621	0,030
	Productivity	-1052,990	0,110	1,367	0,091	0,039
	Average price	0,025	0,692	-3,264E-005	0,669	0,03
Corn	Harvested Area	-177134,947	0,000	232,864	0,000	0,239
	Productivity	-330,204	0,000	0,457	0,000	0,184
	Average price	0,469	0,003	-0,001	0,003	0,114

Source: Prepared by the authors based on data from FUNCEME (2022) and IBGE (2020).

The corn crop showed to be very sensitive to rainfall instability. This information is confirmed in Table 4, when estimating the impact of rainfall considering the residuals of the variables related to production, which proved to be significant.

Conclusions

The distribution of rainfall in Ceará State between 1945 and 2020 showed to be quite unstable. This instability is also reflected in the heterogeneity of the distributions of variables associated with the harvested area, productivity and price of rice, beans, cassava and corn in Ceará. In the particular case of cassava, it was possible to conclude that due to the characteristics of its production, where this crop adapts well to climatic adversities, the interference of rainfall does not significantly influence the decisions and expectations of producers.
The general conclusion is that the rainfall instability observed in Ceará State between 1945 and 2020 played an important role in the behavior of the projections of the variables that define the production of rice, beans, cassava and corn. In view of this, the projections made promote the dissemination of information about the impacts that droughts can cause on rainfed crops,

generally produced for the small family farmers. Having access to, and dissemination of information such as that presented here can help producers to design better planning and decision making strategies in the field, avoiding greater impacts on production and, consequently, on the income of these farmers.

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FORESTRY AND AGRO-FORESTRY

DEEP-PLANTED WHIPS ARE GOOD ALTERNATIVE TO ROOTED CUTTINGS IN THE ESTABLISHMENT OF WHITE POPLAR CLONAL PLANTATIONS

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Abstract

The effect of different planting types and depths on diameter at breast height, plants' height, and survival rate were analyzed in three trials established on soil with a favorable water regime in the first two growing periods. A similar survival rate was achieved by the planting of whips at depth of 2.5m ("deep planting") as by commonly practiced planting of rooted cuttings at depth of 0.8m. The survival rate of whips planted at depth of 2.5m did not decrease significantly during the second growing period. However, better growth in the first two growing periods had been achieved in the plantation established by whips planted at 2.5m than by rooted cuttings planted at 0.8m. Examined clones achieved high survival rate, both in the plantation established by one-year-old whips planted at depth of 2.5m, or by one-year-old rooted cuttings planted at 0.8m. Two-year-old whips achieved higher survival rate and better growth in diameter in the second growing period than two-year-old rooted cuttings, while the difference between those two treatments and the establishment by one-year-old whips was not significant. The survival rate of one-year-old whips planted at 0.8m was significantly lower than the survival rate of one-year-old rooted cuttings planted at the same depth but was not significantly lower than that of one-year-old whips planted at 2.5m. The research should be continued since the establishment of white poplar clones by deep-planted whips could significantly improve white poplar wood products, especially considering the benefits of production of planting material in stool beds in comparison to the production of rooted cuttings.

Keywords: *Populus alba*, deep planting, planting material, stool bed.

Introduction

White poplar (*Populus alba* L.) is adaptable, and fast-growing tree species of the riparian zone. It tolerates dry and salty soils that are marginal for black poplar. It is used as an indicator of biodiversity and is valuable in the restoration of autochthonous riparian ecosystems (Kovačević et al., 2010). Although its hardwood cuttings are generally difficult-to-root, clones, like *Populus alba* L. cl. Villafranca had been selected with favorable rooting potential.
Establishment of poplar plantations by deep planting of one or two-year-old whips (type 1/0 and 2/0, respectively) is the technology that had been proposed by May (1960) based on the experiences with the planting of clones of Euramerican poplar in the valley of river Po in the northern part of Italy as a solution for afforestation of soils with dominant fraction sand. In this case, deep planting allowed contact of the plant with underground water, raising the chances for the success of the plantation establishment (Marković, 1979; Rončević, 1984). Plantation establishment by deep-planted whips had high significance in the introduction of clones of

Eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) in former Yugoslavia in the early seventies of the 20th century. Clones of Eastern cottonwood were interesting for their tolerance to prevalent diseases, especially bark cancer caused by *Dothichiza populea* Sacc. & Briard. However, poor rooting of their hardwood cuttings as well as rooted cuttings raised problems in their nursery production and plantation establishment (Rončević, 1984). Lately, deep planting attracts lots of attention in the melioration of landslides (Phillips et al., 2014) and restoration of riparian vegetation in arid areas (Dreesen and Fenchel, 2008; Dreesen and Fenchel, 2014).

One of the main reasons that slow the expansion of white poplar in forestry of riparian zones is poor rooting of its hardwood cuttings. Although it achieves the best rooting within *Populus* (former *Leuce*) section, it usually is not sufficient and poses a real challenge for common poplar nursery production (Kovačević et al. 2014; Kovačević and Igić, 2018; Nonić et al. 2019; Igić et al. 2020; Kovačević et al. 2020). However, the production of rooted cuttings nowadays is at the core of white poplar forestry practice, since they are now exclusive planting material in the establishment of white poplar plantations.

The aim of this study was to study the possibility of implementation of deep-planted whips’ technology for the establishment of white poplar plantations.

Material and methods

To study the possibility of implementation of whip deep-planting, three experiments were established, described in detail by Kovačević et al. (2021). In all experiments, the survival rate, height, and diameter at breast height were measured after the first and second growing periods. Deep planted whips were planted at 2.5 m planting depth, while in other cases (normally planted) rooted cuttings and whips were planted at the depth of 0.8 m. On site “Kaćka šuma”, four clones were studied (Villafranca, L-12, L-80, and L100) and two planting types, deep-planted one-year whips (4±0.3m high) and normally planted one-year rooted cuttings (2.5±0.3m high). On site “Apatin” clone Villafranca was studied with three planting types: deep-planted one-year (4±0.3m high), two-year (5±0.5m high) old whips, and normally planted two-year (5±0.5m high) old rooted cuttings. And on site “Bačko Petrovo Selo” the clone L-80 with three planting types: deep-planted one-year old whips (4±0.3m high), normally planted one-year old whips (4±0.3m high), and rooted cuttings (2.5±0.3m high). According to Kovačević et al. (2021), in all cases deep-planted plants achieved contact with underground water or at least zone of capillary wetting.

Statistical analysis

Two- and three-way factorial ANOVA block design has been performed depending on the number of factors, as well as Tukey’s test, using the statistical package STATISTICA for Windows version 14 (TIBCO Software Inc., 2020). The survival rate was transformed by arcsine-transformation ($arcsin\sqrt{X}$) to achieve normal distribution of frequencies.

Results and discussion

Effects of differences between examined clones, growing periods, and plant type (deep-planted whips and normally planted rooted cuttings)

To analyze the significance of examined effects in the experiment in "Kaćka šuma", three-way factorial analysis of variance block design was implemented. According to the results, all studied sources of variation achieved significant effect on diameter and height of plants except for interaction growing period × clone in diameter and interactions growing period × clone and planting type × growing period × clone. In the case of survival rate, only the main effects achieved significant effect, suggesting no significant difference in the reaction of studied clones on examined treatments of planting type and growing period (data not shown due to page restrictions).

Generally, after the second growing period, both plating types achieved a high survival rate of more than 90%, although the survival rate was better in treatment established with rooted cuttings planted at depth of 0.8 m (98.9%) than in treatment established with deep-planted whips (92.4%), probably due to the damage achieved by roe deer. In that sense, Kovačević et al. (2021) assume that deep-planted whips are more susceptible to this attack because the part of the shoot at the ground level is more brittle and easy to crack than that of normally planted rooted cutting. There were significant differences between clones at the end of the second growing period, especially in diameter at breast height. Clones L-12 and L-80 achieved greater diameter than L-100 and Villafranca clones in the treatment established with deep-planted whips, while in treatment that was established by normal planting of rooted cuttings clone L-12 achieved greater height than L-100 clone. There were no significant differences in survival rate. It seems that differences in diameter and height are mostly related to the dimensions of plants from the previous growing period, and we expect that they will be greater in the following years (Table 1). The same could be said for two examined ways of planting, except for diameter which did not differ significantly between the two treatments at the end of the first growing period but was significantly greater for deep planting than normal planting treatment at the end of the second growing period 39.7 mm and 30.4 mm, respectively (data not shown due to page restrictions). According to Rončević (1984), the difference in diameter and height between deep-planted whips and normally planted rooted cuttings is expected in favor of rooted cuttings of clones of Eastern cottonwood. The same author also found that this difference sustains for 14 years after the planting. In the experiment in "Kaćka šuma", the favorable growth of diameter of deep-planted whips compared to normally planted rooted cuttings is evident, especially in clones L-100 and L-80. We assume is that the main reason is that the deep-planted whips achieved contact with groundwater or at least with capillary fringe above it, that provided these whips and then survived plants with abundant water supply throughout the vegetation period, while the plants 1/1 planted at 0.8m had to rally only on water gained by precipitation.

Table 1. Tukey's test for diameter at 1.3m, height, and survival rate of plants of white poplar clones by examined planting types after the first growing period in the trial in experimental estate "Kaćka šuma".

Growing season	Planting type	Clone	Diameter at 1.3m (mm)	Height (cm)	Survival rate (%)
First	Plant 1/0 planted	L-100	14.83 i*)	310.81 ef	98.32 abc

	at 2.5m	L-80	21.04 fgh	363.95 cd	97.74 abc
		L-12	22.09 ef	348.49 cde	93.88 abc
		Villafranca	16.28 hi	325.39 def	92.53 abc
	Plant 1/1 planted at 0.8m	L-100	18.26 ghi	292.33 f	99.86 ab
		L-80	20.43 fgh	302.35 ef	100.00 a
		L-12	21.46 fgh	318.12 def	99.43 ab
		Villafranca	21.17 fgh	323.35 def	99.86 ab
Second	Plant 1/0 planted at 2.5m	L-100	36.23 b	420.21 ab	95.83 abc
		L-80	44.07 a	459.15 a	95.26 abc
		L-12	43.94 a	447.47 a	90.02 bc
		Villafranca	34.50 b	415.78 ab	86.72 c
	Plant 1/1 planted at 0.8m	L-100	25.30 de	360.62 cd	100.00 a
		L-80	28.99 cd	380.99 bc	99.43 ab
		L-12	35.06 b	421.71 ab	95.26 abc
		Villafranca	32.09 bc	428.04 ab	98.32 abc

* The differences between values marked with the same letter are not significant at the level $\alpha=0.05$

Effects of the use of two-year-old whips and rooted cuttings

The use of two-year-old plants (rooted cuttings and whips) is of great importance for areas that are occupied by red deer, where robust plants are required to sustain the attack of this game. The trial was protected by a strong 2m high fence, so the effect of the game was not significant. The trial allowed comparison between establishment with deep planting technique, using two-year and one-year-old whips, as well as to compare these results with results gained after establishment with two-year-old rooted cuttings planted at depth of 0.8m, which is usual practice in these conditions. According to analysis of variance, all studied traits were under the significant effect of planting type in Villafranca clone (data not shown due to page restrictions).
The highest survival rate was achieved in treatment established with deep planted two-year whips (98.3% after the second growing period) (Table 2). The difference between survival rate in this treatment and after establishment with deep planted one-year-old whips was not statistically significant. The lowest survival rate was found after regularly practiced establishment with two-year rooted cuttings (69.8% after the second growing period). In the same treatment, the most considerable decrement in survival rate in the second growing period was found, as well as the lowest increment of diameter and height. While the increment of diameter in treatments established with one and two-year whips in the second growing period were almost the same, the height increment in the same period was considerably higher in treatments established with one-year whips. Both treatments established by whips showed superior survival rates and growth in the second growing period. So, these treatments to be implemented in further Villafranca clone plantations in conditions like those in our trail, where the plant’s contact with underground water or at least zone of capillary wetting. However, we assume that high survival rate and larger size of plants two years after the establishment with deep planted two-year-old whips could give an advantage to this treatment, especially in areas that are densely populated with large game animals. The importance of positive effect of the size of deep-planted planting material for its rooting was stressed by Phillips et al. (2014).

Table 2. Tukey's test for diameter at 1.3m, height and survival rate of plants of white poplar clone Villafranca by examined planting types after the first growing period in trial near Apatin

Growing season	Planting type	Diameter at 1.3m (mm)	Height (cm)	Survival (%)
First	Plant 1/0 planted at 2.5m	9.16 e	210.57 d	91.19 abc
	Plant 2/0 planted at 2.5m	31.29 c	523.35 b	99.40 a
	Plant 2/2 planted at 0.8m	45.73 a	626.33 a	78.60 cd
Second	Plant 1/0 planted at 2.5m	16.59 d	332.95 c	88.31 bcd
	Plant 2/0 planted at 2.5m	38.96 b	557.70 b	98.34 ab
	Plant 2/2 planted at 0.8m	48.21 a	641.10 a	69.78 d

Effect of planting of one-year whips at depth of 0.8m

In the trial established near Bačko Petrovo Selo, there were two treatments established with one-year-old whips planted at 2.5m and 0.8m, as well as the regular establishment with one-year rooted cuttings planted at depth of 0.8m. In two growing periods, planting type had significant effect on diameter and survival, but not at height of plants (data not shown due to page restrictions).

Treatments did not differ after the first growing period in examined traits, while after the second growing period diameter in treatment established by whips planted at 2.5m was significantly greater than those planted at 0.8m. Plants in all treatments significantly increased in diameter, but only plants in treatment established by deep-planted whips were significantly higher after the second growing period than after the first period. The additional decrease in survival rate in the second growing period was significant in all treatments except in the treatment established with deep-planted whips. At the end of the second growing season, the survival rate in treatments established by deep-planted whips and by rooted cuttings was around 50%, and in treatment established with whips planted at 0.8m, it was around 33%. However, these differences were not statistically significant (Table 3). Thus, it seems that the success of the establishment by deep-planted whips in this trial is at least at the level of regular practice by rooted cuttings planted at 0.8m. There are indications that the success of the establishment with whips does depend on the depth of the planting, especially regarding the increment of height in the second growing period.

Table 3. Tukey's test for diameter at 1.3m, height, and survival rate of plants of white poplar clone L-80 by examined planting types after the first growing period in the trial near Bačko Petrovo Selo.

Growing season	Planting type	Diameter at 1.3m (mm)	Height (cm)	Survival (%)
First	Plant 1/0 – planting on 2.5m	16.519 cd	2.830 b	67.197 ab
	Plant 1/1 – planting on 0.8m	15.406 d	3.085 ab	80.927 a
	Plant 1/0 – planting on 0.8m	14.204 d	2.783 b	61.061 ab
Second	Plant 1/0 – planting on 2.5m	24.125 a	3.400 a	49.481 bc
	Plant 1/1 – planting on 0.8m	20.615 ab	3.154 ab	56.409 bc
	Plant 1/0 – planting on 0.8m	19.973 bc	3.117 ab	33.852 c

Conclusion

According to available literature, implementation of deep planting of whips in white poplar is relatively rarely studied. That is why most of our experience in deep planting originates from studies in black poplars. Our results suggest that this way of plantation establishment can be used in selected white poplar clones and in favorable soil water status. All trials analyzed in this study suggest that the establishment of plantations of selected white poplar clones by deep-planted whips of selected white poplar clones could be at least as successful the establishment with rooted cuttings planted at 0.8m, which is a regular practice. There are even indications that deep-planted whips could achieve better survival and growth results than rooted cuttings, especially in the case of two-year-old planting material. The additional benefit of the implementation of deep planting in white poplar plantation establishment is relatively simple and probably much cheaper production of whips in stool beds in comparison to the production of rooted cuttings, which is loaded with problems in cuttings' rooting even in selected clones (Kovačević and Igić, 2018; Igić et al., 2021). Thus, studies and efforts in the implementation of deep planting of whips in white poplar plantation establishments should be continued.

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ECOLOGICAL CHARACTERISTICS OF GREEK MAPLE (*ACER HELDREICHII* ORPH.) IN PRIMEVAL FORESTS „PERUĆICA“ AND „BIOGRADSKA GORA“

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Abstract

Greek maple (*Acer heldreichii* Orph.) is subendemic of Balkan Peninsula and tertiary relic, with westernmost distribution border in Bosnia and Herzegovina. This paper deals with ecological conditions (climate, parent rock, soil and vegetation) on its sites in two primeval forests - „Perućica“ on Maglić Mt. within national park „Sutjeska“ in Bosnia and Herzegovina and „Biogradska gora“ on Bjelasica Mt. within national park Biogradska gora in Montenegro. Elevation of Greek maple sites is 1400 m-1700 m a.s.l, aspects are N and NE, and inclination 5-20°. Climate is in both localities moist temperate with cold winters according to Köppen classification, and perhumid according to Thornthwaithe. Geological parent rock is hard limestone on Perućica, and marly limestone and sedimentary breccia on Biogradska gora. Soil type on Perućica is calkomelanosol (leptosol), and calkocambisol on Biogradska gora. Soils are shallow to moderately deep, with mildly acidic to neutral reaction, well supplied with nitrogen and potassium, and poorly provided with phosphorus. Greek maple grows within altimontane beech forest zone on both localities and is codominant species of mixed beech-Greek maple forest community (*Aceri heldreichii-Fagetum* B. Jov. 1957). Based on ecological spectra, this community is mesic according to moist requirements, neutrophilous according to soil reaction, tolerant according to light requirement, mesotrophic according to nitrogen requirement, and according to warmth it is mesothermic. Hemicryptophytes dominate in spectrum of life forms, with high geophytes content. Dominant group in spectrum of floral elements is Central European group, followed by Euroasian, as well as Circumpolar and Cosmopolitan group.

Keywords: *Acer heldreichii, endemic, ecology, Perućica, Biogradska gora.*

Introduction

Balkan Peninsula is floristically the most divesrse and richest part of European continent. Greek maple (*Acer heldreichii* Orph.) is a subendemic of Balkan Peninsula and a tertiary relic. It consists of the two subspecies (van Gelderen *et al*., 2010). *Acer heldreichii* Orph. subsp. *heldreichii* occurs in Balkans in the following countries: Serbia, Bosnia and Herzegovina (only in Republic of Srpska), Montenegro, Northern Macedonia, Bulgaria, Greece and Albania. The other subspecies, *Acer heldreichii* Orph. subsp. *trautvetteri* (Medvedev) Murray occurs in Caucasus area and it is native in the countries: Turkey, Georgia, Armenia, Azerbaijan and Russia. Greek maple is montane species which grows only at elevations between 900 and 2100 m a.s.l. (Alexandrov and Pandeva, 2003).
This paper researches ecological conditions (climatic, orographic, edaphic and vegetational) in which natural Greek maple populations grow in two big primary forests, „Perućica“ and „Biogradska gora“. Primary forest „Perućica“ is located in Bosnia and Herzegovina, on Maglić Mt. within „Sutjeska“ national park, while „Biogradska gora“ is placed in Montenegro on

Bjelasica Mt in national park of the same name. The aim of this research is to contribute to better knowledge of ecological characteristics of Greek maple, which will promote easier gene pool protection and *in situ* conservation of this endemic and relic species, which is protected in Montenegro (Zakon o zaštiti prirode Crne Gore, 2016) and Republic of Srpska (Zakon o zaštiti prirode Republike Srpske 2014). Also, results of this research will facilitate using of this species in forest plantation, since its wood possess similar properties to sycamore maple (*Acer pseudoplatanus* L.) or as an ornamental species, since it has nicely shaped leaves opulently coloured during autumn and decorative reddish fruits (Vukićević, 1996).

Material and methods

Research was made in areas with the highest presence of Greek maple in both primary forests. In Perućica research was done on localities Tunjemir, Prijevor and Crvene Prljage. Coordinates are 43° 18' N lat.; 18° 43' E long. In Biogradska gora research was done on the southern border of the national park above Katun Goleš, on localities Ravni Jeljak and Crvena greda. Coordinates are 42° 53' N lat.; 19° 36' E long. Reseach comprised climatical, orographic, edaphic and cenological characteristics.

Climatic characteristics were determined by using climatic normals for period 1981-2010. for closest sinoptic stations. For Biogradska gora, data is used from sinoptic station Kolašin (944 m a.s.l.), and for Perućica from sinoptic station Žabljak (1450 m a.s.l.). Considering that all researched populations are located on significantly higher elevations than locations of named sinoptic stations, data were corrected. Temperature data were approximated by method of thermic gradients (Kolić, 1988), while precipitation data were approximated by Schreiber method (Milosavljević, 1988). On the basis of climate data, climate clasificcations according to Köpen, Lang and Thornthwaite were produced, as well as de Martonne's aridity index (Gburčik, 1995; Dukić, 1998), and Walter's climate diagrams were drawn.

Researched orographic characteristics included: elevation, aspect and inclination of relief. These parameters were determined using GPS device GARMIN Vista HCX. For investigation of edaphic characteristics two soil profiles were dug on each locality. On the basis of produced soil profiles, determination of types of geological bedrock and soil, as well as determination of physical and chemical soil properties, were made. Analysis of soil characteristics comprised field and laboratory research methods accepted and defined by Serbian Soil Research Society (Jugoslovensko društvo za proučavanje zemljišta 1966, 1997). Detailed morhpogenetical description of soil profiles were done. Soil samples were collected for analysis of standard physical and chemical soil properties which were made in Laboratories for physical and chemical soil properties of University of Belgrade-Faculty of Forestry. Determination of soil taxonomical units were made according to „Classification of soils of Yugoslavia“ (Škorić *et al*., 1985).

Vegetation research was done using standard Braun-Blanquet method (Braun Blanquet, 1964) on the basis of collected 11 phytocoenological relevés (six on Biogradska gora and five on Perućica). Determination of vascular plant species were made using „Flora of Serbia I-X“ (Josifović *et al*., 1972-1977; Sarić *et al*., 1986, 1992; Stevanović *et al*., 2012). On the basis of collected relevés, phytocoenological tables were produced and plant communities separated (Table 1). Names of plant communities were derived according Weber *et al.* (2006) and Tomić and Rakonjac (2013). For established plant communities, spectra of life forms and ecological characteristics (including relation of plants to moisture, soil reaction, nitrogen content in soil, light and temperature), as well as spectrum of floral elements were produced. Spectra of life forms and ecological characteristics were made according to Kojić *et al.,* (1997) and Ellenbeg and Leuschner (2010), while spectrum of floral elements was made according to Gajić (1980, 1984).

Results and discussion

Mean annual temperature on Biogradska gora is 3.7°C, and on Perućica 4.8°C. The warmest month is July with values 11.5°C on Biogradska Gora and 14.4°C on Perućica. The coldest month is January, with values -3.8°C (Perućica) and -3.4°C (Biogradska gora). Average temperatures during vegetation period (April-September) are 8.4°C (Biogradska gora) and 10.4°C (Perućica). Mean yearly precipitation level is 2486 mm (Biogradska gora) and 1494 mm (Perućica). The wettest month is November (355 mm on Biogradska Gora and 215 mm on Perućica). The dryest month is July with values 75 mm (Biogradska gora) and 70 mm (Perućica). Such precipitation distribution is caused by vicinity of Adriatic sea, influenced by Mediterranean climate. This climate is characterized by maritime pluviometric regime with distincltyly dry summer period and very wet winter period. Precipitation during vegetation period is 853 mm on Biogradska gora and 588 mm on Perućica. According to Lang' s climatic classification, yearly values are 672.0 on Biogradska Gora and 317.9 on Perućica, which indicates perhumid climate. However, during vegetation period, situation is much better, with values 101.6 on Biogradska gora, and 56.5 on Perućica. During this period, values are below perhumid level (160) (Unkašević, 2005), which compensates moisture excess during rest of the year and provides growth and development of forest communities. Perhumid climate character on researched localities is also confirmed by Thornthwaite's climate classification, with values 440.7 on Biogradska Gora, and 247.8 on Perućica. According to Koppen climate classification, bot sites have climate type D (temperate dry climate with harsh winters). Analysis of de Martonne's aridity index (181.5 on Biogradska Gora and 101.7 on Perućica) shows that both localities represent typical forest sites where draught does not occur at all. Walter's climate diagrams (Figure 1 and 2) show that both localities are very well supplied with moisture. On both localities perhumid periods exist during most of the year (except in summer), while, on the other hand, neither dry nor moderate dry periods were recorded at all. Analysied climate parameters show that Greek maple is typical forest species and that it occurs in climate conditions very favourable for forest vegetation.

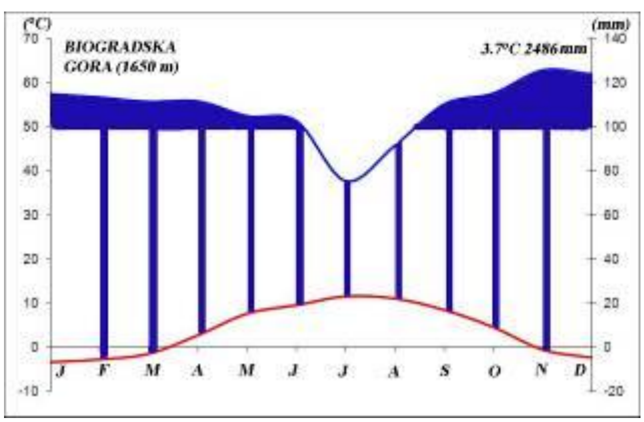

Figure 1. Walter climate diagram for Greek maple population on Biogradska Gora

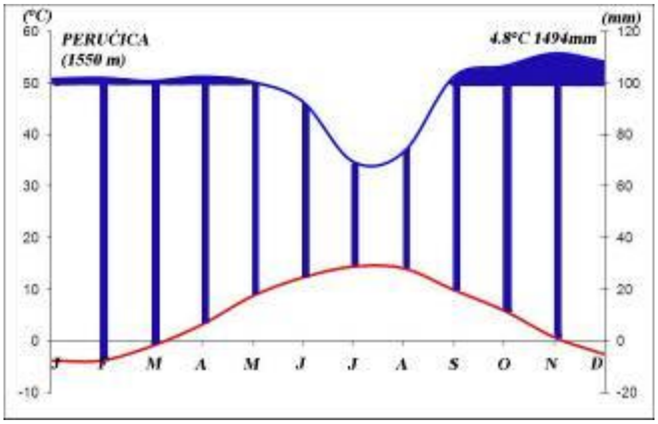

Figure 2. Walter climate diagram for Greek maple population on Perućica

Elevations of researched *Acer heldreichii* Orph. sites on Perućica are 1400-1650 m a.s.l., aspects are northern and northeastern, and inclinations are 5-20°. Elevations on Biogradska Gora are 1600-1700 m a.s., aspect is northern, and inclination 5-10°. These findings are in concordance to Alexandrov and Pandeva (2003), who state that Greek maple is mostly distributed at elevationa 1200-1900 m a.s.l., and to findings of Lakušić (1989), who mentions that this species most frequently grows on northern aspects.

Geological bedrock is on both sites represented by limestones, considering that on Perućica solid limestones occur, while on Biogradska Gora marly limestones and limestone breccias were found. Soils produced on solid limestons are of low productivity, shallow, stony and dry, while marly limestones contain higher content of clay minerals (25-40%), so they are more productive (Nikić and Gajić, 2010).

Analysis of soil profiles shows presence of two soil types, leptosol (calkomelanosol) on both profiles on Perućica and calcocambisol on both profiles on Biogradska gora. Calkomelanosols are shallow soils, rarely over 30 cm deep, and due to little profile depth and decreased water retention capability, their production is low. Calcocambisols are somewhat deeper soils, but rarely over 60 cm, they are well drained thanks to stability and moderate swelling of soil aggregates, so they have better production. They are favourably provided with nutrients, but have insufficient profile depth (Ćirić, 1991; Antić et al., 2007).

Calcomelanosol on Perućica was developed on solid limestone on both analysed profiles. Two subtypes were set apart, cambic calcomelanosol and organic mineralised calcocambisol. Researched soils are shallow, with maximal depth below 40 cm. Topsoil has brownish colour, with crumby structure aggregates. It is interwowen by small roots, and possesses good physical properties. Sand fraction makes 23.3% to 28.4%, and silt and clay fraction 71.6% to 76.7%, so these soils have clayi mechanical composition. Soil reaction is neutral to mildly alkaline (pH 6.9-7.6), stongly saturated by alkalies (>87.5%). Soils are strongly to very strongly humose (humus copntent is 7-13%). C/N ratio (8.3-11.1) indicates production of mull humus. Soils are well supplied with total nitrogen (0.6-0.7%), moderately supplied with easily accessible potassium and poorly supplied with easily accessible phosphorus.

Figure 3. Cambic calkomelanosol(Perućica)

Figure 4. Calkocambisol (Biogradska Gora)

Calkocambisol on Biogradska gora on both profiles was recorded on limestone breccia and marly limestone. Soils are well developed (up to 60 cm deep). A-horizon is shallow (to 15 cm), dark brow, and (B) horizon is well developed (depth above 40 cm), of reddish brown colour. Soils have silty-loamy mechanical composition. Sand fraction comprises 32.0%-60.4%, and silt clay fraction 39.6%-68.0%. Active acidity varies between moderately acidic to strongly acidic (pH 5.3-5.9). Soils are carbonate-free. Alkali saturation ranges 30.0%-67,2%. A-horizon is very rich in humus (14-15%). These soils are well provided with nitrogen (1.9-2.4%), easily accessible potassium (15.3-20.,0% in A-horizont), and poorly supplied with easily accessible phosphorus (1.4%).

Occurence of *Acer heldreichii* Orph. stands on soils of different reaction is in accordance to statement of Lakušić (1989) about its wide amplitude considering soil reaction (pH values between 4 and 7.5). High humus content is consequence of low temperatures and high precipitation level, which causes poorer transformation of organic particles. According to Ćirić (1991) both calcomelanosols and calcocambisols are forest soils of lower productivity, which is one of the reasons that Greek maple is not abundant on researched localities, since this species is mesotrophic (Kojić *et al*., 1997).

On both researched localities *Acer heldreichii* is codominant species of subalpine beech-Greek maple forest plant community (*Aceri heldreichii-Fagetum* B. Jov. 1957) (Table 1). It is in accordance with findings of Perović (2014), that this species predominantly grows as a mixed or dominant species in the subalpine beech belt, and very rarely builds plant communities with other tree species. Within phytocoenological relevés, 60 vascular plant species were recorded, 50 on Biogradska Gora and 36 on Perućica. Greek maple and beech (*Fagus sylvatica* L.) completely dominate in tree layer, while in Perućica occur also indivudual trees of fir (*Abies alba* Mill.) and sycamore maple (*Acer pseudoplatanus* L.) Greek maple and beech are very frequent in the shrub layer also, fir is also frequently present, and on Perućica sycamore maple and alpine honeysuckle (*Lonicera alpigena* L.) occur individually. Most frequent species in ground layer (presence level

IV and III) are: *Adenostyles alliariae* (Gouan) A. Kern., *Glechoma hirsuta* Waldst &Kit., *Allium ursinum* L., *Veratrum album* L., *Poa nemoralis* L., *Anemone nemorosa* L., *Galium odoratum* (L.)Scop., *Dryopteris filix-mas* (L.) Schott, *Aegopodium podagraria* L., *Cicerbita alpina* (L.) Wallr., *Aremonia agrimonoides* (L.) DC and *Sanicula europaea* L.

Table 1. Phytocoenological table for community *Aceri heldreichii-Fagetum* B. Jov. 1957

Plant community	*Aceri heldreichii-Fagetum* B. Jov. 1957											Presence level
Locality	Perućica-Tunjemir, Prijevor					Biogradska gora-Katun Goleš, Crvena greda						
Relevé number	1	2	3	4	5	1	2	3	4	5	6	
Elevation	1650	1650	1600	1600	1550	1700	1700	1700	1650	1650	1650	
Aspect	NE	NE	NE	N	N	N	N	N	N	N	N	
Inclination	20	10	5	10	25	10	10	5	10	10	5	
TREE LAYER												
Canopy	0.9	0.9	0.7	0.8	0.8	0.7	0.7	0.7	0.6	0.8	0.7	
Average height (m)	18	20	21	25	19	23	25	24	22	22	22	
Average diameter (m)	18	12	30	30	20	30	30	35	30	25	25	
Average distance (m)	4	4	5	4	4	5	5	5	5	4	4	
Acer heldreichi	1.1	1.1	1.1	1.1	2.2	2.3	2.2	3.3	3.3	3.3	2.2	**V**
Fagus sylvatica	4.4	4.4	3.3	4.4	3.3	3.3	3.3	3.3	2.2	2.2	3.3	**V**
Abies alba.	+			+	+							**II**
Acer pseudoplatanus	+				2.2							**I**
SHRUB LAYER												
Canopy	0.3	0.1	0.1	0.4	0.3	0.2	0.3	0.4	0.6	0.3	0.4	
Average hight (m)	3	5	3	4	3	2	2	3	3	4	2	
Fagus sylvatica	+		+		3.3	1.1	2.2	2.2	3.3	2.2	1.1	**V**
Acer heldreichii	+				2.2	1.1		2.2	3.3	1.1	1.1	**IV**
Abies alba	2.2	+	+	3.3	+	1.1					2.2	**IV**
Acer pseudoplatanus											1.1	**I**
Lonicera alpigena	1.1											**I**
GROUND LAYER												
Coverage	0.2	0.1	0.7	0.7	0.8	0.8	0.8	0.9	0.7	0.8	0.7	
Adenostyles alliariae				+		2.1	3.3	1.2	+	1.2	3.3	**IV**
Glechoma			+.2	1.2	1.1	1.2	1.2		1.2		1.2	**IV**

hirsuta												
Dryopteris filix mas					+	1.2	1.1	1.1			1.1	**III**
Veratrum album				+		1.1		1.2	+	1.1	1.2	**III**
Galium odoratum				+	+	1.2	1.1	1.2			1.2	**III**
Aegopodium podagraria	+		+	+	+				2.2			**III**
Cicerbita alpina	+.2			3.3		+.2			1.2	+		**III**
Anemone nemorosa							1.2	1.1	2.2	2.3	1.1	**III**
Allium ursinum						4.3	3.3	4.4	1.2	1.2	2.2	**III**
Poa nemoralis						1.2	1.2	+	+.2	2.3		**III**
Abies alba				+	+	+			+	+	+	**III**
Aremonia agrimonoides	1.1				1.2	+		+.2			1.2	**III**
Sanicula europaea		+	+		1.2			1.1	1.2			**III**
Senecio fuchsii						1.1			1.1	+		**II**
Athyrium filix femina					+		+	+				**II**
Prenanthes purpurea	+				1.2					+	+	**II**
Polygonatum verticillatum	1.2				1.1		1.1					**II**
Geranium robertianum					+	+					1.2	**II**
Chaerophyllum aureum	+		3.3			+						**II**
Cardamine enneaphyllos	1.2	+	+	1.2								**II**
Oxalis acetosella				+.2	+.2	+.2						**II**
Arum maculatum						+	+			+		**II**
Anemone ranunculoides						1.1	1.2		1.1		1.1	**II**
Rumex alpinus						+		+	1.2	1.1		**II**
Geranium reflexum						+			1.2	1.2		**II**
Veronica urticifolia				1.2		+			+	1.2		**II**

Hypericum maculatum						+.2			1.1	+		**II**
Gentiana asclepiadea				+					1.2		+	**II**
Plantago reniformis				+					1.2		1.2	**II**
Symphytum tuberosum	1.1	+				1.1						**II**
Geranium sylvaticum	+.2		+	+					+			**II**
Poa alpina	+.2		+	+.2								**II**
Lonicera alpigena	1.1	+			+							**II**
Cardamine bulbifera						2.2	2.2					**I**
Galeopsis tetrachit										+	+	**I**
Isopyrum thalictroides					+				+			**I**
Fagus sylvatica	1.1					2.3						**I**
Paris quadrifolia					1.2	+						**I**
Acer heldreichii			+		+							**I**
Parietaria officinalis						+		+				**I**
Sedum hispanicum						1.3					1.3	**I**
Galium rotundifolium										+	1.2	**I**
Euphorbia amygdaloides	1.1				+							**I**
Viola biflora	+	+										**I**
Polygonatum odoratum	+	+										**I**

Species recorded in single relevé in ground layer: Scrophularia nodosa 1.1 (BG 5), Acer pseudoplatanus + (P 2), Ranunculus lanuginosus 1.2 (BG 1), Hesperis matronalis + (BG 1), Calamintha grandiflora + (BG 1), Myosotis sylvatica 1.2 (BG 1), Fragaria vesca 1.2 (BG 4), Aconitum pentheri + (BG 5), Moehringia trinervia 1.2 (BG 5), Geum urbanum 1.1 (BG 5), Sorbus aucuparia + (P 1), Vaccinium myrtyllus 1.2 (P 4), Dactylorhiza maculata + (P 4), Hieracium murorum + (P 4)

Analysis of ecological spectra shows that this community is typical mesophilous, since predominately mesophilous species make 85% of all vascular plant species. Considering soil reaction, this community is mostly neutrophilous (42% of neutrophilous plant species), with significant participation of basiphilous species (27%). Considering nitrogen requirements, this community is mostly mesotrophic with significant share of eutrophic species (55% mesotrophic, 30% eutrophic species). This finding confirms observation that Greek maple occurs on relatively favourable sites, on more developed soils, relativelly well provided with nutrients. Considering light requirements this community is tolerant-semitolerant (55% of tolerant, 37% of semitolerant species). That is expected in forests where one of the dominant species is beech, since beech is one the most shade tolerant tree species in its range (Houston Durrant *et al*., 2016). According to temperature requirements, the community is mesothermic, where mesothermic plant species participate with 62%. Spectrum op life forms shows that hemycryptophytes are dominant group (55% of plant species), followed by geophytes with 27%, and significant share have phanerophytes also (10%). Dominance of hemycryptophytes is expected, since that life form is dominant in areas within temperate continental climate (Diklić, 1984). It is observed signifinantly higher share of geophytes compared to normal life form spectrum of Serbia, where this life form participates with 9% (Jovanović, 2007). It is caused on one side by low winter temperatures on researched localities, which bring about short vegetation periods, so the accumulation of nutrients in underground parts enables flowering of plants at the begining of vegetation period (Stevanović and Janković, 2014), when temperature conditions are still infavourable for photosynthesis. Also, there is pronounced seasonal light difference in beech forests, which have very shade tolerant character after leaf development, so they contain significant number of ephemorophytes, which finish their life cycle during very short period (Allaby *et al*., 2015). In life forms spectrum, Central European floral group is most frequent (38% of vascular plant species), which confirms mostly mesophilous character of this plant community (Gajić, 1984), and significant participation have floral groups of wide ecological amplitude, Euroasian (20%) and Circumpolar and Cosmopolitan (12%).

Conclusions

Greek maple *(Acer heldreichii* Orph,) in primeval forests Perućica and Biogradska Gora was recorded only in subalpine vegetation zone (1400-1700 m a.s.l.), on cold aspects (N and NE) and relatively flat terrains with mild inclination.

On the basis of Köppen climate classification, researched sites have climate type D (wet temperate climate with harsh winters). Lang's and Thornthwaite's climate classifications show that Greek maple sites on both localities are located within perhumid climate zone. Walter's climate diagrams indicate that both localities are very well provided with moisture. For the most part of the year (except in summer) perhumid periods occur, and on the other hand, neither dry nor semidry periods are recorded at all.

On both localities Greek maple populations are recorded on limestones, with the fact that on Perućica they are found on solid limestones and on Biogradska Gora on marly limestones and limestone breccias. Recorded soil type on Perućica is calkomelanosol, and on Biogradska Gora calkocambisol. Soils are shallow to moderately deep, with clayi to loamy mechanical composition, of acidic to neutral chemical reaction. Soils are well supplied with nitrogen, moderately to well supplied with potassium and poorly supplied with phosphorus. Both soil types belong to less productive forest soils.

Greek maple in two primeval forests Perućica and Biogradska gora occurs predominately in subalpine beech forest zone and presents a codominant species in beech-Greek maple community (*Aceri heldreichii-Fagetum* B. Jov. 1957). This community has hemicryptophyte-geophyte-phanerophyte character according to life forms spectrum. According to moisture requirement it is strongly mesophilous, according to soil reaction it is mostly neutrophilous, according to soil nitrogen content it is mostly mesotrophic. Considering light requirements this community is shade tolerant to semitolerant, and according to the temperature requirements it is mesothermic. Floral elements analysis shows that the Centraleuropean floral group is the most frequent, which indicates mesophilous character of this plant community.

Greek maple is Balkan subendemic and relic species and is protected both in Montenegro and Republic of Srpska. Results of this research will increase knowledge of idioecological and sinecological characteristics of its natural populations, which will enable its more efficient protection and *in situ* conservation of its gene pool, especially in primary forests. Besides, detailed knowledge about ecological characteristics of Greek maple will promote its commercial planting in forestry or as an ornamental species.

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SUPPRESSION OF DUSKY CLEARWING (*PARANTHRENE TABANIFORMIS* ROTT.)

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Abstract

Dusky clearwing (*Paranthrene tabaniformis* Rott.) presents a significant pest in nurseries and juvenile poplar plantations. To study the possible options for its suppression, two different experiments were conducted: (i) use of diflubenzuron (Dimilin SC 48) insecticides with added paraffin oil (Letol EC) and alpha-cypermethrin (Fastac Forst) without added paraffin oil for treatment and (ii) injecting gasoline into the caterpillar holes. First experiment was established at the experimental estate of the Institute of Lowland Forestry and Environment in a two-year poplar plantation as a randomized block design aiming to study the possibilities of control. A handheld sprayer was used for application of insecticides in two terms: on May 20, 2021, and June 17, 2021, by spraying the bark of the tree thoroughly from the ground to the start of the crown. On September 8, 2021, the experiment was evaluated by counting the boreholes made by the larvae of the dusky clearwing. Second experiment included suppression of the dusky clearwing caterpillars by injecting gasoline into the caterpillar holes that was conducted in the second half of July and the beginning of August 2018. The applied insecticide alpha-cypermethrin had an effectiveness of 84.2%, while the usage of diflubenzuron with added paraffin oil had an effectiveness of 83.3%. The injection of gasoline into the larvae holes was 84.8% effective. High efficacy of applied insecticides in preventing plants from attack of dusky clearwing and injecting gasoline into caterpillar holes on plants suggests that these measures might be effective in protection of young poplar plantations against dusky clearwing attacks.

Keywords: Paranthrene tabaniformis, Suppression, Insecticides, Gasoline, Injecting.

Introduction

Caterpillars of dusky clearwing (*Paranthrene tabaniformis* Rott.) cause damages to nurseries and young poplar plantations. Dusky clearwing has a one generation per year and adults swarm from May to August when they lay down their eggs individually on the tree bark (Vujić et al., 1967). Hatched caterpillars build galleries in the stems of young plants or in the branches of older ones and therefore cause physiological weakening of infested plants. Often, at the place of entrance hole of caterpillars there is gall formation and eventual breakage of the plants due to the action of the wind, which causes complete decline of trees. Damages from dusky clearwing can be very significant (Tomić, 1958; Živojinović, 1958; Vujić et al., 1960; 1967). The last large outbreak was recorded in the Forest Estate Sremska Mitrovica in 2017, when several one-year-old plantations were under attack, and the intensity of the attack ranged from 14.7% to 92.9% of plants and control was carried out on a total of 349.9 hectares. For protection, preventive measures are considered in the form of use of healthy planting material and avoiding inducing mechanical damage to plants, because caterpillars often bore at the place of damage. Selection of poplar clones according to which the insect shows a lower degree of preference is very useful as well (Drekić, et al. 2006; 2009). When plants have already been attacked, injection of gasoline or insecticides is applied into the gallery of caterpillar at the summer (Georgopulos, 1956; Tomić, 1958; Vujić et al., 1960; Maksimović, 1964; Jodal, 1967; Vujić et al. 1967). The disadvantage of this method is that it is applied when the damage has already been caused due to the boring of caterpillars. Treating the plants with insecticides to prevent caterpillars from boring has an advantage because it avoids damage. Tillesse et al. (1996) state that chemical protection in nurseries is possible if performed at the right time and repeated throughout the butterfly's flight period, but they do not specify which insecticide can be used. Jodal (1967) mentions the successful treatment of damaged places on plants with the insecticide DDT, which is banned today. Finding effective insecticides to protect poplars in nurseries and young plantations from the attacks of *P. tabaniformis* caterpillars would be a useful complement to existing protection measures. Therefore, the main goal of this research was to test the possibility of using two insecticides in the protection of young poplars from dusky clearwing. In addition, success of injecting gasoline into the caterpillar's gallery as a measure of control of this harmful insect was analyzed.

Material and Methods

The efficacy of two insecticides in the control of *P. tabaniformis* was tested in two years old poplar plantation in the locality Kaćka šuma (N 45º 17´ 09´´; E 19º 54´ 07´´). First application of insecticides was performed on May 20, 2021, and second on June 17, 2021. The treatment was performed with hand sprayer Hipol by spraying the bark of the tree thoroughly from the ground to the start of the crown. The person that applied insecticides used adequate personal protective equipment. During the treatment, the meteorological conditions were without precipitation and without strong wind that would negatively affect insecticide application. The experiment was set up according to a completely random block system in five repetitions. The size of the experimental plot was about 50 plants. The applied insecticides are presented in the Table 1.

Table 1. Insecticides whose efficacy was tested and applied concentration

Insecticide (active ingredient)	Trade name	Applied concentration	Amount of insecticide in 10 l. of emulsion
Diflubenzuron + paraffin oil	Dimilin SC 48 + Letol EC	0,05 % + 1%	5 ml + 100 ml
Alpha cypermethrin	Fastac Forst	1%	100 ml

Addition of paraffin oil (Letol EC) to the insecticide diflubenzuron (Dimilin SC 48) was made with the aim of prolongation of the insecticide`s persistence. Due to the long persistence of the alpha-cypermethrin (Fastac Forst), there was no need of addition of the paraffine oil to this treatment.

The evaluation of the experiment was performed on September 8, 2021, by counting the active boreholes made by caterpillars of dusky clearwing. Efficiency was calculated by the Abbotts formula (Abbott, 1925). In the Forest Estate Sremska Mitrovica at the end of July and the beginning of August 2018, at several sites in one-year plantations of poplar clones, was performed suppression of dusky clearwing by injecting gasoline into the caterpillars' gallery and then closing the entrance with putty. The evaluation of the success of this method was performed at the beginning of September by counting the active and inactive boreholes made by dusky clearwing's caterpillars into which gasoline was previously injected.

Results and Discussion

The average number of boreholes from the caterpillars of dusky clearwing per plant is shown in Table 2. The evaluation results indicate that the number of boreholes was several times lower in treated plants compared to untreated control plants.

Table 2. Average number of galls per plant

Insecticide (active ingredient)	Assessment time	Average number of galls per plant per repetitions					Average number of galls per plant
		I	II	III	IV	V	
Diflubenzuron + paraffin oil	September 8, 2021	0,026	0,053	0,057	0,057	0,048	0,053
Alpha - cypermethrin	September 8, 2021	0,118	0,05	0,023	0	0,03	0,05
Control	September 8, 2021	0,576	0,317	0,256	0,167	0,250	0,317

The efficiency of both applied insecticides was at similar level and amounted 83.3% for the insecticide diflubenzuron with added paraffin oil, and 84.2% for the insecticide alpha-cypermethrin.

Table. 3 Efficacy of apllied insecticides

Insecticide (active ingredient)	Assessment time	Average number of galls per plants	Efficacy
Diflubenzuron + paraffin oil	September 8, 2021	0,053	83,3%
Alpha - cypermethrin	September 8, 2021	0,05	84,2%
Control	September 8, 2021	0,317	-

Given the hidden way of caterpillars' life inside the galleries and the very long period of butterfly flight, it was assumed that satisfactory efficiency would be achieved with minimum of two insecticide treatments, which proved to be true.

Fig 1. Entrance hole and gall of *P. tabaniformis*

The use of pheromone traps for monitoring of the butterflies' flight in nurseries and plantations would be very useful in determining the degree of endangerment and the optimal time of treatment. To prevent damage and reduce protection costs, preventive measures are necessary, such as: destruction of infested plants in nurseries, use of healthy seedlings for establishing of plantation, avoiding mechanical damage to plants and selection of poplar clones to which the insect shows less preference (Tomić, 1958; Jodal, 1967; Vujić et al. 1967; Drekić, et al. 2006; 2009). When, despite the applied protection measures, insects attack plants, it indicates that it is necessary to control them by injecting insecticides or gasoline into the galleries through entrance holes. Due to the intensive attach of the insects, in the summer of 2018, suppression was done in this way in the total of eleven sites managed by FE Sremska Mitrovica aimed to protect the seedlings. To check the efficiency of the implemented gasoline injection into the caterpillars' galleries of the dusky clearwing, an assessment of the effect of the control of this pest at the beginning of September was performed. It was determined that the percentage of dead caterpillars ranged from 60 to 100%. This variation is primarily the result of the quality of work on the application of gasoline in the holes of caterpillars and indicates the need for intensive quality control of work in the field.

Table 4. Results of *P. tabaniformis* control by injecting gasoline into caterpillars' holes

Locality	Percentage of infested plants (%)	Percentage of dead caterpillars after gasoline injection (%)
Morović, Đepuš, odelenje 30	74	60
Višnjićevo, Vranjak, odelenje 21	76	95,7
Višnjićevo, Vranjak, odelenje 29	75	88
Višnjićevo, Vranjak, odelenje 35	71	94,2
Klenak, Senajske bare- Krstac, odelenje 25	45	72
Klenak, Senajske bare- Krstac, odelenje 27	70	92
Klenak, Senajske bare- Krstac, odelenje 31	65	88
Kupinovo, Kupinski kut, odelenje 4	22	100
Kupinovo, Kupinski kut, odelenje 31	48	93

Kupinovo, Kupinski kut, odelenje 33	29	75
Kupinovo, Jasenska - Belilo, odelenje 4	49	75
Average	56,7	84,8

The average efficacy of caterpillar control in this way was 84.8%, which is approximately in line with the results of Jodal (1967), who stated that 82% of caterpillars were destroyed by gasoline. Maksimović (1964) stated the effectiveness of dichlorvos insecticide of 98.9% and phosphamidon insecticide of 99.5% by injecting insecticides into the larvae galleries, but these insecticides are no longer used in Serbia, so today gasoline is the only proven agent of injection.

Conclusions

In protection of poplar from dusky clearwing infestation the efficacy of apha - cypermethrin insecticide was 84.2% while the similar efficacy of 83.3% was achieved by using diflubenzirone with added paraffin oil. The efficacy of gasoline injection into the galleries of dusky clearwing caterpillars averaged 84.8%. The high efficacy of the applied insecticides and the method of injecting gasoline into the caterpillar galleries indicate that these repressive measures can be effectively used in the protection of poplars in nurseries and young plantations from dusky clearwing.

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POPULATION DYNAMICS OF EARLY OAK DEFOLIATORS IN CORRELATION WITH MICRO-CLIMATIC TEMPERATURE CONDITIONS IN KRAGUJEVAC AREA IN SERBIA

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Abstract

Forest dieback that comes in waves since the early 20th century has lately grown into an epidemic, in particular in oak stands. For this reason, research was conducted of the population dynamics of early oak defoliators, which represent a grave danger in oak stands due to their gradogenic attributes. The research was carried out over a 5-year period in oak forests in the area of forest administrations Kragujevac and Gornji Milanovac. The samples used in the research were collected from bottom branches, where Geometridae were found in the largest numbers, as well as from the mid and upper parts of the crowns, where other species were found. Population levels of these pests were presented in laboratory conditions on winter branch samples and in newly foliated stands on site, depending on the basic parameters of the climatic conditions. The greatest deviation of the population level of early oak defoliators was noted in 2018 on all 6 presented localities through the analysis of winter branches and the analysis of their presence in newly foliated stands on site, and it was followed by the highest average air temperature.

Key words: *defoliators, oak, population level, forecast of attacks.*

Introduction

As a result of global climate changes, the enhanced forest protection system is being put in place with the highest degree of urgency. This is particularly important in unstable forest ecosystems (primarily in oak forests) in which defoliation, i.e. reduction in leaf area that most frequently occurs due to defoliating insects, is highly significant (Bosnjak, 2004).
Defoliating insects are prone to occasional and cyclical gradations, and as such able to compromise the production process in forestry and escalate the decline and degradation of forest ecosystems. Gypsy moth used to be considered intolerant to competition by early defoliators, due to which it would start gradations on its own. However, recent studies conducted in Germany as well as in Serbia demonstrate that gypsy moth gradations overlap with gradations of early defoliators, which makes it critical to establish the abundance of these pests.
Damages caused by oak defoliators are manifold. After major attacks by defoliating insects the production of early and late trees decreases, and the same happens in the period following the defoliation. According to research conducted by Simmons et al. (2014), the production of late *Quercus* trees drops by as much as 67% in the year of defoliation, while the production of early trees is decreased by up to 24% in the year following the defoliation. Moreover, Spaić (1986) states that defoliation is in part the cause of low acorn yield, and that rigorous suppression of defoliators in oak seed stands may significantly increase the yield.
Strength of the attack and spread of the pest partially may also depend on the type of host plants, as not all oaks are equally vulnerable to attack by defoliators. For instance, *Quercus petraea* was

found to be more sensitive than *Q. cerris* and *Q. frainetto* (Glavendekić and Medarević, 2010), however the impact of climatic factors remains crucial.

The greatest damage in oak forests is caused by species that show gradogenic attributes, as they occur in mass over certain time intervals. These include species from the family of geometrids (Lepidoptera: Geometridae), tortrix moths (Lepidoptera: Tortricidae) and noctuids (Lepidoptera: Noctuidae). They cause damage not only to leaves, but also to buds and shoots. Fluctuations of their populations are impossible to predict with certainty, and their outbreak does not follow any discernible pattern (Majović and Glavendekić, 2011). Beside gradogenic species, it is important to note the presence of other species whose individual effect is minor, but should under no circumstances be neglected in competition with gradogenic species (Mihajlović and Glavendekić, 2006) as it causes disturbance in the functioning of forest ecosystems (Darr and Coyle, 2021).

It is therefore necessary to conduct regular monitoring of the population level of these pests, based on the principles of forestry diagnostic forecast services as defined by the International Plant Protection Convention (UN) and directives of the European and Mediterranean Plant Protection Organization (OEPP/EPPO), which are incorporated into sectoral laws and bylaws as the obligation of every country (Law on Plant Health and Law on Forests, Rulebook on Lists of harmful organisms and Lists of plants, plant products and regulated objects).

The abundance of the population is determined by several methods: examination of winter branches under laboratory conditions at a constant temperature and air humidity; study of samples from foliated stands in which their numbers depend on climatic conditions; by means of glue boards which trap wingless females going into tree crowns to lay eggs. The degree of danger from defoliation by insects in the following spring (a critical number or threat of heavy defoliation) for winter moths is 1 female per 1 cm of tree diameter in Europe and in Serbia, and 2 females per 1 cm in Croatia (Spaić and Glavaš, 1988). In this paper, the first two methods were used and the obtained results were compared.

Materials and methods

On the basis of the Operative Plan, within the framework of the activity “Establishing the abundance of early oak defoliators from order *Lepidoptera* based on the results of laboratory experiments with winter branch samples, and the prognosis of their harmful effects in natural forest ecosystems”, during the months of January and February the laboratory of the Institute for Forestry (Belgrade, Serbia) conducts the macroscopic analysis of the presence and abundance of individual gradogenic species of early oak defoliators on winter oak branch samples.

Branch samples roughly 70 cm long with buds are cut with pruning shears and packed into nylon bags, which are kept in the refrigerator at 4^0C to prevent hatching of caterpillars prior to the examination. Branches are placed in the laboratory for hatching and examined through a magnifying glass, where each sample undergoes double laboratory analysis.

The method used to establish population density of early oak defoliators in the larvae stadium is rearing winter branch samples in the laboratory (meaning, prior to commencement of vegetation and hatching of caterpillars under natural conditions). One of the disadvantages of this method is difficulty in collecting samples from different parts of the crown. Felling is not suitable due to a large number of trees needed, while climbing to the top is hindered during wintertime. For this reason, in some places the samples were taken from the bottom branches, in which Geometridae are present in the largest number, so the qualitative composition of defoliators does not reflect the actual state in the forest. Moreover, in wintertime it is difficult to differentiate between the vital trees and those in various phases of dieback. Another downside is that it is often impossible to transport the branch samples immediately, so they are kept for days under inadequate conditions which diminishes their vitality and makes many buds dry out, while the caterpillars inside them die due to lack of feed.

Branch samples are reared for a month in glass jars with water, at the room temperature of 23ºC. The samples are controlled daily, and after the first particles of excrement appear at the bottom of the jars, the first larvae of early oak defoliators are collected, determined and recorded. At the end of the laboratory experiment, each sample is once again thoroughly examined, all leaf buds are counted, and on this basis the calculation of the number of leaves in the sample is made (number of buds multiplied by 4).

In their respective studies, Kulfan et al (2019) and Sarvašová et al (2020) emphasize that monitoring the abundance of population of early oak defoliators only during wintertime is not sufficiently reliable to serve as basis for predicting the risk of defoliation the following spring. The samples analyzed under laboratory conditions are therefore only a likely indicator of the future real situation in the field. Given that development of pests directly depends on the climatic conditions during their growth, the exact numbers may be determined with certainty only through examination of foliated stands. An additional control examination of samples from foliated stands was thus performed during the month of May.

The paper presents the results of research of the population level density of early oak defoliators reared under laboratory conditions during winter, at a constant temperature, as well as in the nature (in the field) after the foliation. The presented research used the samples collected from 6 sites in the area managed by the Forest Management Office Kragujevac, and the research spanned a period of 5 years – between 2016 and 2020. (Pest Diagnostic Forecast Service reports 2016 – 2020). The climatic conditions for each year of the research are presented based on the average air temperature during the month of May (at the time of full foliage), and the data were sourced from Meteorological Almanacs of the Republic Hydrometeorological Institute of Serbia and taken at the measuring station Kragujevac. The results of the determined population abundance of early oak defoliators are presented in the table relative to the constant temperature under laboratory conditions during the month of February, and relative to the average temperature in the field during the month of May.

Results and discussion

Population level of early oak defoliators under laboratory conditions and in foliated stands in the field, in the period 2016 – 2020

Based on the data for each year of the research, Table 1 was compiled to provide a comparative view of the mean monthly air temperature and humidity that was constant throughout the laboratory examination of the winter branches (the temperature was 23^{0}C and the relative air humidity was maintained at 80%), however in the field, spring climatic conditions were changeable depending on the weather conditions during the year, which undoubtedly impacted the development of the examined pests in the spring.

Table 1. Population level of early oak defoliators reared in the laboratory on winter branch samples and in foliated stands in the field in the area of Forest Management Office Kragujevac, subject to basic parameters of climatic conditions, in the period 2016 – 2020.

Coordinates, Name of location (FMU, section)	**Month, year**	**Mean monthly air temperature (^{0}C)**	**Number of caterpillars per 1000 leafs**			
			Tortricidae	Geometridae	Other	Total
4888000;7509000 FMU Rogot, 8/a	Feb 2016	23.0	3.2	22.2	0.0	25.1
	May 2016	15.6	0.0	0.0	0.9	0.9
	Feb 2017	23.0	0,0	0.0	0.0	0.0
	May 2017	12.2	0.8	0.8	0.0	1.6
	Feb 2018	23.0	37.8	0.0	0.0	37.8
	May 2018	19.0	2.1	0.0	0.0	2.1
	Feb 2019	23.0	0.0	0.0	0.0	0.0
	May 2019	14.7	0.5	0.0	0.0	0.5
	Feb 2020	23.0	1.2	0.0	0.0	1.2
	May 2020	15.8	0.0	0.0	0.0	0.0
4884000;7466000 FMU Rudnik I, 105/6	Feb 2016	23.0	2.4	2.8	0.0	5.2
	May 2016	15.6	1.5	0.0	1.5	3.0
	Feb 2017	23.0	0.0	0.0	0.0	0.0
	May 2017	12.2	0.0	0.0	0.0	0.0
	Feb 2018	23.0	27.7	0.0	0.0	27.7
	May 2018	19.0	0.7	0.0	0.0	0.7
	Feb 2019	23.0	0.0	2.1	4.1	6.2
	May 2019	14.7	6.0	0.0	1.3	7.3
	Feb 2020	23.0	1.2	0.0	0.6	1.8
	May 2020	15.8	1.1	0.0	0.0	1.1
4883000;7467000 FMU Rudnik I, 104/a	Feb 2016	23.0	19.3	12.1	0.0	31.4
	May 2016	15.6	2.3	0.0	1.5	3.8
	Feb 2017	23.0	0.0	0.0	0.0	0.0
	May 2017	12.2	3.6	2.1	0.7	6.4
	Feb 2018	23.0	0.0	0.0	0.0	0.0
	May 2018	19.0	2.9	0.0	1.1	4.0
	Feb 2019	23.0	0.0	0.0	0.0	0.0
	May 2019	14.7	3.4	1.1	2.2	6.7
	Feb 2020	23.0	0.5	0.0	0.0	0.5
	May 2020	15.8	1.1	1.1	0.0	2.2
4881500;7459300 Rajac-Ostrvica, 57/a	Feb 2016	23.0	7.5	2.5	0.0	10.0
	May 2016	15.6	0.0	0.0	4.8	4.8
	Feb 2017	23.0	0.0	0.0	0.0	0.0
	May 2017	12.2	0.8	0.0	1.6	2.4
	Feb 2018	23.0	130.4	0.0	86.9	217.3
	May 2018	19.0	0.9	0.0	0.0	0.9
	Feb 2019	23.0	0.0	0.0	0.0	0.0
	May 2019	14.7	0.0	0.0	0.7	0.7
	Feb 2020	23.0	0.0	1.2	0.0	1.2
	May 2020	15.8	0.0	0.0	0.0	0.0
	Feb 2016	23.0	0.0	5.1	0.0	5.1

4873000;7454000 FMU Vujan-Rozanj, 64/a	May 2016	15.6	0.0	0.0	0.0	0.0
	Feb 2017	23.0	0.0	0.0	0.0	0.0
	May 2017	12.2	8.0	2.0	4.0	14.0
	Feb 2018	23.0	0.0	0.0	0.0	0.0
	May 2018	19.0	0.7	0.7	0.7	2.1
	Feb 2019	23.0	0.0	0.0	0.0	0.0
	May 2019	14.7	2.4	0.0	1.2	3.6
	Feb 2020	23.0	0.0	0.0	0.0	0.0
	May 2020	15.8	0.0	0.0	0.0	0.0
4892000;7459300 FMU Rudnik II, 76/6	Feb 2016	23.0	0.0	0.0	0.0	0.0
	May 2016	15.6	0.0	0.0	0.0	0.0
	Feb 2017	23.0	0.0	0.0	0.0	0.0
	May 2017	12.2	3.7	0.0	0.9	4.6
	Feb 2018	23.0	0.0	0.0	0.0	0.0
	May 2018	19.0	4.7	0.9	1.9	7.5
	Feb 2019	23.0	0.0	0.0	0.0	0.0
	May 2019	14.7	2.3	0.0	0.0	2.3
	Feb 2020	23.0	7.4	0.0	0.0	7.4
	May 2020	15.8	0.0	0.0	0.0	0.0

In the presented period of research, 2016 to 2020, the average temperature variation during foliation ranged between 12.2 and 19.0^{0}C, while over the duration of the experiment in the laboratory in wintertime the temperature was maintained at a constant 23.0^{0}C.

Deviations in the prognosis of abundance of early oak defoliators for each year of the research based on the laboratory examination relative to their abundance in the field during spring of the same year (period 2016 – 2020)

As previously stated, the analysed samples of winter branches are only a likely indicator of the real situation in the field, and given that development of pests directly depends on the climatic conditions during their growth, the exact numbers may be determined with certainty only through examination of foliated stands. For this reason, they were re-examined and counted during foliation in May.

The results presented in Table 1 clearly demonstrate that the greatest deviation in the level of population of early oak defoliators through the analysis of winter branches (February) and the analysis of their presence in foliated stands in the field (May) was recorded in 2018 in all 6 presented sites. In one site the deviation is drastic (217.3 versus 0.9), medium in two sites (37.8 versus 2.1; 27.7 versus 0.7), while in the remaining 3 sites the deviation is low (0.0 versus 2.1; 0.0. versus 4.0; 0.0 versus 7.5). The deviation in 2018 in all presented sites showed greater abundance of the pest population in winter branch samples. That same year, at the time of foliation, the highest average air temperature was recorded (19.0^{0}C), which alongside all other factors did not favour the development of the pests.

According to the reference sources (Fält-Nardmann et al, 2016), changes of climatic conditions may ease changes in the pest range and spread, but may also have a negative impact on herbivores insects in case of disturbance in the locally adapted synchronization between the insect phenology and the phenology of their host plants. The capacity of a pest species to colonize new areas depends on its ability to adjust the time of phenological events in its bionomia.

In the year 2016, a slight increase of abundance was found in Forest Management Office Kragujevac, Forest Management Unit (FMU) Rudnik I, section 104/a, with the presence of 19.3 Geometridae caterpillars and 12.1 caterpillars of other species, which in total amounted to 31.4 caterpillars per 1,000 leaves. This led to conclusion that in this site the spring of 2016 a mild crown lightening may be expected, which did not happen as their total abundance decreased to 3.8 caterpillars. In spring 2016 in the area of FMO Kragujevac, the average defoliation amounted to 5 to 10%. Among other harmful insects and diseases, the presence of oak flea beetles, weevils, powdery mildew, leaf miners and gall wasps *Cynipidae* was found. These insects partially contributed to defoliation of the leaf mass along with early oak defoliators, while powdery mildew occurred in a narrow scope and had no effect on the increment of the stands.

Laboratory analysis of samples from the area of FMO Kragujevac in 2017 and 2019 did not detect any increase in the abundance of early oak defoliators, which was confirmed in the spring, and defoliations were very low (up to 5%). In FMU Rudnik I, section 105/b, the examination conducted in the spring 2017 uncovered severe damages of the leaf mass caused by frost (picture 13) and new leaf mass was in the process of forming at the moment of the examination, while among harmful insects and diseases the examination found oak flea beetles, leaf miners, gall wasps *Cynipidae,* and powdery mildew. In 2019, in the FMU Rogot, weevils were found to be present in large numbers, which contributed to defoliation

In 2018, laboratory analysis established a significant increase of the abundance of early oak defoliators in FMO Milanovac, FMU Rajac-Ostrvica, section 57/a, with great predominance of defoliators from the Tortrix month family (Tortricidae) – 130.4 caterpillars per 1,000 leaves. The increased numbers of these pests were also found in FMO Kragujevac, FMU Rogot, section 8/a in particular, where once again Tortrix moths dominated – 37.8 caterpillars per 1,000 leaves. It was therefore presumed that stronger crown lightening would occur in the spring, however it did not happen and the abundance of these pests was reduced to only 0.9 caterpillars per 1,000 leaves, which caused no significant defoliation of the leaf mass. In the spring of 2020, defoliation did not exceed 10% and mostly remained in the range from 2 to 5%.

In 2020, a slight increase of the abundance of early oak defoliators was found in FMO Milanovac, FMU Rudnik II, section 76/b, with dominant defoliators from the Tortrix moth family (Tortricidae) – 7.4 caterpillars per 1,000 leaves. It was again presumed that mild crown lightening would occur in the spring, however it did not happen as in the spring their abundance was reduced to the extent that no caterpillars were found during the examination of samples from foliated stands (0.0) (picture 14). In the spring of 2020, defoliation did not exceed 5% and mostly remained in the range from 0 to 2%.

Taking into account that in the presented research period 2016 – 2020 the presence of caterpillars of early oak defoliators in the area managed by FMO Kragujevac was below the threshold of harm and the damages to the leaf mass were minimal, no additional measures of protection were applied other than those prescribed that are regularly implemented every year.

Conclusion

Some of the main pests in our most valuable oak forests include large and small winter moth, oak leafroller, oak sawfly, and lackey moth, which together with other species of minor significance form the complex of “early oak defoliators”.

Early oak defoliators show gradogenic attributes, as they occur in mass over certain time intervals. They cause damage not only to leaves, but also to buds and shoots. Fluctuations of their populations are impossible to predict with certainty, and their outbreak does not follow any discernible pattern. The samples analyzed under laboratory conditions are only a likely indicator of the future real situation in the field, given that development of pests directly depends on the climatic conditions during their growth and the exact numbers may be determined with certainty only through examination of foliated stands. Therefore, besides the examination of winter branches and rearing of pests under laboratory conditions, the prognosis of their abundance necessitates the examination of stands once they are foliated, which in Serbia’s climate means during the month of May.

The main goal of the examination and control of abundance of the pest population in stands is to determine their presence and identify the degree of danger to which the forests are exposed.

In oak stands, beside gradogenic species, there is a significant presence of other species whose individual effect is minor, but should under no circumstances be neglected if they occur in competition with gradogenic species as it causes disturbance in the functioning of forest ecosystems.

The population level of early oak defoliators largely depends on climatic factors, primarily the temperature at the time of foliation. The greatest deviation in the level of population of early oak defoliators through the analysis of winter branches (February) and the analysis of their presence in foliated stands in the field (May) was recorded in 2018 in all 6 presented sites. In one site the deviation is drastic (217.3 versus 0.9), medium in two sites (37.8 versus 2.1; 27.7 versus 0.7), while in the remaining 3 sites the deviation is low (0.0 versus 2.1; 0.0. versus 4.0; 0.0 versus 7.5). The deviation in 2018 in all presented sites showed greater abundance of the pest population in winter branch samples. That same year, at the time of foliation, the highest average air temperature was recorded (19.0^{0}C), which alongside all other factors did not favour the development of the pests.

The work on researching the presence of defoliators and their abundance continues in order to ensure implementation of the required measures of protection in a timely manner and to prevent greater damage to our most valuable oak forests.

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BIOPOTENTIAL OF INTRODUCED FALSE INDIGO AND ALBIZIA WEEVIL IN HOST PLANT CONTROL AND DURATION OF ITS DEVELOPMENT STAGES IN SOUTHERN REGIONS OF PANONIAN BASIN

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Abstract

The paper present results of the entomological experimental studies of the biological, ecological and (bionomic) insect performances such as seasonal adaptation of introduced monophagous false indigo and albizzias weevil's *Acanthoscelides pallidipennis* Motschulsky. and *Bruchidius terrenus* (Sharp), Coleoptera: Chrysomelidae: Bruchinae, to phenological phases of aggressive invasive host plant *Amorpha fruticosa* L. on territory of Republic of Serbia with special attention on assessing and monitoring of new formed and detected inter species relations between autochthons parasite wasps from fauna (Hymenoptera: Chalcidoidea) and herbaceous seed weevil beetle. During 15 years (2006-2021) on approximately 30 localities data analyses were done for observed experimental host plants from samples with statistical significance. Status of genera from families Hymenoptera: Chalcidoidea.: Pteromalidae and Eulophidae after intensive investigations has been trophicly identified. Recorded seed pest species of *A. fruticosa*, or *A. julibrissin* (Fabales: Fabaceae) was introduced in Serbia, and planted as ornamental trees, also were put undergo different kinds of laboratory and field research tests during this period in a goal of collecting data about lasting each of develop stage of their seed beetles. Field generations in different stages were also monitored by continuous infested seed collecting and its disection. Established host plant-seed predator linkage was observed in correlation with different environment parameters, especially water level fluctuations in bank corridor formation stands and riparian cultures.

Keywords: *pods, Amorpha, Albizia, weevil, Chalcidoid wasp, herbivores, Serbia.*

Introduction

The genera Bruchidius (Coleoptera: Chrysomelidae: Bruchinae), which is described by Schilsky in 1905, is distributed in the old world and includes about 300 seedbeeetle species (Hoebeke at al, 2009). Bruchine are economically important as pests of agricultural storage products; for example (Ebadollahi et al., 2013; Loni & Panahi, 2015). Larvae of Bruchidius species for host plants have leguminous plants, family (Fabaceae). *Amorpha fruticosa* and *Albizzia julibrissin* (Fabales: Fabaceae) was introduced in Serbia, and planted as ornamental trees (Vukićević, 1996, Jovanović, 2000). Duration of certain developmental stages of *Acanthoscelides pallidipennis* in laboratory conditions (in days) were measured by several methodologies(dissection, and one flacon one seed raising).During the research since 2012 in Serbia, the species *Bruchidius terrenus* (Sharp), which are parasitized by *A. julibrissin* seeds, and their parasitoids (Gagić-Serdar et al., 2012; Gagić-Serdar et al., 2013) had been recorded for the first time (Figure 1 and Figure 2.)

Materials and methods

The collected Populations (Figure 1) were cultivated under controlled conditions in order to obtain data on the length of each development stage. In addition to the development stages of seedbeetles, development stages of their parasitoids have also been recorded, which are further grown in plastic boxes with mesh and petri dish for the purpose of determination (Figure 9). The discovery of randomly selected pods revealed seed infestation according to Tuda's methodology and associates (2001). Parasitoids are determined by using interactive act keys like De Graham (1969); Trâpicéyn (1989); Medvedev (1978) & Bouček (1991) by prof. dr Ljubodrag Mihajlović.

Results and discussion

Since start of research many numbers of species of parasitic wasps had been determined and registered as autochthonous fauna; they were determined as the natural enemies of granivorous *Acanthoscelides pallidipennis* (Motschulsky) *and* Bruchidius *terrenus* (Sharp). Duration of certain developmental stages of *Acanthoscelides pallidipennis* in laboratory conditions (in days). The parasitoid complex consist primary of both are the *Dinarmus acutus* (Hymenoptera: Chalcidoidea: Pteromalidae) and an entirely new species of Eurytomidae (Hymenoptera: Chalcidoidea) (Figure 3-8). The adaptation process is still ongoing; parasitoids are indigenous species, trying even to be synchronous with development stages of Bruhinae. *D. acutus,* which is fed inside the pods, is fed in the larvae within the originally infested healthy generative material of the silk tree, and is considered to be solitary ectoparasitoid. Nevertheless, they were found by numerous species of Eulophidae (Hymenoptera: Chalcidoidea) as the base, as parasitoids of the second or third order-hyperparasitoids: Eulophid genera *Aprostocetus* and *Tetrastichus*. Nothing less important for the abundance of germination of seeds is also mentioned parasitoids that have about 15-20% of the total number of infested seeds. Information on the, biology, identity, phenology, distribution and impact of silk tree key parasitoid species in Serbia still need to be investigated in the future.

Figure 1. Beetle *Bruchidius terrenus* after ecclosion, 2017, site Ruma (Original)

Figure 2. Darker appearance of the adult elitrae cover of *Bruchidius terrenus,* site Novi Sad, 2014. (Original)

Figure 3. parasitic, wasps fam. Eurytomidae (Hymenoptera: Chalcidoidea; Eurytomidae), by *Albizia* weevil (Original)

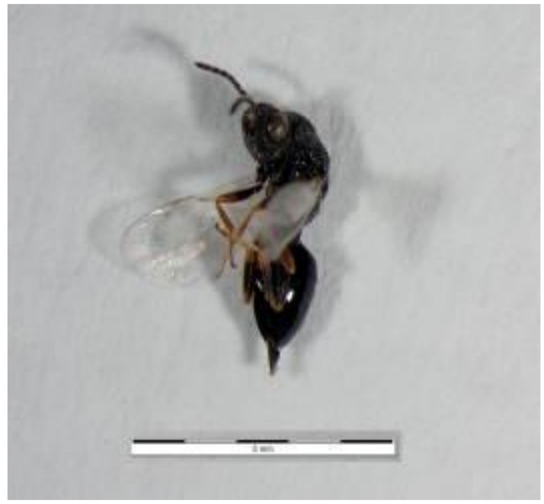

Figure 4. parasitic, wasps fam. Eurytomidae (Hymenoptera: Chalcidoidea; Eurytomidae), by *Albizia* weevil (Original)

Figure 5. Parasitic wasp family (Hymenoptera: Chalcidoidea: Eupelmidae), *Eupelmus vesicularis, Albizia*’s Weevil (Original)

Figure 6. Parasitic wasp family (Hymenoptera: Chalcidoidea: Eupelmidae), *Eupelmus vesicularis, Albizia*’s Weevil (Original)

Figure 7.
Parasitic wasps family Pteromalide, *Dinarmus acutus* (Hymenoptera: Chalcidoidea: Pteromalidae) *Albicija*’s weevil(Original)

Figure 8.
Parasitic wasps family Pteromalide, *Dinarmus acutus* (Hymenoptera: Chalcidoidea: Pteromalidae) *Albicija*’s weevil(Original)

Figure 9. Adults of *Bruchidius terrenus Albicija*’s weevil, flew out of the legume pods (seeds) of *Albizia jullibrisin* collected in Ruma Deteline site, parasitoids, and details in appearance compared of adults and green pods with damage and emerging wholes (Original)

Table 1. Duration of certain developmental stages of *Acanthoscelides pallidipennis* in laboratory conditions (in days)

Year of research	Egg stage	Larval stages	Pupa stage	Imago stage	Total life cycle duration
2007	11 - 14	25 - 33	7 - 9	15 - 26	58 - 72
2008	12 - 14	18 – 20	8 - 11	14 - 19	52 - 64
2009	10 - 15	15 - 19	8 - 15	12 - 17	45 – 61
2010	11 – 15	17 – 21	7 – 11	15 – 17	50 – 64
2011	10 – 14	20 – 22	8 – 14	12 – 14	50 – 64

Conclusions

Parasitoids that are shown and photographed in this paper, the first time were recorded and found as *Amorpha fruticosa* and *Albizia jullibrisin* weevil parasitoids and they are indigenous species that have adapted to non-native host (host plant as non-native seed beetle). Duration of certain developmental stages of *Acanthoscelides pallidipennis* in laboratory conditions (in days) were measured by several methodologies(dissection, and one flacon one seed raising). That’s opens many questions about their bionomy and ecology which is in adaptive, denizen state.

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PROPERTIES AND POSSIBILITIES OF FORESTATION OF NON-AGRICULTURAL SOIL IN THE AREA OF SREM IN SERBIA

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Abstract

The paper presents the characteristics of soil in the lowland agricultural area of Srem in Serbia, which is not used for agricultural production. The research was carried out on three areas in the area of the village Surduk with an area of 16.69 ha. Three pedological profiles were opened in the study area and their external and internal morphology was described. Samples of disturbed soil were taken from pedological profiles and standard physical and chemical analyzes were performed. The type of carbonate chernozem soil on the loess plateau was determined. The granulometric composition of the soil indicates a slightly higher share of total clay in relation to the total sand, and the texture class of loam predominates. Carbonation increases with depth and the examined soil belongs to strongly carbonate, neutral to weakly alkaline reactions. The humus content is the highest in the powerful humus horizon, ranging from 2.06 to 2.54%, and can be classified as moderate humus soil. Having in mind the favorable granulometric composition, the amount of humus and the stable crumbly structure, this soil has a very favorable water-air regime. The examined soil is of great production value for raising hardwood forest plantations.

Keywords: *Non-agricultural soil, afforestation, Srem.*

Introduction

The area of Srem is mostly a lowland area, where fertile areas that are used exclusively for agricultural production predominate. Forest areas in Srem are represented in the area around river watercourses and in the hilly area of Fruška gora. The area of AP Vojvodina itself is very little covered by forest, ie the entire AP Vojvodina has only 6.37% of forest according to Vlatković (1981). Since these are agricultural areas that are very little covered by forest, it is necessary to use all areas that are not used for agricultural purposes to raise forest plantations in order to increase the forest cover of this area (Pekeč et al. 2013). By raising forests on agricultural land, in addition to increasing forest cover, a multiple effect is achieved in the form of ecological contribution and in the appearance of the landscape according to Knežević (2011) as well as a positive impact on health by various phytoncides from forest trees according to Zorić et al. (2020). One of such areas that is not used for agriculture can be found in the area of the village of Surduk, while there are arable land around. The surveyed areas were used as illegal landfills. The characteristics of the soil were examined on the mentioned areas in order to correctly determine the types of trees that correspond to the given edaphic conditions during the future afforestation of this area. Potential forest vegetation according to Tomić et al. (2011) in the area of the Pannonian forest-steppe zone, there are no climatogenic forests on larger areas, but rather "foresters" - smaller stands of various xerothermic communities oaks. Apart from the edaphic conditions, the choice of species should be harmonized with the temperature conditions and water balance, which according to Kostić et al. (2019) significantly affect tree growth, especially at a time of current climate change that poses a challenge to the forestry sector according to Alexandrov and Iliev (2019).

Material and method

The paper investigates the soil on three plots that are not used for agricultural production, and which is located in the area of Srem (Serbia) in the area of Surduk village. The altitude of the examined soil is from 96 to 103 m, and the total area of all three plots is 16.69 ha. Three pedological profiles were opened in the test area P1 (N 45^0 04$^{'}$ 12,16$^{''}$ E 20^0 18$^{'}$ 15,60$^{''}$), P2 (N 45^0 04$^{'}$ 7,42$^{''}$ E 20^0 18$^{'}$ 43,26$^{''}$) i P3 (45^0 04$^{'}$ 40,35$^{''}$ E 20^0 18$^{'}$ 21,41$^{''}$). External and internal profile morphology is described. Samples of disturbed soil were taken from open pedological profiles and the following physical and chemical analyzes of soil were performed:

Mechanical composition, B-pipette method with preparation of samples for analysis in sodium pyrophosphate according to Bošnjak et al. (1997), and the texture class of the soil was determined by the Tommerup classification.

CaCO3 content was determined volumetrically using a Scheibler calcimeter;

pH value was determined in soil suspension with water, potentiometrically;

Humus content according to Tyurin, modified by Simakov (1957);

Based on the characteristics of the examined soil, a proposal for possible afforestation was given.

Picture 1 Location of pedological profiles (Source: Google Earth 2021)

Results and discussion

Analyzing the granulometric composition of the examined soil (Table 1), it can be seen that the smallest share is the fraction of coarse sand, on average 0.75-1.17%, while the fraction of fine sand predominates (44.26-48.64%), followed by the powder fraction (27.56-31.77%). and the clay fraction has a lower share ranging from 22.69-23.21%. The ratio of average values of total sand is slightly lower in relation to the total clay and ranges from 45.01 to 49.67% to 50.33 to 54.99%. The textural class of the examined soil is loamy, while one surface horizon is classified as sandy clay loam.

Table 1. Granulometric composition of soil

Profile number	Horizon	Depth (cm)	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Total sand (%)	Total clay (%)	Texture class
P1	A	0-80	2.97	46.67	27.68	22.68	49.64	50.36	Loam
	AC	80-100	0.34	42.11	31.92	25.64	42.44	57.56	Loam
	C	100-200	0.21	47.95	32.08	19.76	48.16	51.84	Loam
	Average	0-200	1.17	45.58	30.56	22.69	46.75	53.25	Loam
P2	A	0-70	1.49	44.31	28.24	25.96	45.8	54.2	Loam
	AC	70-100	0.17	42.71	33.52	23.6	42.88	57.12	Loam
	C	100-200	0.59	45.77	33.56	20.08	46.36	53.64	Loam
	Average	0-200	0.75	44.26	31.77	23.21	45.01	54.99	Loam
P3	A	0-70	1.58	54.82	15.84	27.76	56.4	43.6	Sand clay loam
	AC	70-100	0.97	45.71	33.56	19.76	46.68	53.32	Loam
	C	100-200	0.53	45.39	33.28	20.8	45.92	54.08	Loam
	Average	0-200	1.02	48.64	27.56	22.77	49.67	50.33	Loam

According to the chemical composition of the examined soil (Table 2), it can be stated that the average carbonate content is from 16.76 to 18.20% and increases with depth. According to the classification (Belić et al. 2014), the examined soil is highly carbonate. The reaction of the soil determined in water ranges on average from 7.39 to 7.72, and with regard to the growth of carbonates and the pH value, it increases with depth. According to the classification (Belić et al. 2014), the examined soil is neutral to slightly alkaline. The humus content is the highest in the strong humus horizon, where it ranges from 2.06 to 2.54%, and according to the classification according to Belić et al. (2014) it can be classified as moderate humus soil, while with depth the humus content decreases significantly. (0.30 to 0.57%) in the C horizon, so the average values of humus for the entire profile are from 1.04 to 1.21%.

Table 2. Chemical composition of soil

Profile number	Horizon	Depth (cm)	$CaCo_3$ (%)	pH (in H_2O)	Humus (%)
P1	A	0-80	10.26	7.45	2.54
	AC	80-100	21.25	7.46	0.44
	C	100-200	18.76	7.51	0.36
	Average	0-200	16.76	7.47	1.11
P2	A	0-70	12.34	7.31	2.06
	AC	70-100	21.17	7.30	0.76
	C	100-200	21.09	7.57	0.30
	Average	0-200	18.20	7.39	1.04
P3	A	0-70	4.96	7.41	2,38
	AC	70-100	22.56	7.85	0.68
	C	100-200	22.89	7.90	0.57
	Average	0-200	16.80	7.72	1,21

All three pedologically described profiles are of the same morphological structure: A-AC-C, with a pronounced powerful humus A horizon with a depth of 70 to 80 cm. The deeper parts of this soil are the transitional AC horizon at depths of 70-80 to 100 cm and the light parent substrate, ie the C horizon at depths of 100 to 200 cm. The systematic unit of soil of all three examined pedological profiles is identical and according to the map of the soils of Vojvodina (1: 50.000), the type of soil prevails in the entire examined area: carbonate chernozem on a loess plateau. According to the classification of Yugoslav soils, (Škorić et al. 1985), it is about soil from the order of automorphic soils, class of humus-accumulative soils, soil type chernozem, subtype on loess and loess-like sediments, carbonate variety, medium deep form. Due to the granulometric composition, this type of soil has a very small share of the coarse sand fraction, while the share of the fine sand fraction predominates. The powder fraction is also significant and has a large share in the total granulometric composition, and unlike the powder, this soil contains a smaller share of the clay fraction. The ratio of total sand to total clay varies and the content of total clay is generally slightly increased. The chemical properties of this type of soil are characterized by relatively high carbonate content, which grows along the depth of the profile. The reaction of the soil in the water is in accordance with that, and with the increase of carbonate in depth, the alkalinity increases. The humus content of these soils is good to moderate in the surface humus horizon, and with depth there is a large drop in humus content. Having in mind the favorable granulometric composition, the amount of humus and the stable crumbly structure, this soil has a very favorable water-air regime. Chernozem is a soil of high production potential that is mostly used for agricultural production, and by nature there are smaller oases under forest vegetation (Živkovic et al. 1972). For raising and growing forests in the area of Ravni Srem, human action is necessary, but as close as possible to natural processes, so that we imitate nature, and not drastically disturb it (Rađević et al. 2020). Looking at the characteristics of the soil in the examined area, it can be stated that this soil is of great production value and as such has a great production potential for raising hardwood forest plantations, which will increase the level of forest cover in this area.

Conclusion

The paper presents the characteristics of the soil of the agricultural area of the lowland part of Srem which is not used for agricultural production. The determined soil of the examined area belongs to the order of automorphic soils, class of humus-accumulative soils, type of chernozem soil. According to the examined physical and chemical properties, this soil has a very favorable water-air capacity and is highly productive for raising hardwood forest plantations, which will increase the percentage of forest cover and achieve multiple effects in the form of ecological contribution of the examined area.

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CHANGES IN THE FLORISTIC COMPOSITION OF BEECH FORESTS (FAGUS SYLVATICA L.) AT TWO SITES IN SOUTHEASTERN SERBIA OVER A PERIOD OF 14 YEARS

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Abstract

European beech forests are generally poor in species. However, the composition and richness of vascular plants in these forests vary with region and site. Numerous factors can cause changes in the floristic composition and diversity of these forests over time, one of which is the type of management practice. The paper presents the results of research on the floristic composition and diversity of beech forests at two sites in southeastern Serbia (MU "Lomnička Reka" and MU "Bukovik II") over a period of 14 years. The number and cover of all plant species were recorded in the selected sample areas of each locality in 2008 and 2021. The studied beech stands grew in similar environmental conditions – on dystric cambisol and at the altitude ranging from 685 to 735 m. A total of 35 plants were registered at both sites in the period from 2008 to 2021. It was found that the average number of species per plot increased slightly in the research period at both sites. The number and cover of the species that indicate faster decomposition of organic matter and the formation of milder forms of humus such as *Festuca drymeia* Mert. &Koch, *Asperula odorata* L. and *Mercurialis perennis* L. did not change significantly over time. The most significant changes were related to the species that hinder natural regeneration, such as *Rubus hirtus*, whose increased presence was registered after those stands had been thinned, which resulted in the canopy opening.

Keywords: Beech forests, diversity, vegetation changes, forest management.

Introduction

Recent decades of vegetation studies related to forest ecosystems have revealed significant changes in plant diversity, extinction of rare and endangered species, increase in nitrophilic and acidophilic species, as well as drought-resistant species (Jantsch et al., 2013). Climatic changes (Walther et al., 2002; Baeten et al., 2010), air pollution (van Dobben and de Vries, 2010) and forest management methods have been cited as the major factors causing the changes in the vegetation of forest ecosystems. Forest management is one of the primary drivers of diversity and may enhance or reduce biodiversity depending on the applied measures (Kutnar et al., 2015; Horvat et al., 2017; Lelli et al., 2019). The diversity of ground vegetation is an important indicator of habitat quality and at the same time a measure of the impact of management on the environment. This is the reason why the composition, condition, or change of vegetation cover over time are key factors in various environmental studies and nature protection programs.
Beech forests are the most widespread in Serbia and cover 660,400 ha, 29.4% of the total forest area (Banković et al., 2009). Beech forests live in various environmental conditions, from the

submontane zone to the montane-subalpine zone. Apart from the broad climate amplitude, the beech is characterised by a wide edaphic amplitude. It grows on acidic silicate, basic, ultrabasic and carbonate substrates. The composition and richness of vascular plants in beech forests are highly variable among European regions and habitats, but European beech forests are generally poor in species. The aim of this research was to determine the change in the floristic composition and diversity of beech forests at two localities in southeastern Serbia over a period of 14 years, i.e. before and a couple of years after the implementation of silvicultural measures.

Material and Methods

The research was conducted in beech stands at two localities in southeastern Serbia – Veliki Jastrebac (MU "Lomnička Reka") and Bukovik (MU "Bukovik II"). The studied forest complexes are state-owned and managed by the "Srbijašume" state enterprise. The most important data are shown in Table 1. The localities are characterised by similar soil and climate conditions. The studied beech stands grow on district cambisol at an altitude of 685-735 m.

Table 1. Basic data on research plots in beech stands

	Altitude	*Aspect*	*Soil type*	*Age*
MU “Lomnička Reka”	735	W	Dystric cambisol	80-100
MU “Bukovik II”	685	E	Dystric cambisol	60-80

Floristic sampling was done on a floristically homogeneous surface area with a plot size of 900 m^2 (30 x 30 m). A complete floristic list of all vascular plants (tree, shrub, and herb layers, separately) was recorded for each plot using the Braun-Blanquet scale (Braun-Blanquet, 1964). The transformation of abundance and cover estimation of each species within phytosociological relevés was performed according to the Van Der Maarel method (1979). Species diversity was measured using the *Shannon Wiener* diversity index (H’) (Shannon and Weaver, 1963); the Pielou Index was used for the estimation of species evenness (1975). JUICE 7.0 software was used to calculate all indices (Tichý, 2002). The spectrum of floral elements was determined according to the systematisation of geo-floristic elements by Gajić (1980); the spectrum of life forms and the indicator values of plants and ecological optimums were determined using the method of Kojić *et al.* (1997).

Results and Discussion

A total of 35 plant species were registered in the period from 2008 to 2021 at both studied localities (Table 2). Apart from the species typical of beech forests, the presence of the invasive species *Erigeron annuus* (L.) Pers. was also registered at the selected locality in the MU "Bukovik II" during the observation in 2021.

The average number of species in both studied stands ranged from 19 to 22 species per plot (Table 3), which was consistent with previous studies on the diversity of beech forests up to about 800 m (Ujházyová et al., 2016; Stajić et al., 2021). The number of species increased slightly from 2008 to 2021. To a certain degree, it was caused by the canopy opening that resulted from the silvicultural measures implemented in the stands. Light availability may affect the species richness in the herb layer (Axmanová et al., 2012; Dormann et al., 2020). The canopy opening in these stands was partly induced by the action of an unfavorable abiotic factor (i.e. ice breakage) that hit most of eastern Serbia in 2014.

Table 2. Floristic composition of studied beech stands

Species	*2008*	*2021*	*2008*	*2021*
	MU "Bukovik II"		MU "Lomnička Reka"	
Abies alba Mill.			+	+
Acer platanoides L.	+	+	+	+
*Acer pseudoplatanus*L.	+	+	+	+
Arum maculatum L.			+	+
Asarum europaeum L.			+	+
Asperula odorata L.	+	+	+	+
Circaea lutetiana L.		+		+
Dipsacus pilosus L.		+		
Dryopteris filix-mas (L.) Schott	+	+		+
Epilobium angustifolium L.	+			
Euphorbia amygdaloides L.	+			
Fagus sylvatica L.	+	+	+	+
Festuca drymeia Mert. &Koch	+	+		
Fragaria vesca L.	+	+		
Fraxinus ornus L.			+	+
Geranium robertianum L.	+	+		+
Glechoma Waldst. & Kit.		+		
Hedera helix L.			+	
Lamium galeobdolon (L.) Crantz	+	+	+	+
Lathyrus venetus (Miller) Wohlf.		+		
Mercurialis perennis L.			+	+
Mycelis muralis (L.) Dum.	+	+		+
Polygonatum odoratum (Mill.) Druce			+	
Polygonatum verticillatum (L.) All.	+			
Polystichum aculeatum (L.) Roth	+	+	+	+
Populus tremula L.				
Prunus avium L.	+	+		
Pulmonaria officinalis L.	+		+	
Rubus hirtus Waldst. & Kit.	+	+	+	+
Ruscus hypoglossum L.	+	+		+
Stellaria media (L.) Vill.	+			
Sambucus nigra L.			+	+
Erigeron annuus (L.) Pers.		+		
Viola alba Bess.			+	+
Viola sylvestris Lam.	+	+		

The number and cover of the species that indicate faster decomposition of organic matter and the formation of milder forms of humus such as *Festuca drymeia* Mert. &Koch, *Asperula odorata* L. and *Mercurialis perennis* L. did not change significantly over time. The most significant changes were related to the species that hinder natural regeneration, such as *Rubus hirtus* Waldst. & Kit., whose increased presence was registered after the stands had been thinned, resulting in the canopy opening. Due to the dense canopy closure in the beech stand of the MU "Bukovik II", the presence of species that hinder natural regeneration, such as *Rubus hirtus* Waldst. & Kit. was not significant. On the other hand, the entire area of the beech stand in the MU "Lomnička Reka" was covered with this species, as a consequence of the intensive stand canopy opening.

The value of the Shannon Wiener diversity index ranged from 2.17 to 2.46; its value also increased in the period from 2008 to 2021, following the increase in the number of species.

Table 3. Species richness, Shannon Wiener diversity index (H') and species evenness

Forest community type	*Year*	*Species richness*	*Shannon Wiener diversity index (H')*	*Evenness*
MU "Bukovik"	2008	19	2.23	0.73
	2021	22	2.46	0.75
MU "Lomnička Reka"	2008	19	2.17	0.69
	2021	20	2.38	0.75

The spectrum of floral elements (Tables 4 and 5) shows that the Central European range type dominates in the studied beech forest communities. The share of plants of xerothermophilic character (Sub-Mediterranean and Balkan range types) increased compared to 2008 from 15.8% to 20% in the MU "Lomnička Reka" and 23.8% in the MU "Bukovik II". At the same time, the share of mesophilic plants (Central European and Sub-Atlantic range types) decreased from 57.9% to 50% in the MU "Lomnička Reka" (Tables 4) and from 47.4% to 42.9% in the MU "Bukovik II" (Tables 5). Such spectrum indicates the change in the site regime of temperature and water partly caused by the canopy opening in both stands. A slightly higher share of circumpolar plant type was also registered compared to 2008.

Table 4. Spectrum of floral elements – MU "Lomnička Reka"

Cumulative range types	2008		2021	
	Share (%)			
Sub-Mediterranean	10.5	15.8	15.0	20.0
Balkan	5.3		5.0	
Central European	52.6	57.9	45.0	50.0
Sub-Atlantic	5.3		5.0	
Eurasian	15.7	21.0	10.0	20.0
Cosmopolitan	5.3		10.0	
Circumpolar	5.3	5.3	10.0	10.0

Table 5. Spectrum of floral elements – MU "Bukovik II"

Cumulative range types	2008		2021	
	Share (%)			
Pontic	-	15.8	9.5	23.8
Sub-Mediterranean	10.5		9.5	
Balkan	5.3		4.8	
Central European	47.4	47.4	38.1	42.9
Sub-Atlantic	-		4.8	
Eurasian	15.8	17.3	14.3	19.1

Cosmopolitan	10.5		4.8	
Circumpolar	10.5	10.5	9.5	9.5
Adventive	-		4.8	4.8

The mean indicator values of environmental factors (Table 6) show that the studied beech stands grow in almost the same environmental conditions. The mean indicator values changed slightly over time in both stands. There is an evident change in the mean value of the environmental index obtained for temperature, whose value increased in both stands from 3.16 to 3.43 (MU "Bukovik II") and 3.21 to 3.35 (GJ "Lomnička Reka"). This result is in line with the analysis of the spectrum of floral elements, which revealed an increased share of plants of xerothermophilic character in the recent period.

Table 6. Average values of environmental indices

Year	Moisture	Soil reaction	Nitrogen	Light	Temperature
MU “Bukovik II”					
2008	3.11	3.16	3.11	2.63	3.16
2021	3.10	3.24	3.00	2.62	3.43
MU “Lomnička Reka”					
2008	3.05	3.32	2.95	2.16	3.21
2021	3.15	3.30	3.05	2.15	3.35

Conclusions

Research of the floristic composition and diversity of beech forests, which was conducted over a period of 14 years, showed that the average number of species increased slightly from 2008 to 2021 in both studied stands. This increase resulted from the canopy opening that was partly caused by silvicultural measures implemented in the stands and partly by the unfavorable influence of an abiotic factor (ice breakage) that hit the area of eastern Serbia in 2014. The analysis of the spectrum of floral elements revealed an increased share of plants of xerothermophilic character compared to 2008, i.e. a decrease in the share of mesophilic plants (Central European and Sub-Atlantic range types). Such spectrum indicates the change in the site regime of temperature and water partly caused by the canopy opening in both stands. This was further confirmed by an increase in the mean values of the environmental factor for temperature.

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THE VIABILITY OF BEECH SEEDS (*FAGUS SYLVATICA*) ORIGINATING FROM THE REPUBLIC OF MOLDOVA UNDER HIGH POSITIVE TEMPERATURES

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Abstract

Under the conditions of the ongoing climate change, the ripening seeds of the beech *Fagus sylvatica* L. in the Republic of Moldova during the autumn period are exposed to long-term effects of the high temperatures, which may adversely affect their germination. The purpose of our research was to study the effect of different doses of supraoptimal temperatures on the viability and germination of *Fagus sylvatica* seeds harvested in 2021 from Hîrjauca, the Republic of Moldova. The viability of beech seeds was determined by two rapid tests: a) evaluation of enzyme activity by 1.0% of 2,3,5-triphenyltetrazolium chloride (TTC); b) forced germination by 1% hydrogen peroxide (PH), which have the good correlation (Pearson coefficient 0.8281). Initial viability of beech seeds was 80.0 by TTC and 79.0% by PH test. The beech seeds were exposed to short times (30, 45, 60 min) heat at 50ºC and 60ºC. The significant decrease in viability by 39% was establish after seed treatment at a temperature of 60ºC for 30 min ($LSD_{0.05}$=16.58, $p<0.05$). At the same time the roots length of survived seeds germinated during seven days in hydrogen peroxide slightly increased from 0.46 cm to 0.53 cm ($LSD_{0.05}$=0.07, $p>0.05$). The heat treatment at minimal tested dose (50ºC for 30 min) before the stratification led to a decrease in viability and germination rate of beech seeds. After two months of germination in coconut substrate, the emergence was only about 18.5%. The roots length of survived seeds after heat treatment did not differ significantly from intact seeds ($LSD_{0.05}$= 0.34, $p>0.05$).

Keywords: *Fagus sylvatica, seed, viability, germination, high temperatures.*

Introduction

The beech forests of the Republic of Moldova grow on the border of the southeastern distribution area of *Fagus sylvatica* in Europe. It is known that population boundaries are determined by two main factors: environmental conditions and gene flow limits (Zhivotovsky and Osmanova, 2020). Environmental conditions are the main factor in the life of a population, which determines its phenotypic appearance and genetic profile as a result of evolution. Habitat change is one of the most important reasons for the extinction of populations and species. For this reason, climate fluctuations can have a significant impact on the number of adapted and survived beech plants in new condition.

As a result of global and regional climatic changes in the territory of the Republic of Moldova in the last two decades, there has been a noticeable shift in weather and climate conditions. Every year there is an increase in the average temperature in the autumn-winter period with a decrease in the amount of precipitation. So, for example, in 2020, the average air temperature for the autumn season was +12 ... +14 ºC across the territory of the Republic of Moldova, which exceeded the norm by 3.0-4.0 ºC, and in the winter season of 2019-2020 – by +1.5...+3.5 ºC, which was 4.5-5.5 ºC above the norm. On average, once every 10 years in the northern regions of the republic and once every 4 years in the rest of the territory, there is no steady drop in air temperature in winter below 0 ºC. The amount of precipitation during the autumn season averages 100-135 mm across the territory, or about 20% of the annual norm. During the winter season, an average of precipitation equal 85-110 mm falls on the territory, or 16-20% of the annual amount (http://moldova.pogoda360.ru, http://old.meteo.md).

The ripening of *Fagus sylvatica* seeds in the conditions of the Republic of Moldova occurs on in the period from September to October, when daytime air temperatures can often exceed +20 ... +25 ºC and even reach +30 ºC; at average daily temperatures during this period +14 4 ºC and +8.7 ºC at night). Therefore, after ripening, the seeds are exposed to supraoptimal temperatures for a long time, which can adversely affect their viability and germination. Many researchers are studying the effect of different storage temperatures on the viability of beech seeds (Gugala, 2002; Pukacka and Wrjkiewicz, 2003; Ratajczak and Pukacka, 2005; Procházková and Bezděčková, 2008; Bezděčková et al., 2013; Bezděčková and Matějka, 2015). It has been established that an increase in storage temperature leads to a decrease in seeds viability. Previously, we showed that the procedure for determination of seed viability using hydrogen peroxide can be apply for rapid evaluation of effect caused by supraoptimal temperatures on germination capacity (Elisovețcaia et al., 2021).

The purpose of our research was to study the influence of supraoptimal temperatures and different exposure times on the viability and germination capacity of seeds of *Fagus sylvatica* originating from the Republic of Moldova by hydrogen peroxide test and germination through stratification.

Materials and Methods

The experiments were carried out in the Laboratory of Natural Bioregulators of the Institute of Genetics, Physiology and Plant Protection, Republic of Moldova during 2021-2022. The seeds of European beech *Fagus sylvatica* L. (Fagaceae) were collected in the autumn of 2021 from beech stand Hîrjauca in the Republic of Moldova. The beech seeds were dried until they reached a moisture content of 8-10% and were stored at the temperature of +4±1ºC in polyethylene bags, placed in plastic containers.

Treatment of seeds by supraoptimal temperatures

After quality appreciation by rejection of empty and damaged seeds, the seeds were selected for experiments by calibration, thus that the seeds weight in each repetition was approximately the same; the weight deviation varied within ± 1%.

Each variant consisted of 30 seeds on 4 replications. The seeds were soaked in distilled water and infused for 24 hours at room temperature (+22±1 °C). Further, the seeds in the experiment were exposed to temperatures at t=+50 and t=+60 °C during different exposure times (τ=30, 45 and 60 minutes) in a water bath (JB Academy Unstirred Water Baths JBA5, UK). The viability seeds, germination capacity and roots length were studied.

The viability of seeds of Fagus sylvatica

The viability of seeds was determined by two test using the 2.3.5-triphenyltetrazolium chloride (TTC) solution (Kerkez *et al.*, 2018) and hydrogen peroxide (HP) solution (Sharma and Sibi, 2020). Root length (in cm) of germinated seeds in the HP test was measured.

The viability of seeds in TTC test was classified according to standard methods in dependence of the seed staining (Kerkez *et al.*, 2018; Elisovețcaia *et al.*, 2020). However, some seeds after heating had a lighter shade of the inner region of the cotyledons compared to the outer regions. Therefore, when the inner part of the cotyledons was significantly lighter than the rest of the areas and occupied an area of more than 1/3 of the cotyledons, the seeds were considered unviable. If, with a well-colored root, the inner slightly colored area of the cotyledons occupied less than 1/3 of the area, the seeds were classified as conditionally viable (Fig. 1).

a) b)

Figure 1. Conditionally viable seeds of *Fagus sylvatica* in the TTC test in the variants of processing with supraoptimal temperatures: a) seed with bright red staining of the outer areas; b) partially stained seed in horizontal cut may produce either normal or abnormal seedling.

The germination test

The germination test was carried out in accordance with the recommendations of the International Seed Testing Association (ISTA, 2006). Seeds stratification was passed at moisture content of 30% and temperature of +4±1 °C until germination. Each variant consisted of 100 seeds on 4 replications, the weight was equal to 27.60±0.29 g for 100 seeds. The seeds were soaked in distilled water and infused for 24 hours at room temperature (+22±1 °C). Then, the seeds in the experiment were exposed to supraoptimal temperature of 50 °C for 30 min in a water bath (JB Academy Unstirred Water Baths JBA5, UK). After that, the seeds were mixed with pre-moistened coconut substrate (100 g of moistened substrate for each repetition). The mixture was placed in nylon bags and the seeds were stratified at a temperature of +4±1 °C until germination. During the experiment, the coconut substrate with seeds was periodically moistened as needed and the seeds germination was monitored. The appearance of beech seeds with the roots longer than 0.3 cm they were considered as germinated. The total percentage of germinated seeds and the length of roots were determined.

Statistical analysis

The data analysis was performed in Excel and Statgraphics Plus 5.0 programs. Values were presented as the mean of four replicates by standard deviation.

Results and Discussion

The viability of freshly harvested seeds (origin Hîrjauca, Republic of Moldova), determined by tetrazolium (TTC) and peroxide (HP) tests, averaged 80.0 and 79.0%, respectively. The values obtained by the two tests correlate well with each other, the Pearson correlation coefficient was 0.8281.

It was established that under the influence of abiotic factor – seed treatment at a temperature of 50 ºC for 30, 45 and 60 minutes, the viability of seeds, determined by TTC and HP tests, fluctuated insignificantly compared to the control (Table 1). At the same time, seed treatment at

a temperature of 60 ºC for 30 minutes led to a significant decrease in viability – by 39% compared to the control ($LSD_{0.05}$= 16.58, $p<0.05$).
The roots length of germinated seeds on the 7th day of germination in 1% hydrogen peroxide solution slightly increased with increasing temperature and exposure time from 0.46 cm in control to 0.53 cm in the variant of heating at 60 ºC for 30 min ($LSD_{0.05}$ = 0.77, $p>0.05$) (Table 1).

Table 1. Changes in the viability and roots length of beech seeds (*Fagus sylvatica L.*) originating from the Republic of Moldova (Hîrjauca) depending on temperature and exposure time

Variants	Parameters of seed treatment		Germination, %		Roots length, cm
	temperature, ºC	exposure times, min	TTC test	HP test	
Control	-	-	79.00	80.00	0.46
Variants of seed treatment with supraoptimal temperatures	50	30	82.00	83.00	0.49
	50	45	84.00	90.00	0.49
	50	60	83.00	91.00	0.50
	60	30	40.50	41.00	0.53
DEM $_{0.05}$			12.78	16.58	0.07

Previously, we found that the effect of a temperature of 40 ºC for 30 and 60 minutes on beech seeds obtained from Ukraine (Ivano-Frankivsk, harvested in autumn 2020) led to a significant decrease in viability – from 85.01% to 71.88 and 69.54%, respectively (Elisovetcaia et al., 2021). In the case of seeds from Hîrjauca (harvested in autumn 2021) treated at a temperature of 50 ºC for 30-60 minutes, there was no decrease in viability when determining TTC and HP tests. These results can be explained as specific response of the beech seeds of the Moldavian population from Hîrjauca to heat treatment. Nevertheless, it should be noted that some deviations were found in the physiological state of treated seeds in comparison with the control seeds. Thus, in the variants with heating, some evenly stained seeds in the TTC test had a lighter shade of the inner region of the cotyledons compared to the outer regions (Fig. 1). At the same time, part of the seeds definitely died after heating and had a yellowish-whitish shade of dead areas (Fig. 2).

Figure 2. Partially stained non-viable seed *Fagus sylvatica* in the TTC test (outside and sectional view).

When performing the HP test, the following signs were also noted: the cotyledons of the seeds in the variants with high temperature treatment had “scalded” areas even with well-sprouted roots (Fig. 3). Therefore, it is difficult to draw unambiguous conclusions about the real viability of seeds treated with supraoptimal temperatures, based only on the results of these two tests.

Figure 3. Non-viable (non-staining) *Fagus sylvatica* seeds in the TTC test (outside and sectional view)

The TTC and HP methods of determining viability described above have been used so far for seeds that have been stored for a long time at temperatures not exceeding, as a rule, +25 ºC. We did not find the results of similar studies for possible comparison with ours. The next step of ours researches was to evaluate what extent these methods (TTC and HP) reflect the real state of heated seeds. For this purpose, the seeds exposed to supraoptimal temperature were stratified in optimal conditions and the germination capacity were determined.

Previously, we found (Elisovetcaia et al., 2021) that in optimal conditions of stratification the majority of the seeds germinated during two months. Therefore, seed germination was counted after two mounts of stratification. It has been established that the seeds subjected to heating in a water bath at a temperature of 50 ºC for 30 minutes before stratification germinated two times slower than the control seeds. On the 62^{nd} day of stratification in samples with treated seeds were only an average of 18.5% germinated seeds, or two times less than in the control samples. Roots length was 0.76 cm in the experiment and 0.92 cm in the control. Statistical analysis of the data did not reveal a significant difference in root length between both variants ($LSD_{0.05}$= 0.34, $p>0.05$). Further stratification of seeds (on day 74) showed that this pattern persists: 79.0±4.3% germinated in the control, while in the variant with heating only 57.0±6.6%. The difference in germination capacity between control seeds and seeds treated by supraoptimal temperature was significant ($t=5.5880$; $p=0.0014$). At the same time, the length of the roots of germinated seeds in the experiment did not differ significantly from the control ones and reached 1.37 and 1.40 cm, respectively ($LSD_{0.05}$= 1.52, $p>0.05$).

Thus, despite the fact that the viability of seeds exposed to supraoptimal temperature of 50 ºC during 30 to 60 minutes, determined by the TTC and HP tests, remained at the control level, seed germination capacity is significantly delayed in the process of stratification: in 1.4-2.0 times in comparison with control seeds. At the same time, the effect of high temperature (50 ºC) on the seeds before stratification does not significantly affect the root length of germinated seeds.

Conclusions

The beech seeds origin Hîrjauca, Republic of Moldova exposed to high temperatures before stratification revealed a good resistance. More than half of the seeds (57.0±6.6%), exposed to heating at 50ºC for 30 min, germinated after 2.5 months of stratification. In addition, it was found that heating by this temperature does not affect the lengths of roots in surviving seeds. Thus, we suppose that the increase in average daily temperatures observed in recent decades in the autumn-winter period in the Republic of Moldova has an insignificant effect on the viability and germination of seeds of *F. sylvatica* origin Hîrjauca, Republic of Moldova.

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AGROFORESTRY AND PUBLIC PRIVATE PARTNERSHIP: A CASE STUDY OF AGROSILVICULTURE COMMUNITY GROWERS IN MPUMALANGA 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, public-private partnerships involve collaboration between a government agency and a private-sector company that can be used to finance, build, and operate projects, such as agroforestry/agriculture projects. In this case, a collaboration exists between Mountain to Ocean (MTO) (Private Company) and Agricultural Research Council (ARC) (Government Research Agency) to implement an agroforestry project in Mpumalanga Province, South Africa. The objective of the study was to determine the socio-economic characteristics of the agrosilviculture community growers (ACG) and to link the agrosilviculture community growers (ACG) to the market. A purposive sampling technique was used to select 143 ACG who were spread on the 37.2 ha MTO Forest land, and each allocated a 2601m^2 area of land for production during October 2021. Quantitative and qualitative designs were used as a questionnaire, stakeholder’s discussion and field observations were part of the data collection. The ARC team conducted tele-interviews on ACG before harvest (17 – 20 January 2022) and the second tele interviews on after harvest scenario will be conducted during June/July 2022. The socio-economic data was coded, captured, and analysed using Statistical Package for Social Science. The ACG also emphasized that they went to MTO plantation in search of its good climate including rainfall and as it would improve their livelihood through income generation, job creation and food security. This before harvest scenario was well presented and received by the MTO and potential groundnut market. In addition, the potential market agreed on supporting the ACG with offtake agreements. It is thus recommended that the establishment and expansion of agroforestry should be carried out and intensified in the Mpumalanga Province.

Keywords: *Agrosilviculture Community Growers, Socio Economic, Public Private Partnerships, Mountain to Ocean (MTO), Ehlanzeni District, Mpumalanga Province, and 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 (Nair, 1993). Properly managed agroforestry system provides multiple benefits and contribute to improved livelihoods and income generation. Agroforestry systems are also area and climate specific hence, it is key to develop agroforestry systems that are locally relevant and consider the biophysical and socio-economic context on a case-by-case basis (Mercer and Miller, 1998). South Africa is known as a semi – arid country vulnerable to water stress, particularly drought. According to ARC (2020), Mpumalanga Province annual rainfall varies from 400 mm in the lowveld to more than 1000 in most of the escarpment area. Substantial areas receive 600-700 mm. Likewise summer temperatures vary from cool in high-lying plateau and escarpment areas to hot in the lowveld (T_{max} in January 21-22 and 33-34°C respectively, with substantial areas between 31 and 32°C). Winter temperatures vary from cold in high-lying escarpment areas to mild in the lowveld (T_{min} in July 1-2°C and 9-10 respectively). Evaporation is moderately high in the lowveld at 900-1100 mm for the summer months of October to March. Frost does not occur regularly in the lowveld. The threshold for rainfall agriculture is averaged at 250 mm annually. In terms of forestry, the plantation forests of South Africa use just 3% of the country's total water resources and rainfall needs to be higher than 750 mm per annum to sustain commercial forestry.

Agriculture in Ehlanzeni District has annual turnover of R1, 5 billion consisting of subtropical fruit farming and processing, avocados, bananas, mangoes, litchis, macadamia nut, sugar cane cultivation by large and smallholder farmers and sugar production at two mills producing nearly 20% of South Africa’s sugar (EDM, 2014). Ehlanzeni produces 16% of South Africa’s citrus crop and export 70% (EDM, 2014). The Agricultural research Council (ARC) is currently involved in an agricultural and agroforestry project in the Ehlanzeni district in Mpumalanga Province where a comprehensive socio-economic assessment was conducted. In addition, there is a great interest in agroforestry practice among the smallholder farmers and community growers in the Ehlanzeni and Gert Sibande Districts (Maponya et al. 2021a). In the present study, research was conducted with the overall aim to determine the status of the agroforestry practice in terms of socio economic and food security. The major objectives were: (1) To identify and describe the socio-economic characteristics of the selected agrosilviculture community growers. (2) To determine the before harvest food security status of the agrosilviculture community growers.

Material and Methods

Study Area

A total of 143 agrosilviculture community growers participated in the study and were spread as follows: The agrosilviculture community growers were spread on the MTO plantation.

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 questionnaires were developed, and pilot tested with researchers working on community development within the Agricultural Research Council (ARC). The ARC VIMP farmer support, commercialisation and enterprise development team conducted tele-interviews on 143 community growers before harvest (17 – 20 January 2022) and furthermore analysed 143 community growers’ socio-economic characteristics and food security before harvest (24 – 3 February 2022).

Sampling Procedure and Analytical Technique

A purposive sampling technique was used on selected 143 agrosilviculture community growers in the Ehlanzeni district. These agrosilviculture community growers were spread on the 37.2 ha Mountain to Ocean (MTO) Forest land and each agrosilviculture community grower was allocated an area of land as follows for production (in m^2): 37.2*10000 m^2 (372 000m^2) of land to 143 growers, with each grower receiving 2601m^2 (372 000m^2/143) during October 2021. The list of agrosilviculture community growers were supplied by the MTO stakeholder relations office and the sample size was agreed with the stakeholder. A rule of thumb was applied, which is the minimum selection of 10% of the population and it is considered as a good sample size. The eucalyptus trees were then integrated with Groundnuts. Furthermore, data collected was analysed quantitatively using the Statistical Package for Social Sciences (SPSS) windows version 21. Descriptive analysis was conducted.
In addition, 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 consumed inadequate diet or ate food that they did 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 on a continuous basis 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 security. 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.

Average Monthly Rainfall Approach

Decadal (ten-day period) 1km x1km surfaces were created from rainfall data (1920 – 1999) downloaded from the AgroMet databank at the Agricultural Research Council- Soil, Climate and Water (ARC-SCW) (South African Weather Service and SCW weather stations) from stations with a recording period of 10 years or more. Regression analysis and spatial modelling were utilized considering topographic indices such as altitude, aspect, slope, and distance to the sea during the development of the surface.

Results and Discussion

Agrosilviculture Community Growers Socio-Economic Characteristics

The majority of agrosilviculture community growers interviewed were female. According to Table 1, 68% of women were interviewed as compared to 32% males. In terms of educational attainment (Table 1), 79% of growers had lees grade 7 education and 21% of growers had matric education. As indicated in Table 1, all hundred and forty-three growers were full time engaged in agrosilviculture practice. The community growers indicated that they received training from Small Enterprise Development Agency (SEDA) to educate them about co-operatives. The community growers were then registered as co-operatives through SEDA with the assistance from MTO stakeholder relations team. In addition, the community growers indicated that for agroforestry practice they relied mostly on their indigenous knowledge system (IKS). Results on land acquisition (Table 1) indicated that the growers were allocated land by MTO Forestry for production. The age distribution of the growers indicated that the majority were in the age group of >60 (60 %). As indicated in Table 1, there is 2% of youth involvement, 36 – 45 (2 %) while 46 – 60 had 36%. This situation is worrisome and indicates the urgent need to attract young generation into agroforestry as an important priority. The same trend of youth involvement was observed in the Limpopo Province (Maponya et al., 2019 and Maponya et al., 2020 and Maponya et al., 2021a, Maponya et al., 2021b, Maponya et al., 2021c and Maponya et al., 2022). The agrosilviculture community growers farming experience is spread across 1 – 5 years (2%); 6 – 10 years (2%); 11 – 20 years (52%) and 21 – 49 years (60%).

Table 1: Ehlanzeni District Agrosilviculture Community Growers Selected Socio Economic Characteristics

Variables	Community Growers	% Community Growers Socio-Economic Characteristics
Province		
Mpumalanga	143	100
District		
Ehlanzeni	143	100
Local Municipality		
Bushbuckridge	143	100
Gender		
Female	97	68
Male	46	32
Total	143	100
Age Categories		
18 – 35	3	2
36 – 45	3	2
46 – 60	52	36
>60	85	60
Total	143	100
Level of Education		
Less Grade 7	113	79
Matric	30	21
Post Matric	0	0
Other	0	0
Total	143	100
Employment Status		

Full Time Community Grower	143	100
Total	143	100
Language		
SiSwati	143	100
Total	143	100
Land Acquisition		
MTO Land	143	100
Total	143	100
Farming Experience		
1 - 5	3	2
6 - 10	3	2
11 - 20	52	36
21 - 49	85	60
>50	0	0
Total	143	100
Training Provided		
Yes	143	100
Total	143	100
Training Service Provider		
Small Enterprise Development Agency (SEDA)	143	100
Total	143	100

Agroforestry sites rainfall availability future estimates

According to ARC – SCW (2020), The three estimate rainfall show the 33rd percentile, median (50th percentile) and 67th percentile. To explain what these maps depict, one can consider the 33rd percentile. *If there were 100 years of recorded data arranged in sequence from dry to wet, then the 33rd percentile would be the value of the 33rd year. In other words, the chances are good to exceed this rainfall, or the chances are small that you will have less rain*. The model estimated annual rainfalls for the broad study area at 801 – 1000+ mm for the 33rd percentile; 901 – 1000+ at the 50th percentile and +1000mm at the 67th percentile. These agroforestry sites will allow for timber production and will thus support agroforestry / timber based mixed farming systems.

Agrosilviculture Community Growers Before Harvest Food Security Status

Food Accessibility Before Harvest

In terms of food accessibility before intervention)*:*143 community growers indicated that they do not have resources like land to grow or access food and Table 2 also indicated the food insecurity levels among community growers (143 community growers' moderately food insecure). Furthermore, the community growers indicated that they resorted to monotonous diets because it is all that they can afford. For instances, 143 community growers sometimes must eat fewer meals in a day, 143 community growers sometimes have to eat a smaller meal, 143 community growers sometimes eat some foods that really did not want to eat. This food access situation is worrying however the community growers indicated they don't go day and night without eating.

Table 2. Ehlanzeni District Agrosilviculture Community Growers Food Security Levels & Extent of Food Insecurity Before Harvest

Variable	Category	Community Grower	Total
Food Security Level	Food Secure	0	0/0%
	Food Insecure	143	143/100%
Extent of Food Insecurity	Mild	0	0/0%
	Moderate	143	143/100%
	Severe	0	0/0%

Food Availability Before Harvest

In terms of food availability before intervention: Most of the community growers (77 often and 66 never) indicated that their food runs out before they get money to buy more. Quite several community growers (101 sometimes and 42 never) cannot afford to eat enough food every day. The situation in the Limpopo and Mpumalanga Provinces indicated that the community growers do not have economic access to grow or purchase food and food do not last until month end. As a result of the situation, majority of community growers often feels hungry (103 sometimes and 40 always) and their children cannot get enough to eat (40 sometimes and 103 always). According to Maponya et al. (2022) and Coates et al. 2007, some of the coping strategies community growers used against food availability includes: getting food on credit from local shops, remittances, social grants, food parcels, food support from neighbours ect.

Food Utilisation and Diversity Before Harvest

In terms of food diversity, all the 143 community growers 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 pertaining to 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 findings of the study show that agroforestry can help to bridge the gap between agriculture and forestry by creating integrated systems that fulfil both environmental and socioeconomic goals, as well as food security. Furthermore, public-private partnerships involve collaboration between a government agency and a private-sector company can be used to finance, build, and operate projects, such as agroforestry/agriculture projects. In this case, a collaboration exists between Mountain to Ocean (MTO) (Private Company) and Agricultural Research Council (ARC) (Government Research Agency) to implement an agroforestry project in Mpumalanga Province, South Africa. In conclusion, the study established the before harvest agrosilviculture community producers' food insecurity status and it also showed that an enabling environment can be created for market access. Furthermore, the study proposes that agroforestry be promoted because it has the potential to increase productivity, hence enhancing farmer income and food security. Recognizing and addressing the primary elements that influence farmer participation in agroforestry, both socioeconomic and biophysical considerations, is critical for agroforestry adoption. As a result, it is recommended that agroforestry be expanded throughout South Africa, as it contributes to food security and sustainability.

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PUPA MORTALITY OF *THAUMETOPOEA PITYOCAMPA* DENIS & SCHIFFERMÜLLER, 1775 (LEPIDOPTERA: NOTODONTIDAE) IN ALGERIA

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Abstract

The pine processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) (Lepidoptera: Notodontidae) is one of the most serious pests of pine and cedar forests in Southern Europe and North Africa. In northern Algeria outbreaks occur in Aleppo pine (*Pinus halepensis* Miller) forest particularly in the semi-arid area and in Atlas cedar forest (*Cedrus atlantica* Manetti) in sub-humid elevation area. This work, carried out in northeastern and northwestern Algeria, aimed at studying pupal mortality of this pest on two host species, *C. atlantica* and *P. halepensis*. Three types of antagonist factors were assessed: (i) natural mortality (dried pupae), (ii) mortality caused by parasitoids and (iii) diseases. For this goal, 1382 mature larvae at the end of their activity were collected and examined. The overall mortality rate of pupating larvae of *T. pityocampa* due to several factors ranged from 13.7% to 31.1%. Mortality of *T. pityocampa* caused by parasitoids ranged from 7.0% to 11.9%. The mortality due to mycoses was limited between 2.7% to 6.3%. Three parasitoids species belonging to the Diptera have been identified: *Exorista segregate* (Rondani, 1859), *Phryxe caudata* (Rondani, 1859) (Tachinidae) and *Hemipenthes* sp. (Bombyliidae). The primary parasitoid *E. segregata* was the most abundant comprising almost 65% of the total emerging parasitoids. To this, we note the presence of the entomopathogenic fungus, *Beauveria bassiana* (Deuteromycotina: Zygomycetes) which, despite its low presence, can be used in an alternative biological control.

Key words: *Thaumetopoea pityocampa, coniferous forests, pupal mortality, parasitoid.*

Introduction

The pine processionary moth (PPM) *Thaumetopoea pityocampa* (Denis & Schiffemüller, 1775) (Lepidoptera: Notodontidae) is considered the most important forest pests in the Mediterranean Basin and other Southern European areas (Palacio et *al*., 2012; Jacquet et *al*., 2013; Li et *al*., 2015; Campoa et *al*., 2021; Camarero et *al*., 2022). This pest is oligophagous on *Cedrus* and *Pinus* species, both native and artificial stands (Ayache et *al*. 2021; Rahim et *al*., 2021a).
Thaumetopoea pityocampa has a univoltine lifecycle. The adults emerge, mate and lay eggs in a single clutch on pine needles or underside of cedar twigs in July at colder sites and in August at warmer sites. Each egg batch contain, on average, in the northern regions of Algeria 220 eggs covered with protective scales which are produced by the female (El Mokhefi et *al*., 2021). Embryonic development lasts for 30-45 days, the larvae live in aggregations and form a conspicuous white nest on the top of the trees where they develop until springtime. The mature larvae leave the tree in a single file, head-to-tail procession, searching for soil sites suitable for pupation. Some pupae have a prolonged diapause lasting 1-6 years which is conditioned by the surrounding environment (Zamoum, 1998).

Currently, a latitudinal and altitudinal expansion of the range of the PPM have been attributed to climate warming (Robinet et *al*., 2007; Roques, 2015; Bourougaaoui et *al*., 2021). In Algeria, outbreaks occur in Aleppo pine (*Pinus halepensis* Miller) forest in the semi-arid area and Atlas cedar forest (*Cedrus atlantica* Manetti) in sub-humid elevation area (El Mokhefi et *al*., 2016). The expansion areas of this species in Algeria was favored by the recent large afforestation program of Barrage Vert (plantation of pure stands of Aleppo pines to serve as a barrier to desertification) along the Saharian Atlas (Zamoum, 1998). These Outbreaks cause significant radial growth losses, lead to tree dieback after repeated defoliation, or make trees more susceptible to secondary pests or pathogens attack (Sbabdji et *al*., 2009; Sbabdji and Kadik, 2011; Jacquet et *al*., 2012). Additionally, this moth can affect humans and animals, because of the release of microscopic urticating hairs (setae) present from L3, which are responsible for respiratory problems, skin lesions of varying severity and allergic reactions (Roques, 2015; Battisti et *al*., 2017).

Natural enemies (i.e., predators, parasitoids and pathogens) play a vital role in regulating many harmful insect pests (*e.g.* Rahim et *al.,* 2016; Alhadidi et *al*., 2018; Francis et *al*., 2020; García et *al*., 2021; Sebti et *al*., 2022). All over the Mediterranean area, many natural antagonists, including parasitoids (Buxton, 1990; Tarasco, 1995; Schmidt et *al*., 1999), pathogens (Topkara et *al*., 2022; Dannon et *al*., 2020) insectivorous birds, such as the great tit (*Parus major* L.) (Pimentel and Nilsson, 2007; Del Pino and Variada, 2009; Barbaro et *al*., 2013), Tettigonidae (Gonzalez-Cano, 1981; Hódar et *al*., 2013), and ants (Way et *al.,* 1999) might have a role in controlling PPM. These natural enemies attack all life stages of the pest, i.e., embryos in eggs, any of the all instar larvae, during pupation processions, or adults (Battisti et *al*., 2015a; Rahim et *al*., 2021b). Given the recent increase in the frequency of *T. pityocampa* outbreaks in multiple pine and cedar forests of Algeria and the potential for future outbreak events due owing to climate warming (Paritsis and Veblen, 2011; Robinet et *al*., 2013; De Boer and Harvey, 2020), our objectives are (1) to identify the natural enemies attacking pupae of *T. pityocampa* in Atlas cedar and Aleppo pine forests in northern Algeria, and (2) to determine the contribution of parasitoids diversity and various factors to pupae mortality. The data collected from the biological material analyzed provides opportunities for biological control in these fragile forest environments.

Materials and methods

Sampling sites

The study was conducted in 2020 in two plantations forests located in northern Algeria: Halouane and Tadjenanete (table 1). The distance between the two forests sites is about 200 km.

The first site, Halouane is a pure Atlas cedar (*Cedrus atlantica* Manetti) plantation (age of trees: 35 years) located in Djurdjura National Park (140 km southeast of Algiers), covering an area of almost 8000 ha. The locality is under a subhumid bioclimate, with average temperatures of 3 °C in winter and 20 °C in summer, and an average annual rainfall of 1300 mm.

The second site, Tadjenanet is a pure planted stand of Aleppo pine (*Pinus halepensi*s Miller) (age of trees: 30 years) located in Mila region (500 km southeast of Algiers), covering an area of 300 ha. This area is located in a semi-arid bioclimate, characterized by average annual temperatures of 9 °C and 28 °C in winter and summer, respectively and an average annual rainfall of 400 mm.

Table 1. Main environmental characteristics of the studied sites.

Site	Latitude (N)	Longitude (E)	Elevation (m)	Topog. Orientation	Slope (%)	Host plant	Tree height (m)
Halouane	36°27'	3°56'	1233	Northeast	13.2	*C. atlantica*	3-8
Tadjenanete	36°07'	6°00'	842	Southeast	2.1	*P. halepensis*	2-6

Population density of T. pityocampa

The winter nests made by *T. pityocampa* larvae overwintering on colonised trees counted. Egg batches of *T. pityocampa* are small, inconspicuous and difficult to detect them in the canopies of trees, whereas the winter nests built by late-instar larvae are easy to detect even at low population. *Thaumetopoea pityocampa* density was therefore estimated as the mean number of the larval nests (winter nests) per tree. The number of winter nests per tree was estimated on each site on 35 trees along a linear transect (about 300 m long) (Jactel et *al*., 2006). Trees were carefully visually inspected for the presence of nests under tree canopies in all directions and was carried out in January, when the formed nests on the trees were more easily visible (Trematerra, 2016).

Sampling of larvae of T. pityocampa

For each location, mature larvae (fifth instar) of *T. pityocampa* searching for pupation into the soil were collected on a weekly basis during January-March. This period corresponds to the pupation procession and beginning of pupation phase of the pest.
The collections of mature larvae were made randomly along access roads at forest margins and extended up to one hundred meters into the forests, depending on the size of the *T. pityocampa* populations. The areas of collection extended over 5-6 ha. In total, 996 larvae were collected, either with or without visible evidences of parasitism. Field-collected larvae were reared in groups of 50 individuals in cylindrical plastic containers (20 x 20 x 25 cm) containing soil, covered with a nylon tulle netting of 1.5 mm and reared at 28±2°C until parasitoids emerged or until hosts died or adult moths emerged.

Statistical analysis

The percentage parasitism was calculated as number of insects in a sample from which parasitoids had emerged divided by the total number of insects in the sample. In order to compare the density of *T. pityocampa* (tents/per tree) and pupal mortality between sites, one-way ANOVA test was carried out after assessing for normality and homoscedasticity of the data. Chi-square (χ^2) test of the equality of distributions was used to compare the frequency of the parasitoid species between sites. Statistical analyses were carried out using the SPSS v25 (IBM Corp., Armonk, N.Y., USA, 2017).

Results and discussion

Population density

The population density of *T. pityocampa* (mean number of nests/tree) varied significantly between the two sites ($F_{1, 68}$ = 289.87, P<0.01). The mean (±SD) density of winter nests was higher in the Aleppo pine forest of Tadjenanete (6.09±1.29) than in cedar forest of Halouan (1.74±0.78). Forest parameters such as stand height, age and tree density was the most important factors determining population density of *T. pityocampa*. Régolini et *al*. (2014) found that *T. pityocampa* tent density decreased significantly with stand density. Geri and Miller (1985) showed that low density of *T. pityocampa* are associated with old natural forest sites that are characterised by a greater tree height.

Mortality factors

The overall mortality rate of pupating larvae of *T. pityocampa* is 21.3% in cedar forest of Halouan, and from 30.8% in the Aleppo pine forest of Tadjenanete, with high significant differences between sites ($F_{1, 25} = 16.59$, $P<0.01$) (Table 2).
Mortality of *T. pityocampa* caused by parasitoids was 11.9% in the Aleppo pine forest of Tadjenanete and 7.0% in cedar forest of Halouan, with high significant differences between the two sites ($F_{1, 25} = 23.82$, $P<0.01$).
Fungal infections rates differed among the two sites ($F_{1, 25} = 15.28$, $P<0.01$), being higher in Tadjenanete, 6.3% then in Halouan, 2.7%.
Mortality of *T. pityocampa* caused by undetermined factors, which could not be attributed either to pathogens or parasitoids was 11.6 and 12.6% in Halouan and Tadjenanete, respectively. No difference in the rate of mortality caused by unknown factors was detected in across the two sites.

Table 2. Survival and mortality of *T. pityocampa* pupating larvae.

Site	Total number of individuals	Emerged adults % (N)	Pathogens % (N)	Parasitoids % (N)	Unknown % (N)	Total mortailty % (N)
Tadjenanete	969	69.2 (671)	6.3 (61)	11.9 (115)	12.6 (122)	30.8 (298)
Halouane	413	78.7 (325)	2.7 (11)	7.0 (29)	11.6 (48)	21.3 (88)
Overall	1382	72.1 (996)	5.2 (72)	10.4 (144)	12.3 (170)	27.9 (386)

The highest mortality caused by the parasitoids in the Aleppo pine forest of Tadjenanete, the site with the highest PPM abundance density could be interpreted as a simple increase in probability of encountering the larvae by the parasitoids. According to Hall et *al*. (2019), the effectiveness of parasitoids depends on population density of the insect pests, with the highest percentages of parasitism recorded during the outbreak. A lower and moderate levels of parasitism of several insect herbivore is commonly observed in non-outbreak periods (Mills and Kenis, 1991; Quayle et *al*., 2003; Zovi et *al*., 2006; Paritsis et *al*., 2012; Rahim et *al*., 2016; Hall et *al*., 2019). The average total parasitism we found in our study ranged from 7.0-11.9% and indicates that parasitoids contribution to *T. pityocampa* mortality is low, compared to 43.9% in sub-Saharan region of Algeria (Zamoum et *al*., 2007).

Parasitoid complex: species composition and relative abundance

During the research period pupae of *T. pityocampa* proved to be affected by three parasitoid species: *Exorista segregata* Rondani 1859, *Phryxe caudata* Rondani 1859 (Diptera Tachinidae)

and *Hemipenthes* sp. (Diptera Bombyliidae). The frequency of the parasitoid species was not significantly varied between the two collection sites (χ^2=5.018, *P*=0.658).
The relative abundance of the parasitoids emerging from pupa *of T. pityocampa* is presented in Table 3. Tachinid flies were the dominating parasitoids in both sites, with a total of 132 individuals (91.7% of the total emerging parasitoids) emerged. The parasitism for both sampled sites was largely driven by *E. segregata*, accounting for about 61% of the total emerged parasitoids, followed by *P. caudata*, accounting for 27.6 and 31.3% of all the parasitoids in Halouan and Tadjenanete, respectively. The bombyliid *Hemipenthes* sp. was present only in the Aleppo pine forest of Tadjenanete, accounting for 1.7% of all the parasitoids. In addition, there were 8.7% of unidentified dipterous parasitoids, which pupated but did not emerge as adults.

Table 3. Insect parasitoids of *T. pityocampa* and their relative abundance.

Site	Parasitoid species	Emergence period	N	Relative abundance %	Mortality %
Tadjenanete	*E. segregata*	June-September	67	58.3	9.6
	P. caudata	June-September	36	31.3	3.7
	Hemipenthes sp.	August	2	1.73	0.2
	unidentified diptera	-	10	8.7	1.0
Halouan	*E. segregata*	June-September	21	72.4	5.1
	P. caudata	June-September	8	27.6	1.9

Family Tachinidae (Diptera) occupies an important place among the parasitoids, not only by the richness of species and wide geographical distribution but also because they parasitize a large number of economically important insect pests. Late instar larvae and pupae are mostly impacted by the tachinid flies. Tachinid parasitoid contribute to the collapse of several lepidopteran pests (Fuester et *al*., 1988; Pemberton et *al*., 1993; Alalouni et *al*., 2013; Rahim, 2016; Hammami et *al*., 2019).
The tachinid *E. segregata* is a highly polyphagous and polyvoltine parasitoid of Lepidoptera. This parasitoid is known from many Mediterranean countries as parasitoids of *Thaumetopoea* species. *E. segregata* was found to attack *Thaumetopoea ispartaensis* Doğanlar & Avci, 2001 (Avci and Kara, 2002), *T. pityocampa* (Pekel, 1999; Aytar and Turgut, 2021), *Thaumetopoea wilkinsoni* Tams, 1926 (Wilkinson, 1926; Erkaya, 2020; Aytar and Turgut, 2021), *Thaumetopoea solitaria* Freyer 1838 (Halperin, 1990). In Algeria, *E. segregata* was also found to attack the cedar processionary moth *T. bonjeani* (Rahim, 2016) and the gypsy moth *Lymantria dispar* Linnaeus 1758 (Lepidoptera Erebidae) (Mecellem and Chakali, 2021).
Phryxe caudata is a typical parasitoid that depends on the processionary moth species. This tachinid is known from many Mediterranean countries as the most prevalent parasitoid of PPM (Haleprin, 1990; Robinet et *al*., 2012; Battisti et *al*., 2015b; Bonsignore et *al*., 2015; Tarasco et *al*., 2015; Zamoum et *al*., 2017; De Boer et *al*., 2020). *P. caudata* has two generations per year, with the first emerging in spring from mature larvae and the second in summer-autumn from pupae (Buxton, 1990). This parasitoid was found to attack *T. ispartaenis* (Avci and Kara, 2002) and *T. wilkinsoni* (Aytar and Turgut, 2021).

The genus *Hemipenthes* is known to parasitize *T*. pityocampa in Europe. *Hemipenthes velutina* Meigen, 1820 and *Hemipenthes morio* Linnaeus, 1758 was the main parasitoid emerged from *T. pityocampa* (Tarasco et *al*., 2015; Rubin, 2000). In Algeria, the parasitoid *Hemipenthes* sp. is reported for the first time as a parasitoid of *T. pityocampa*.
The entomopathogenic fungi, *Beauveria* sp. was another factor of pupal mortality of *T. pityocampa.* Beauveria species attack many insect species worldwide (Sevim et *al*., 2010). This fungus killed larvae and pupae of many *Thaumetopoea* species, with *Beauveria bassiana* being the most common one (Halperin, 1990; Battisti et *al*., 2000; Mirchev et *al*., 2012). Currently, *B. bassiana* is used in forest protection as classical or augmentation biological agents (Pilarska et *al*., 2018).

Conclusion

The composition of natural enemy associated to *T. pityocampa* was relatively low in Algeria. Four species of natural enemies of the *T. pityocampa* were found: three of parasitoids and one species of fungus. In order to understand the dynamics in population fluctuations of this insect in cedar and pine forests, in our future work we will focus on assessing temporal variability in mortality caused by multiple natural enemies that attack eggs, larvae, pupae and adult stages across larger areas of the geographic distribution of *T. pityocampa*.

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ECOLOGICAL ASSESSMENT OF SOIL CO_2 EMISSIONS IN POST-AGROGENIC SUCCESSION OF FALLOW OVERGROWTH BY FOREST ON PODZOLUVISOLS

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Abstract

Global climate change is one of the key environmental problems of the XXI century. Deforestation and reforestation are the most important changes in land use that determine the intensity of soil CO_2 emission. About 30 million hectares of agricultural land are not used in Russia since 1990-s, and most of them are in the southern taiga zone with Podzoluvisols. Fallow lands are the main territorial reserve for both increasing agricultural production and ecological restoration and expansion of the RF natural forest framework. There are results of Podzoluvisols CO_2 emission monitoring within post-agrogenic succession of fallow overgrowth by forest in the representative southern-taiga zonal ecosystems of the Central Russia. Five sites with succession stages from a perennial meadow to 100-year spruce forest are located compactly (within 300 m transect) on geomorphologically comparable slope landscapes in the Central Forest Reserve. The intensity of the soil CO_2 flux, temperature and soil moisture were determined weekly in the warm period of the year and once a month in the cold period. A mobile infrared gas analyzer Li-820 was used to analyze the soil CO_2 fluxes. Year-round studies were conducted in conditions of the biosphere reserve - regional southern-taiga reference object. They revealed statistically significant seasonal and succession tenfold and two - fold dynamics of soil CO_2 emission of Podzoluvisols in the main environmental types of the southern-taiga land-use. It should be considered in strategic land-use planning to achieve the stated goal in Glasgow of a phased transition to C-neutral development of the RF regions.

Keywords: *Post-agrogenic succession, Fallow overgrowth by forest, Soil CO_2 emission, Podzoluvisols, Russia.*

Introduction

Global climate change is one of the key environmental problems, the level of awareness of the importance of which is rapidly increasing in the XXI century (IPCC, 2013; 2021). One of the main causes of climate change is an increase in the concentration of greenhouse gases in the atmosphere and, above all, CO_2 (Kudeyarov, 2014; Buchkina et al., 2013). The ecological functions of soils play an important role in regulating the CO_2 content in the atmosphere.
The intensity of soil CO_2 emissions depends on many factors: air temperature, soil temperature and moisture, vegetation state, soil type, and is subject to high seasonal and inter-seasonal dynamics (Zadorozhny et al., 2010; Stepanov, 2011; Valentini and Vasenev, 2015). Land use changes, including deforestation and restoration of forests, is the most important factor determining the intensity of soil CO_2 fluxes (Stepanov, 2011).
Currently, about 30 million hectares of originally agricultural land are not used in Russia, and most of them are in the southern-taiga zone. At the same time, abandoned arable lands, or fallows, are the main territorial reserve both for increasing agricultural production and for

ecological restoration and expansion of the natural land framework of the regions (Valentini and Vasenev, 2015).

Despite the well-known studies and generalizations on the assessment of the carbon balance of fallow ecosystems [Kudeyarov, 2014; 10], the annual dynamics of soil CO_2 emissions in the conditions of regionally typological variants of comparable forest, fallow and newly forest-overgrowing ecosystems has not been studied enough. In addition, many investigations belong to the forest-steppe zone or the zone of mixed forests, and limited number of studies were carried out in chronosequence sites with a reliable justification of their soil's morphogenetic comparability.

Our research was carried out in the conditions of the Central-Forest State Natural Biosphere Reserve which is representative for the southern taiga ecosystems of the Russia Central region.

The ecosystems of this reserve are traditionally the site of stationary studies of soil cover and vegetation representative for background southern-taiga biocenoses (Zheltukhin and Zheltukhina, 2007; Vasenev, 2008, 11)

Materials and methods

The Central-Forest State Natural Biosphere Reserve was established in 1931 to preserve and study the undisturbed areas of the southern taiga in the center of the European part of Russia. The reserve is located in the Nelidov and Andreapol districts of the Tver region (56°30' N; 32°55' E), occupying an area of about 24.4 thousand hectares in the southwestern part of the Valdai Upland (http://www.clgz.ru). Already at the time of its creation, the first director of the reserve G.L. Grave designated the research of the forest ecosystems dynamics, determined by both natural and anthropogenic factors, as a priority task of the studies in it [11].

The research plots include the comparable to each other sites of meadow perennial fallow and four stages of its reforestation up to spruce forest older than 100 years (Fig. 1). All of them compactly (within 300 m) located in the geomorphologically similar areas, with the same position in the relief (the middle part of the long slope of the western exposure) and zonal sod-pale-podzolic soils (Podzoluvisols).

1 2 3 4 5

Fig. 1. Succession sites of comparable different age forest-overgrowing fallows:

1) *0-moment with meadow perennial fallow – 1-st stage,*
2) *10-15-year birch trees with seldom aspen and single spruce undergrowth – 2-nd stage,*
3) *20-30-year pole-size birch, aspen inclusion and seldom spruce undergrowth – 3-d stage,*
4) *50-60-year birch forest with aspen inclusion and spruce undergrowth – 4-th stage,*
5) *older than 100-year spruce forest with seldom old birch and aspen inclusion – 5-th stage.*

Monitoring of soil CO_2 emission was carried out in the period from June 2016 till May 2018, in a 5-spatial repetition for each studied area. The measurements were running using the method of ground exposure cameras and a mobile infrared gas analyzer Li-820. In parallel, air temperatures, soil temperature and moisture were measured with mobile electronic sensors: a Checktemp thermometer (Hanna) and an SM300 moisture sensor (Eijkelkamp) – in repetition from 3 to 5 (in case of pronounced variation) for each chamber, with averaging for topsoil of 0-10 cm. At the same time, an exposure chamber (diameter 20 cm, height 15 cm) was hermetically installed on pre-installed bases in the soil (diameter 20 cm, incision depth 7 cm), connected to a mobile IR gas analyzer Li-820 by incoming and outgoing air hoses. As a rule, observation was carried out for 5-15 minutes until a uniform increment of CO2 concentration in the chamber by more than 100 ppm is recorded.

Results and discussion

Monitoring measurements of soil CO_2 emission showed the pronounced seasonal dynamics and increased spatial variability of CO_2 fluxes with maximum ranges of spatial-temporal variability for meadow perennial fallow (Fig. 2) – more than 50 g CO_2 m^{-2} day^{-1} between the average value of summer maximum (55.42 g CO_2 m^{-2} day^{-1}) and winter minimum (0.89 g CO_2 m^{-2} day^{-1}). Seasonal dynamics of soil CO_2 fluxes was largely determined by seasonal variability of air temperature, soil temperature and moisture (Fig. 3)

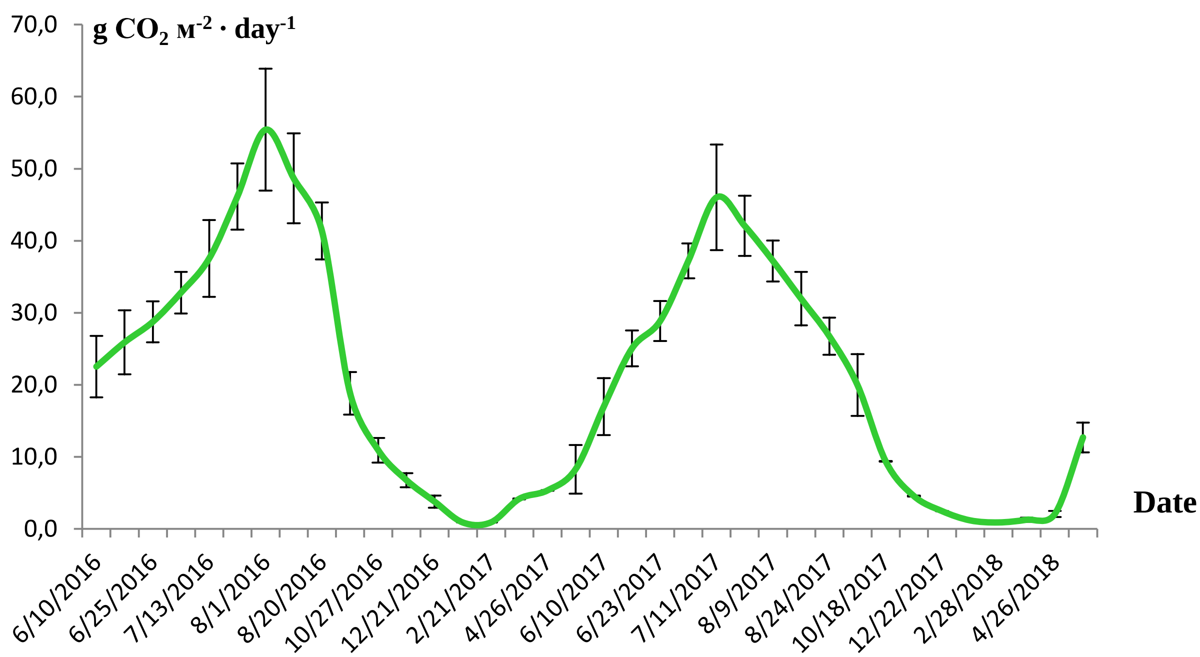

Fig. 2. Seasonal dynamics of CO_2 emission (μ±σ) of sandy loamy Podzoluvisol in the meadow perennial fallow.

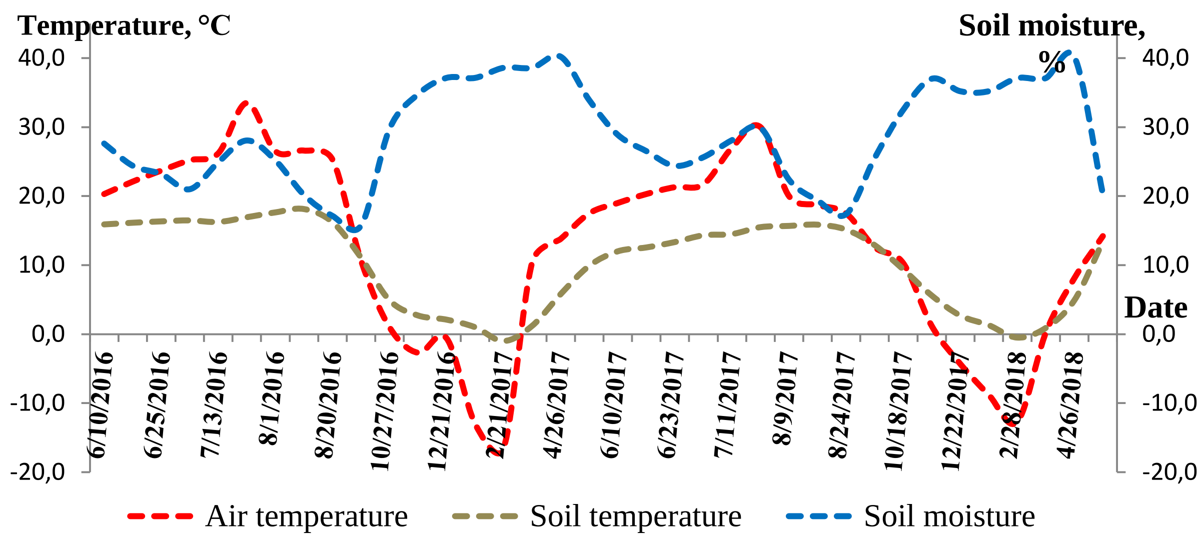

Fig. 3. Seasonal dynamics of air temperatures, topsoil temperature and moisture of sandy loamy Podzoluvisol in the meadow perennial fallow.

The year-round monitoring of the seasonal dynamics of soil CO_2 emission within the succession chronosequence of the reforesting of different ages showed a gradual decrease in its summer intensity under the reforestation: from 55-56 g CO_2 m^{-2} day^{-1} in early August 2016 for meadow perennial fallow to 36-37 g CO_2 m^{-2} day^{-1} for fallow overgrown with birch at the age of 20-30 years (3-d stage of the succession), and down to 27-28 g CO_2 m^{-2} day^{-1} in spruce forest older than 100-year with seldom old birch and aspen inclusion (5-th stage of the succession) (Fig.4).

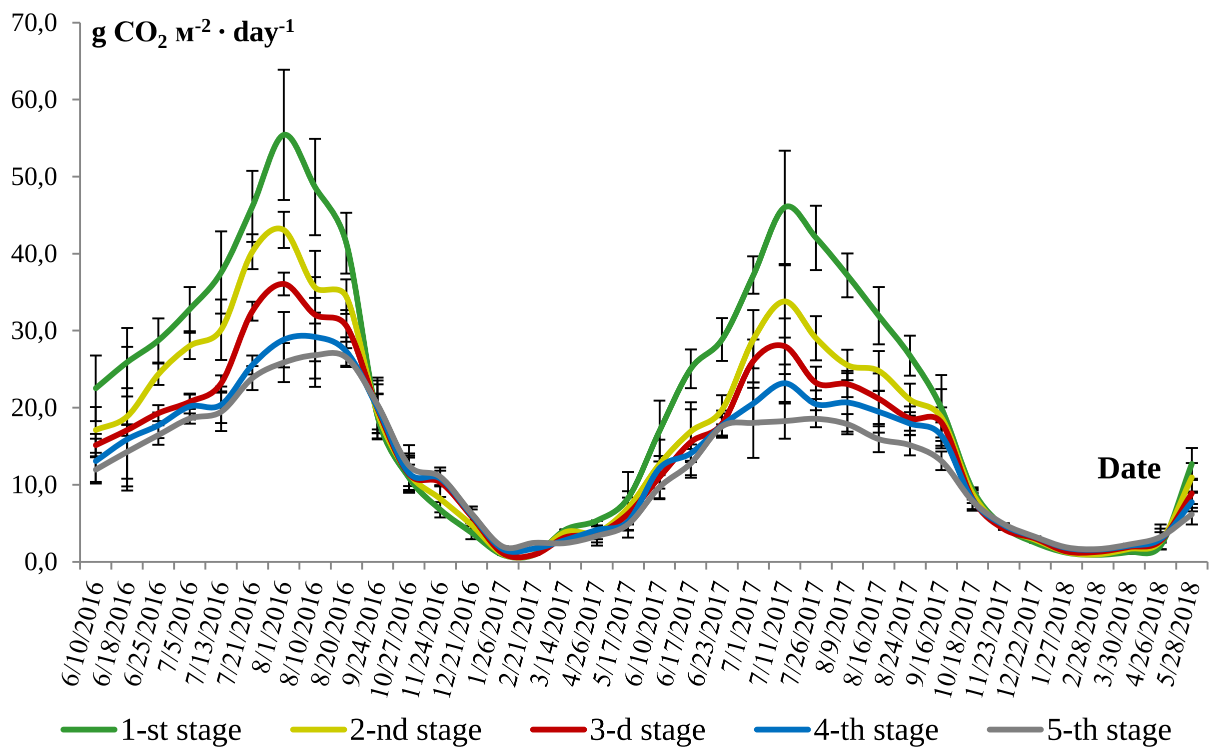

Fig. 4. Seasonal dynamics of CO_2 emission ($\mu\pm\sigma$) of sandy loamy Podzoluvisol within the reforestation succession.

The main ecological factor affecting the rate of soil CO_2 fluxes is the age of the renewed forest: a decrease of almost 2 times the soil CO2 fluxes in the ecosystem of the spruce forest aged over 100 years relative to the meadow perennial fallow. The main ecological factors determining the seasonal dynamics of soil CO_2 emission, which are confirmed by correlation and regression analyses of their influence within each season and stage of the reforestation succession (Table 1, 2), are soil temperature (K_{TS} from 0.77 to 0.99), air temperature (K_{TA} from 0.42 to 0.99), as well as soil moisture in the spring and autumn periods (K_{WS} from -0.55 to -0.98).

Table 1. Analysis of the influence of ecological factors: air temperature (T_A), soil temperature (T_S) and soil moisture (W_S) – on the seasonal dynamics of soil CO_2 emission in the meadow perennial fallow

Season	Regression equation, g CO_2 m^{-2} day^{-1}	K_{TA}	K_{TS}	K_{WS}	R^2
Summer	CO_2 = -62.01 + 0.99T_A + 4.57T_S + 0.30W_S	0,74	0,77	-0,26	0,89
Fall	CO_2 = 10.52 – 0.77T_A + 2.50T_S – 0.51W_S	0,88	0,96	-0,97	0,99
Winter	CO_2 = 85.92 + 0.27T_A – 1.56T_S – 2.13W_S	0,66	0,81	-0,87	0,96
Spring	CO_2 = 13.19 – 0.01T_A + 0.49T_S – 0.33W_S	0,93	0,97	-0,98	0,99

Table 2. Analysis of the influence of ecological factors: air temperature (T_A), soil temperature (T_S) and soil moisture (W_S) – on the seasonal dynamics of soil CO_2 emission in the stages of the reforestation succession

Season	Regression equation, g CO_2 m^{-2} day^{-1}	K_{TA}	K_{TS}	K_{WS}	R^2
20-30-year pole-size birch, aspen inclusion, and seldom spruce undergrowth – 3-d stage					
Summer	CO_2 = -31,41 – 0,05T_A + 2,98T_S + 0,74W_S	0,50	0,81	-0,06	0,82
Fall	CO_2 = -4,51 – 0,89T_A + 2,91T_S – 0,15W_S	0,82	0,93	-0,61	0,99
Winter	CO_2 = -8,11 – 0,21T_A + 3,92T_S + 0,34W_S	0,50	0,90	-0,86	0,94
Spring	CO_2 = 5,30 – 0,02T_A + 0,51T_S – 0,13W_S	0,97	0,99	-0,94	0,99
Older than 100-year spruce forest with seldom old birch and aspen inclusion – 5-th stage					
Summer	CO_2 = -9,57 + 0,20T_A + 1,56T_S + 0,13W_S	0,52	0,83	-0,36	0,84
Fall	CO_2 = -34,95 – 3,21T_A + 6,40T_S + 0,41W_S	0,92	0,97	-0,55	0,99
Winter	CO_2 = 61,25 – 0,26T_A + 2,69T_S – 2,15W_S	0,42	0,81	-0,81	0,99
Spring	CO_2 = 1,87 – 0,01T_A + 0,36T_S + 0,01W_S	0,99	0,99	-0,95	0,99

In the autumn, against the background of a decrease in air and topsoil temperature, there is a gradual decrease in the intensity of soil CO_2 fluxes – down to a sharp attenuation in winter: to

1.58 g of CO_2 m^{-2} day^{-1} for the meadow perennial fallow in 2018. The intensity of soil CO_2 fluxes closely depends on the weather and climatic conditions of a particular season of the year. For example, in the conditions of the season with elevated air and soil temperatures (2016), the intensity of soil CO_2 fluxes increases, and in the conditions of the season with lowered air and soil temperatures (2017), the intensity, on the contrary, decreases.

Conclusion

The conducted studies of the seasonal dynamics of soil CO_2 fluxes within the reforestation succession show an increase in the level of "closure" of the local biological carbon cycle as the formation of a forest phytocenosis close to the zonal background. In this case the intensity of soil CO_2 fluxes in the summer months is no more than 40% of the soil CO_2 fluxes in the meadow perennial fallow. The statistically significant dependences of soil CO_2 fluxes on air temperature, soil temperature and soil moisture confirmed by the results of regression analysis (R^2 from 0.81 to 0.99) allow one to predict the seasonal and interseasonal dynamics of soil CO_2 emission in conditions similar to the studied areas and, with successful verification, can extend to a significant part of the fallow with overgrowth by forest in the southern taiga zone of the Central Region of Russia. It should be considered in strategic land-use planning to achieve the stated goal in Glasgow of a phased transition to C-neutral development of the RF regions.

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SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL) VARIABILITY IN THE AREA OF EASTERN SERBIA ACCORDING TO LEAF MORPHOLOGICAL TRAITS

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Abstract

In order to preserve available sessile oak genepool in the area of eastern Serbia and to control usage of genetic resources, intrapopulation and interpopulation research has been conducted on the basis of leaf morphological traits. In July of 2020, 86 trees of phenotypically highest quality and good health condition were selected in three populations, being the carriers of sessile oak (*Quercus petraea* (Matt.) Liebl) reproductive material production, from which undamaged leaves were collected. On the basis of 11 measured morphometric parameters and one derived relation on the sample of 50 leaves per tree, the intrapopulation and interpopulation variability have been determined. The descriptive and multivariate statistic methods were used in the research. Research results indicate high variability of investigated populations. The least variable trait was the length and width relation of a leaf (CV = 13.24%), while the most variable trait was leaf area (CV = 45.39%). Populations were statistically significantly different in all examined leaf morphological traits ($p<0.01$; $\alpha = 0.05$) according to analysis of variance. Although interpopulation variability was statistically significant, the level of intrapopulation variability was significantly higher (43.6-57.3%) in relation to the interpopulation differentiation (7.2-18.6%). According to obtained results, it can be stated that available sessile oak genepool is characterized by a satisfying level of genetic variability. The obtained results should be accepted as the preliminary ones, which are presenting a good basis for the long-term preservation and ecological adaptability improvement and evolutionary potential of the sessile oak population, by implementing adequate in situ and ex situ conservation measures.

Keywords: *Sessile oak, population, genepool, variability.*

Introduction

Sessile oak (*Quercus petraea* /Matt./ Leibl.) represents autochthonous European species, of the economically very important genus Quercus. In the forest fund of the Republic of Serbia it represents the most valuable oak species, after the common oak. In the total volume, sessile oak shares 5.9%, i.e. it is distributed over 173.200 ha. Sessile oak forests in Serbia are located within a special oroclimatogenic altitudinal zone, above the climatogenic forests of Hungarian and Turkey oak. It can mostly be found on the warmer, southern expositions within the alliance of *Quercion petraeae-cerris* Lakš. et Jov. 1980. Forest complex occupies the upper part of the hill belt and the lower part of the mountain belt, from an altitude of 300 m to 1300 m (Stojanović, Lj., et al., 2005). Pure stands are presented with 57,5%, and mixed stands with 42,5%. Coppice forest are dominant on cc 75% of the total area (Banković et al. 2009).
Anthropogenic influence on Serbia’s forests is mostly affecting oak forests, which are being cleared mostly for the purpose of creating agricultural lands. In the last few decades, the occurrence of die-back of stands, groups and single trees is detected on almost whole species

areal, and its cause is still not studied enough and systematized. This negative occurrence is most likely caused by the influence of complex of factors with cumulative effect (Marinković, P., et al., 1990). The results of the research of potential causative agents are suggesting that sessile oak die-back is a consequence of a global climate change, sessile oak forests population structure change, air pollution, plant diseases, insects gradations, etc. (Isajev, V., et al. 2005).

Genus Quercus is characterized by common hybridization between individual members, and differentiation and speciation processes are still not finished. Sessile oak, as most of the other oak species, has a haploid number of chromosomes 12, while the polyploidy occurrence is very rare, ranging around 0,5 %. Sessile oak is characterized by great individual and group variability, which is confirmed by provenance tests and offspring tests (Isajev, V., et al. 2005). The variability of sessile oak could be regarded as adaptive and neutral variability complex (Šijačić-Nikolić et al., 2009). It was reported in seedling growth, tree forming, tolerance to powdery oak mildew infections and insect attack. In the biochemical research of different provenances fruits, there have been determined significant differences in the agglutinin content, which can be useful for easier identification of oak provenances (Jovanović, M., Tucović, A. 1975). A significant differences exist also within provenances, as within individual trees. Morphological traits of trees and acorns are under significant genetic control. Regarding the occurrence of hybridization process, in the scientific literature there are data about the occurrence of spontaneous hybrids among several oak species, described under different names: *Quercus petraea* /Matt./ Leibl. and *Quercus robur* L.; *Quercus petraea* / Matt./ Leibl and *Quercus dalechampii* Ten; *Quercus petraea* /Matt./ Leibl and *Quercus polycarpa* Schur. The offspring of the cited parental species is characterized by expressed variability of quantitative and qualitative traits. It is not morphologically homogeneous, rather the hybrids are phenotypical mosaics with intermediary traits of parental species (Viscosi et al., 2012). The great changeability of morphological and physiological traits of spontaneous hybrids creates considerable difficulties in the forest reproductive material production and usage of available sessile oak genepool (Isajev, V., et al. 2005).

Preservation of biodiversity represents one of the most important assignments of modern man and society. In order to reach harmony in human and nature relations, it is necessary to harmonize the preservation of biological diversity and its utilization. Due to damages and alterations that seized many ecosystems to a large extent, achieving the balance of these two processes seems very distant. The ecosystem preservation of further degrading by controlled utilization would be considered a success in given conditions. The term biodiversity preservation means controlled and sustainable genetic resources utilization, restoration of degraded ecosystems, natural habitats, and permanent protection of outstanding natural values. Vitality and survival of forest woody species natural populations in changed environmental conditions depends on the degree of their genetic variability, as the basis for adaptation and unhindered evolution (Šijačić-Nikolić, M., Milovanović, J. 2012). The occurrence of a high degree of intrapopulation variability is a common trait of majority of forest trees (Bogdan, S., 2009). In order to increase the certainty of natural regeneration processes, taking appropriate measures for the preservation and survival existence it is necessary to have its genetic variability knowledge.

The aim of this research was the determination of level and pattern of phenotypic variability of leaf morphological traits in sessile oak natural populations in eastern Serbia and therefore contribute to enlightening natural populations variability in the part of its natural areal. The obtained results can represent the basis for further research of genetic diversity, beginning of breeding species processes, and providing guidelines for the genepool conservation.

Materials and methods

In July of 2020, for the purpose of genetic variability determination and genetic resources estimation on the area of eastern Serbia, 86 sessile oak (*Quercus petraea* (Matt.) Liebl) trees in three populations were selected and sampled. For the research purpose the representative trees of top-quality phenotype were chosen as the carriers of reproductive material of good health production. The leaves were collected in the full development stage, from 4 to 6 m in height, from short, fertile shoots of the outer part of the canopy (leaves of sun), from the southeastern side. There has been collected 60 to 70 fully developed, undamaged leaves, per each tree. After leaf collecting, they were herbarized. Morphometric leaf characteristics were determined on the sample of 50 leaves per tree. The measured morphometric leaf parameters are: LA – leaf area (cm^2); LPE – leaf extent (cm); LW – leaf width on its widest part (mm); LW25 – leaf width on 25% of its length (mm); LW50 – leaf width on 50% of its length (mm); LW75 – leaf width on 75% of its length (mm); LL – leaf length (mm); LL25 – leaf length on 25% of its width; LL50 – leaf length on 50% of its width; LL75 – leaf length on 75% of its width; PL – petiole length; LL/LW – leaf length and width relation.

On the basis of measured values, the descriptive statistic was performed and morphological leaf traits were described by descriptive statistical indicators: arithmetic mean (x), minimal value (min), maximal value (max), standard deviation (sd), and coefficient of variability (cv). In the purpose of determining intrapopulation and interpopulation variability, there has been used univariate analysis of variance (ANOVA). The analyzed variability factors were population and tree, with the tree factor nested within the population factor. REML method (Restricted Maximum Likelihood Method) was used in order to obtain insight into the abundance of a certain researched sources of variability in the total variance (within the tree, between the trees among the population, between the populations). All referred statistical analyses were done by statistical program STATISTICA 7.0 (StatSoft Inc. 2004).

Results and Discussion

Results of sessile oak leaf morphological parameters descriptive statistics on the population level are presented in table 1. The least variable parameter was leaf length and width ratio (CV = 13.24%), while the most variable trait was leaf area (CV = 45.39%). The average value of leaf area at the population level is 41.28 cm^2, and it ranges from 145.73 cm^2 to 7.6 cm^2. The highest average value of leaf length and width ratio is 2.81, and the lowest is 0.69, with the average value being 1.52.

Table 1. Basic indicators of descriptive statistics of leaf morphological parameters at the level of populations

Trait	No. of specimens	Mean value	Minimum	Maximum	Standard deviation	Coefficient of variation
LA (cm2)	4300	41,28	7,60	145,73	18,38	45,39
LPE (cm)	4300	31,56	11,21	76,88	9,51	24,11
LW (mm)	4300	59,25	19,03	156,77	15,89	23,56
LW25 (mm)	4300	47,10	13,52	145,51	14,71	29,71
LW50 (mm)	4300	53,45	16,55	137,79	15,31	28,28
LW75 (mm)	4300	41,59	7,42	124,56	13,82	31,14
LL (mm)	4300	98,63	38,85	185,35	22,18	21,16

LL25 (mm)	4300	73,44	23,90	143,33	17,51	21,06
LL50 (mm)	4300	97,36	7,05	181,36	22,81	20,42
LL75 (mm)	4300	74,54	25,84	138,24	17,48	23,19
LL/LW	4300	1,52	0,69	2,81	0,22	13,24
PL (cm)	4300	2,14	1,02	4,42	0,47	21,18

Legend: LA - leaf area (cm^2); LPE - leaf extent (cm); LW - leaf width on its widest part (mm); LW25 - leaf width on 25% of its length (mm);LW50 - leaf width on 50% of its length (mm); LW75 - leaf width on 75% of its length (mm); LL - leaf length (mm); LL25 - leaf length on 25% of its width; LL75 - leaf length on 75% of its width; LL/LW - leaf length and width relation; PL – petiole length;

Table 2. Results of univariate analysis of variance (ANOVA).

Trait	Within populations			Between populations
	I	II	III	
LA	p<0,01	p<0,01	p<0,01	p<0,01
LPE	p<0,01	p<0,01	p<0,01	p<0,01
LW	p<0,01	p<0,01	p<0,01	p<0,01
LW25	p<0,01	p<0,01	p<0,01	p<0,01
LW50	p<0,01	p<0,01	p<0,01	p<0,01
LW75	p<0,01	p<0,01	p<0,01	p<0,01
LL	p<0,01	p<0,01	p<0,01	p<0,01
LL25	p<0,01	p<0,01	p<0,01	p<0,01
LL50	p<0,01	p<0,01	p<0,01	p<0,01
LL75	p<0,01	p<0,01	p<0,01	p<0,01
LL/LW	p<0,01	p<0,01	p<0,01	p<0,01
PL	p<0,01	p<0,01	p<0,01	p<0,01

Results of conducted analysis of variance (ANOVA) are demonstrating that the trees among population are notably different on the level of significance of 0.01 for all observed morphological leaf traits (table 2). Populations are pointing to statistically significant differences, on the level of significance of 0.01 for all observed leaf morphological traits (table 2).
In the table 3 the share of certain variability sources in the total variability for all researched leaf morphological traits is represented. Leaf variability among tree occupies the greatest share of total variability, while the interpopulation variability is lower than intrapopulation variability.

Table 3. Variance components.

Trait	Effect (%)		
	Population	Tree/population	Within the tree
LA	7,22	35,71	57,07
LPE	8,74	36,72	54,54
LW	8,23	29,84	61,93
LW25	10,11	36,41	53,48
LW50	9,87	39,41	50,72
LW75	12,35	32,42	55,23
LL	10,75	33,55	55,7
LL25	8,75	26,76	64,49
LL50	9,46	29,44	61,1
LL75	11,74	26,55	61,71
LL/LW	13,33	29,42	57,25
PL	9,45	31,52	59,02

The research of genetic variability of sessile oak populations that are covering the majority of natural areal has shown the clinal variation trends, decreasing of variability with increasing of researched area (Zanetto, A., Kremer, A. 1995). Sessile oak genetic variability examinations on the territory of the Republic of Serbia, on the basis of chloroplast genome molecular markings, have reported the existence of five different haplotypes, for now. The most abundant is haplotype 1 and its presence is confirmed in all parts of Serbia. Due to the presence of this single haplotype, Vojvodina, northwest Serbia, Šumadija, eastern Serbia, southeast Serbia, central Serbia and northeast Serbia, including the Majdanpek domain, are considered a homogeneous unity (Šijačić-Nikolić et al., 2009). However, in addition to genes, plant morphological traits are also affected by environmental factors (Barkoulas et al., 2007), so the high morphologic difference of oak leaves is also influenced by environmental factors and phenotypic plasticity (Viscosi, Fortini, 2011). Namely, some morphological differences might be caused by the fact that each genotype adapts to its own environment (Abrams, M. D. 1990). Certain studies have shown how evident this relation is, on the example of certain traits variation that are associated with latitude and altitude (Kleinschmit J. 1993). The morphological variability phenomenon indicates adaptation to local climatic conditions, which is confirmed by a significant correlation with climate parameters, and there is no connection with the population distance (Jurkšiene, G., Baliuckas, V., 2014). On the other hand, some investigated morphologic traits were variable among the populations without expressing any geographic trends. In addition to direct sunlight exposure, morphological variability can be influenced also by water stress and soil nutrient content (Carter, S.P., et al. 1987).

A leaf is an important plant organ that acts in plant communication with the external environment, exchange of water and air, as well as the primary site of photosynthesis. Leaf morphological traits variations are therefore directly affecting the physiological and biochemical plant processes, that are also reflecting plant adaptive strategies in resource acquisition (Yao-Qi, Zhi-Heng, 2021). The white oaks group is characterized by enlargement of leaf size in the shadow, and leaf dimensions are closely related to the length of the lamina, the width of the sinuses, and the width of the lobes. Also, leaf shape analysis of natural populations suggested a correlation between the depth of leaf lobes and temperature, precipitation, and, less often, height (Viscosi et al., 2009). The conducted research on the sessile oak leaf samples from natural populations of eastern Serbia indicates a high rate of examined morphological traits variability. Popović et al. (2020) studied sessile oak intrapopulation variability on the territory of AOB ”Avala” and reported also a satisfying level of genetic variability of the given location, on the basis of the same morphological traits.

From the preservation perspective, great genetic variability among tested populations is encouraging, but adequate ecological management is necessary for the in situ populations preservation (Bruschi, P., et al. 2003). If excessive logging were to occur, causing the population size to decrease, the risk of extinction would increase (Gilpin, M.E., Soule, M.E. 1986). Higher extinction risk exists also in populations of smaller sizes, where the decreased genetic diversity could be the result of genetic drift. In subsequent generations, the loss of heterozygosity, as a result of genetic drift and inbreeding, may lead to reduces fitness, in these otherwise stable populations (Bruschi, P., et al. 2003). Peripheral or isolated populations that are located on habitats differing in soil, climate, and competitors, may be the source of new adaptations, and conservation efforts should be directed toward such populations (Lessica, P., Allendorf, F.W. 1995). In order to preserve such populations, appropriate ex situ conservation measures should be taken, using generative and vegetative propagation methods, Propagation from seed is more justified, since it is the least harmful option for existing populations and it would include the widest range of genetic variability (Bruschi, P., et al. 2003).

Conclusion

On the basis of published research, it can be concluded that in the examined sessile oak populations the high variability rate of the researched leaf morphological traits is determined. The acquired results are the basis for the continuation of research that is necessary to be performed in order of giving guidelines and recommendations for the preservation and directed utilization of sessile oak genetic resources in the area of eastern Serbia.

The analyzed trees should be registered as the objects of on situ conservation and simultaneously they can serve as the source of reproductive material for artificial forest establishing, or as a help to natural regeneration of pure or mixed sessile oak stands. In addition, they can serve as source of planting material for establishing object ex situ conservation, which will have great importance for sessile oak genetic resource preservation in this area.

For complete study of sessile oak variability in the range of its natural areal, the research should be extended by molecular markers utilization.

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ECOSYSTEM SERVICES AND EUROPEAN GREEN INFRASTRUCTURE STRATEGY: THE ROLE OF THE URBAN AND PERI-URBAN FORESTS

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Abstract

The European Green Infrastructure Strategy is a "strategically planned network of natural and semi-natural ecosystems with other environmental features designed and managed to offer a wide range of ecosystem services." On the one hand, a framework that incorporates biodiversity conservation in green infrastructure is being promoted. On the other hand, the interest of researchers, professionals, and decision makers in the structural and functional connectivity of urban and peri-urban forests and in the quality of their environment is also demanded. These forests play a basic role in environmental improvement, as well as in the biodiversity conservation (air pollution removal, carbon storage, erosion control, hydrological regulation, ecological connectivity…). To this end, these "ecosystems" must face a triple challenge: i) a changing environment; ii) a lack of tools to assess environmental perturbations due to its proximity to urban areas; iii) a social perception favourable. This work is based on the knowledge to plan the urban and peri-urban forests in an environment where pressure on them should be regulated. Our aim is to generate a debate analysing their main functions in sustainable development studying a particular case: Green Belt of the Lugo city (Spain). This question is complex because it involves different aspects –social, economic, and environmental–, being necessary a coordination between all stakeholders. Urban and peri-urban forests and open green spaces have an increasingly strategic importance to improve our quality of life. In fact, evidence indicates that the presence of natural assets within an urban context contributes to improving it. In addition to the essential environmental services such as purification of air and water, noise reduction, or stabilization of the microclimate, green areas provide social services crucial to the habitability of our cities and the welfare of its inhabitants.

Keywords: *Green infrastructure, Biodiversity conservation, Stakeholders, Urban world.*

Introduction

Green infrastructure is a well-planned network of natural and semi-natural areas with other environmental characteristics designed and managed to provide a wide range of ecosystem services such as water purification, air quality, space for recreation and climate mitigation and adaptation (European Commission, 2013a; Civic and Siuta, 2014). This network is constituted by green (land) and blue (water) spaces if the aquatic ecosystems are considered and other physical features in terrestrial (including coastal) and marine areas. Green infrastructure is present in rural and urban settings and can improve environmental conditions and so citizens' health and quality of life. It also supports a green economy, creates job opportunities, and enhances biodiversity. The Natura 2000 network constitutes the backbone of the EU Green Infrastructure (Hermoso *et al.*, 2020). Green infrastructure planning is an effectively tested instrument to provide environmental, economic, and social benefits through natural solutions. In many cases, it can

reduce dependence on *“grey”* infrastructure that can be damaging to the environment and biodiversity, and often more expensive to build and maintain (Mell, 2015).
The European Commission has developed a Green Infrastructure Strategy. This strategy aims to ensure that the protection, restoration, creation, and enhancement of green infrastructure become an integral part of spatial planning and territorial development whenever it offers a better alternative, or is complementary, to other standard choices (European Commission, 2013a). Green Infrastructure encompasses natural and man-made structures and solutions which facilitate the flow of ecosystem services from nature to people and preserve our natural capital. It promotes the use of marginal land as well as making use of all possible land for biodiversity. In terms of environmental protection, it addresses the problems of habitat loss and habitat fragmentation by enhancing ecological connectivity. However, biodiversity is only one of the focuses of green infrastructure. (Civic and Siuta, 2014).
The European Commission emphasizes natural solutions not only protect and enhance the environment but also contribute to sustainable socioeconomic growth by increasing societal well-being and promoting smart growth. Natural solutions have been shown to be cheaper and long-lasting than conventional approaches (Breuste *et al.*, 2015). In this context, urban and peri-urban forests assume a key role in environmental recovery as well as in the biodiversity conservation (Berglihn and Gómez-Baggethun, 2021). Therefore, our objective is to study the main functions of these forests in sustainable development in a particular case: Green Belt of the Lugo city (Spain). This issue is multifaceted since it involves different aspects –social, economic, and environmental–, being necessary a coordination between all stakeholders.

Material and Methods

Ecosystem services, green infrastructure, and urban forests

Ecosystem services are the benefits that flow from nature to people (European Commission, 2013b). Natural ecosystems are multifunctional –they can provide a wide range of services simultaneously. The range and flow of these benefits depends largely on biodiversity and ecosystem conditions. A network of healthy ecosystems often provides cost-effective alternatives to traditional *“grey”* infrastructure, offering benefits for EU citizens and biodiversity. This is why the EU promotes the use of nature-based green and blue infrastructure solutions (European Commission, 2013a). Hence, green infrastructure constitutes a particularly valuable tool for environmental protection and climate change adaptation in a multi-stakeholder setting (Civic and Siuta, 2014).
Urban and peri-urban forests, defined as tree dominated ecosystems in and near human settlements, are important components of healthy and liveable cities. They play a key role in defining sense of place, sustaining environmental quality, and improving well-being in and around those places where people live today (FAO, 2018). These forests and open green spaces have a strategic importance to enhance our quality of life. In addition to essential environmental services such as purification of air and water, noise reduction, or stabilization of the microclimate, green areas provide social services crucial to the habitability of our cities and the welfare of its inhabitants (Berglihn and Gómez-Baggethun, 2021).

Study area and green infrastructure

Lugo is a municipality in the northwest of Spain with a population of around 100,000 inhabitants and a population density of 295 inhabitants/km^2. The climate is oceanic with a certain

continental influence. The municipal capital occupies about 8 km^2, of which, 0.5 km^2 are green areas unevenly distributed. The urban core is densely occupied by administrative, cultural and sports facilities. However, it generally has few trees on the streets and avenues, as well as poor infrastructure of public parks.
On the contrary, in the surrounding areas of the urban nucleus, the houses usually have small garden plots or dedicated to vegetable gardens for self-consumption. A short distance from the urbanized areas, and even interconnected with them, there are several large parks and a riparian forest along the Miño, Rato and Fervedoira rivers that form the Lugo Green Belt (Figure 1) (De la Sota *et al*., 2019). These green infrastructures play a fundamental role in the conservation of biodiversity, but not unique. They are also of great importance in the protection of the environment where the problem of habitat loss and fragmentation is addressed by improving ecological connectivity (Civic and Siuta, 2014; Breuste *et al*., 2015), as well as in the adapting of urban areas and their surroundings to climate change.

Figure 1. Lugo Green Belt (Source: https://www.lugobiodinamico.eu/)

Methods: program of actions and performances in urban and peri-urban forests

The methodology followed in the program of actions and performances in urban and peri-urban forests aims to implement an innovative urban planning strategy. It consists of carrying out different forestry treatments and intermediate-scale urban management works in neighbourhoods and/or residential areas, as well as in the urban environment. These interventions are planned to achieve resilient urban areas adapted to the consequences and effects of climate change.

In this way, the aim is to conceive global change, at the same time, as a challenge and an opportunity, not as a threat. The purpose is to improve the quality of urban spaces and the growing need for regeneration of their environment.

Results and Discussion

Forestry measures in urban and peri-urban forests: biodiversity conservation

Urban forestry is a specialized field of forestry that it has as objective the reforestation and forest management of urban and peri-urban forests. The main purpose of urban forestry is to take advantage of the current and potential contribution of tree stands to the well-being of the urban population, from the physiological, sociological, and economic point of view. In a broader sense, the concept of urban forestry refers to a sustainable management system. This system also includes the municipal watersheds, wildlife habitats, outdoor break and recreation opportunities, landscape design, municipal waste recovery, tree maintenance and conservation, and even the potential production of wood fibres or other non-wood forest products as raw materials (Kuchelmeister and Braatz, 1993).

Urban ecology is defined as an emerging science that includes the human factor as the main agent of change in urban ecosystems. Studies on urban biodiversity have addressed different topics in recent years, especially the goods and services that are generated as a CO_2 sink, surface runoff, pesticide pollution, biological conservation...

Several authors highlight the use of gradients as a methodology to analyse the distribution and dynamics of urban flora and fauna. Every day the value of urban green areas, biodiversity and urban ecology is shown in an increasingly populated world (Padullés *et al*., 2015).

Biological diversity is an indicator of the state of ecosystems that is used in environmental conservation and management studies. The urban and peri-urban system and its landscape boundaries is a heterogeneous ecosystem that presents an important variety of small-sized areas that have a biocenosis and particular ecological and environmental conditions. Boada and Capdevila (2000) developed a pedagogical methodology in order to interpret and disseminate the environmental values of considering the city as an ecosystem. This urban biodiversity analysis methodology can be extrapolated to different urban systems through the characterization of biotopes, adapting to their corresponding context.

Figure 2. Riparian peri-urban forest of Miño river in the surroundings of Lugo city, Spain. The forest is part of the Green Belt which by walks, paths and walkways joins the natural-cultural resources linked to the Miño, Rato and Fervedoira rivers.

Monitoring of results: new alternative ways in the context of climate change

The green infrastructure actions in the urban and peri-urban forests of the study area were completed with the monitoring of the obtained results at a physical, socioeconomic, and environmental levels. The procedures developed could serve as an example for their application in other medium-sized municipalities and local entities, like the city of Lugo. This will make it possible to offer new alternative forms of action in the context of climate change and the consequences derived for urban environments.

Climate change is considered one of the main causes of biodiversity loss, together with others such as the reduction or loss of habitats, overexploitation, pollution, and the increase in invasive alien species (Mauree *et al*., 2019). Ecosystems and their services gradually change over time and can adapt to small disturbances naturally. However, climate change supposes a variation of environmental conditions so drastic and rapid that the ecosystems cannot assimilate it, suffering a significant imbalance. The impact of this phenomenon is causing biodiversity to be lost at the highest rate of the last 65 million years (Moreno and Ruiz, 2016). One of the main consequences that the ecosystems are suffering is the modification of the cycles of nutrients and water, as well as their distribution. Another important effect is the alteration of habitats and their repercussion on the landscape.

Conclusions

Urban and peri-urban forests and their landscape limits are vital areas for biodiversity conservation. They perform multiple functions: recreational activities, sports practice, enjoying nature close to an urban environment..., including the contribution to energy sustainability, due to its particular microclimate. They are ecosystems with their own dynamics, providing benefits to people and wildlife, and playing a key role in improving green infrastructure. Considering the New Urban Agenda methodology adopted at the United Nations Conference held in Quito in 2016, these spaces are not just simple aesthetic features of the landscape, but also drivers of development. The implementation of green infrastructure actions aims to carry out an urban planning strategy to undertake an effective adaptation to climate change based on urban forestry actions in the municipality of Lugo, Spain. Emphasize that the knowledge to plan these spaces must be the basis of the research. Understanding how these forests benefit people has increased to include different environmental, social, and economic viewpoints. Therefore, it is a multifaceted topic, where suitable coordination between all implicated stakeholders is essential.

The reciprocal dependence between two environmental issues as important as climate change and biodiversity is evident. However, while the first is widely recognized by society, in general the level of concern about biodiversity loss remains poor. This may be because nature conservation is only associated with aspects such as the protection of species, leaving aside the important role that conservation biodiversity must combat climate change. The terrible consequences that are already taking place on nature and ecosystems due to global warming, as well as the importance of contributing to reducing said effects, highlight the crucial role that biodiversity plays in mitigating it.

Despite this, still many countries and development cooperation agencies continue to consider urban forestry only as an activity oriented towards aesthetic purposes, the practice of which is desirable but not necessarily essential. Multi-purpose urban forestry is not recognized as an effective and efficient tool from the socioeconomic point of view to improve the environmental conditions and aesthetic appearance of cities, as well as to maintain and conserve biodiversity. This would also help to alleviate poverty of local populations, especially in developing countries.

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CONSERVATION OF BIODIVERSITY IN URBAN FORESTS AND ITS LANDSCAPE BOUNDARIES

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Abstract

Urban forests and their landscape boundaries have diverse economic, environmental, and social functions from provide to the people with leisure and recreation opportunities, the likelihood of practicing different sports and the well-being that produces enjoy nature near to urban environment to the contribution to energy saving through its microclimatic effect. Also, these forests play a key role in improving green infrastructure for the development sustainable of the cities. Urban forests have to face a multiple challenge: i) a modified and degraded environment, ii) a lack of performances to assess ecological-environmental problems due to their proximity to urban world, and iii) a mostly positive public perception.
The knowledge to suitably plan its possibilities should be the basis of the study given the increase in urban pressure on them. Our aim is to generate a logical discussion analysing the role of urban forests like areas of particular significance for biodiversity conservation in the Lugo municipality, Spain. Scientific understanding of how urban forests and green spaces benefit people has grown in recent years to include social, environmental, and economic aspects. However, there is a considerable delay in the political action in a lot of municipalities. Urban forests and their landscape can be regarded as green infrastructure. Research has shown that the benefits of these forests are optimized through a long-term management for maximum efficiency. Awareness about forest resources and land use enables planning for multi-functional use of urban lands to multiply economic returns. For instance, land that is dedicated to other infrastructures, such as power lines, could be managed to grow products for nearby neighbourhoods from fuelwood to food. In Japan, urban green spaces are managed for both recreational use and as areas for disaster relief services if ever needed.

Keywords: *Leisure activities, Social ecosystem services, Cultural landscapes, Rural cities.*

Introduction

Urban forests defined as tree dominated ecosystems in and near human settlements are important components of healthy and habitable cities. In our increasingly urbanized world, they play a vital role in defining sense of place, sustainable environmental quality, and improving well-being in and around those locations where most people live today (Endreny, 2018). On the one hand, the importance of urban forests is recognized by a growing number of international organizations and science-policy initiatives at the 2030 Agenda for Sustainable Development (2015). It is highlighted by the Sustainable Development Goal 11 of the United Nations General Assembly, which calls for *"making cities and human settlements inclusive, safe, resilient and sustainable"*. In order to reach Goal 11, the following successes would need to be achieved by 2030 (United Nations General Assembly, 2015): 1) ensure access for all to adequate, safe, and affordable housing and basic services and upgrade slums; 2) provide access to safe, affordable, accessible,

and sustainable transport systems for all, improving road safety, notably by expanding public transport, with special attention to the needs of those in vulnerable situations, women, children, persons with disabilities and older persons; 3) enhance inclusive and sustainable urbanization and capacity for participatory, integrated and sustainable human settlement planning and management in all countries; 4) strengthen efforts to protect and safeguard the world’s cultural and natural heritage; 5) significantly reduce the number of deaths and the number of people affected and substantially decrease the direct economic losses relative to global gross domestic product caused by disasters, including water-related disasters, with a focus on protecting the poor and people in vulnerable situations; 6) reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management; 7) provide universal access to safe, inclusive and accessible, green and public spaces, in particular for women and children, older persons and persons with disabilities.
On the other hand, considering the New Urban Agenda methodology adopted at the United Nations Conference held in Quito in 2016, urban forests are not just mere aesthetic features of the landscape, but promoters of development. The New Urban Agenda represents a shared vision for a better and more sustainable future. It presents a paradigm shift based on the science of cities; it lays out standards and principles for the planning, construction, development, management, and improvement of urban areas. It incorporates a new recognition of the correlation between urbanization and development. It underlines the linkages between urbanization and job creation, livelihood opportunities, and improved quality of life, which should be included in every urban renewal policy and strategy (United Nations General Assembly, 2016). The interrelation between the New Urban Agenda and the 2030 Agenda for Sustainable Development is therefore evident, especially in Goal 11 on sustainable cities and communities.
In this context, urban forests assume an important role in environment improvement and maintaining as well as in biodiversity conservation (Berglihn and Gómez-Baggethun, 2021). In addition, urban forests provide multiple ecosystem services for city residents, including water quality, opportunities for recreation and tourism, climate regulation and habitat provision. Despite growing scientific attention to urban forests, not too many comprehensive assessments of their ecosystem services have been carried out to date (Boada and Capdevila, 2000; Camps-Calvet *et al*., 2016; Berglihn and Gómez-Baggethun, 2021). Our objective is to study the role of urban forests as areas of exceptional importance for biodiversity conservation in Lugo municipality, Spain.

Material and Methods

New opportunities and challenges for increasing urban forests

Growth in urban populations creates opportunities for urban forests to deliver ecosystem services critical to human welfare and biodiversity. Our challenge is to expand urban forests and give communities, particularly vulnerable ones, a healthier life (Endreny, 2018). A sustainable cycle is possible for expanding urban forests, with benefits paying for management, and new forests advancing research to maximize services. The field of urban forestry will grow with that of urban science, which is poised to grow rapidly, generating discoveries at the socioecological system nexus critical to sustainability (Acuto *et al*., 2018). Linking urban forestry to ecological engineering provides an opportunity to focus on building with nature to achieve renewably

powered and systems-based self-designs that satisfy human needs and advance ecosystem conservation (Endreny, 2018).
Two developments have been key to the growing attention paid to urban forests in the science and policy agendas over recent years. First, urban forests provide multiple ecosystem services for city dwellers, including climate regulation, both locally and globally, air purification, runoff control, food provision, energy and water supply, disaster risk reduction, and a range of intangible benefits and cultural values, including recreational opportunities, aesthetic values, inspiration and education, stress relief, social cohesion, and sense of place and community. Second, urban forests are increasingly recognized for their potential in providing *“nature-based solutions”*, i.e. actions to protect, sustainably manage, and restore ecosystems, that address societal challenges, simultaneously providing human well-being and biodiversity benefits (Berglihn and Gómez-Baggethun, 2021).

Study area, urban forests, and green infrastructure
Lugo is a municipality in the northwest of Spain with a population of around 100,000 inhabitants and a population density of 295 inhabitants/km^2. The climate is oceanic with a certain Mediterranean influence. The municipal capital occupies about 8 km^2, of which, only 0.5 km^2 are green areas unevenly distributed. The urban centre is densely occupied by administrative, cultural and sports facilities, as well as by emblematic historical buildings and constructions. The Roman wall that surrounds the old city of Lugo stands out, being the only one in the world that remains whole with a length of 2 km. For this reason and because of its mysterious beauty, it is a World Heritage Site. Legend has it that the Romans built the wall to protect not a city but a forest, the *“Sacred Forest of Augustus”*, in Latin *“Lucus Augusti”*.
In general, trees on streets and avenues are insufficient, as well as the infrastructure of public parks. However, from a few years now, the municipal government has been carrying out new plantations of tree species typical of each site, in addition to increasing the number of public gardens and parks. On the contrary, in the surrounding areas of the urban nucleus, the houses usually have small garden plots or dedicated to vegetable gardens for self-consumption. A short distance from the urbanized areas, and even interconnected with them, there are several large parks and a riparian forest along the Miño, Rato and Fervedoira rivers that form the Lugo Green Belt (Figure 1) (De la Sota *et al*., 2019). This green infrastructure plays a vital role in the conservation of biodiversity, but not unique. It is also of great importance in the protection of the environment where the problem of habitat loss and fragmentation is addressed by improving ecological connectivity (Civic and Siuta, 2014; Breuste *et al*., 2015), as well as in the adapting of urban areas and their surroundings to climate change.

Figure 1. Riverside forest and viewpoint along the Rato river.
(Source: https://www.caminandoentresenderos.com)

Development of sustainable cities: performances in urban forests
A decline in the structure and function of urban forests would endanger the current focus on sustainable city development. The program of actions and performances in Lugo urban forests aims to implement an innovative urban planning strategy. It consists of carrying out different forestry treatments and intermediate-scale urban management works in neighbourhoods and/or residential areas, as well as in the urban environment. These interventions are planned to achieve resilient urban areas adapted to the consequences and effects of climate change. In this way, the aim is to conceive global change, at the same time, as a challenge and an opportunity, not as a threat. The purpose is to improve the quality of urban spaces and the growing need for regeneration of their environment.

Results and Discussion

Forestry and ecology in urban forests: biodiversity conservation
Urban forestry is defined as the planting, maintenance, care, and protection of tree populations in urban settings. And the role of trees is an essential function of city planning and urban infrastructure. Planned connections of green spaces encompass not only parks and gardens, but also landscaped boulevards, river and coastal promenades, greenways, and even simple street-side tree boxes. All of this requires strategic planning and a skilled workforce. The main objective of urban forestry is to take advantage of the current and potential contribution of tree stands to the well-being of the urban population, from the physiological, sociological, and economic point of view (De la Sota *et al*., 2019). The concept of urban forestry refers to a sustainable management system. This system also includes the municipal watersheds, wildlife habitats, outdoor recreation opportunities, landscape design, tree maintenance and conservation, afforestation to promote the green economy, and even the production of forest products as raw

materials (Figure 2) (Kuchelmeister and Braatz, 1993). As we seek remedies for the climate crisis, urban forestry will continue to be a key component to any strategy looking to maximize the benefits that trees provide.

Within the science of ecology, urban ecology is defined as the study of structure, dynamics, and processes in urban ecological systems. It is the study of the relationships of human and nonhuman organisms in urban areas, the interactions of these organisms with the native and built physical environment, and the effects of these relationships on the fluxes of energy, materials, and information within individual urban systems and between urban and nonurban systems. Urban ecological systems include individual organisms, populations, communities, and landscapes, as well as buildings and infrastructures (Pickett and Cadenasso, 2012).

Studies on urban ecology have addressed different topics in recent years, especially the goods and services that are generated as a CO_2 sink, surface runoff, pesticide pollution, biological conservation…Several authors highlight the use of gradients as a methodology to analyse the distribution and dynamics of urban flora and fauna. Every day the value of urban green areas and urban ecology in an increasingly populated world is shown (Padullés *et al*., 2015).

Biological diversity is an indicator of the state of ecosystems that is used in environmental conservation and management studies. The urban forests and its landscape boundaries is a heterogeneous ecosystem that presents an important variety of small-sized areas that have a biocenosis and particular ecological and environmental conditions. Boada and Capdevila (2000) developed a pedagogical methodology in order to interpret and disseminate the environmental values of considering the city as an ecosystem. This urban biodiversity analysis methodology can be extrapolated to different urban systems through the characterization of biotopes, adapting to their corresponding context.

Figure 2. LIFE Lugo+ Biodynamic Project: afforestation to promote the green economy (Source: https://concellodelugo.gal)

New alternative ways in the context of climate change

The actions in the urban forests of the study area were completed with the follow-up of the results at a physical, socioeconomic, and environmental level. The developed methods could serve as an example for their application in other medium-sized municipalities such as Lugo. This will allow offering alternative forms of action in the context of climate change as well as in the derived effects for urban environments. Climate change is one of the main causes of biodiversity decrease together with the reduce or loss of habitats, overexploitation, pollution, and the increase in invasive alien species (Mauree *et al*., 2019). Ecosystems and their services gradually change over time and can adapt to small disturbances naturally. However, climate change supposes a drastic and rapid alteration of environmental conditions, so that ecosystems cannot assimilate it, suffering a significant imbalance. The impact of this phenomenon is causing biodiversity to be lost at the highest rate of the last 65 million years (Moreno and Ruiz, 2016). One of the main effects that ecosystems are suffering is the modification of the water and nutrient cycles as well as the habitats that make up the landscapes. There is no doubt that the actions developed to mitigate emissions and increase urban resilience will effectively contribute to biodiversity conservation.

Conclusions

Urban forests and their landscape limits are vital areas for biodiversity conservation. They perform multiple functions: recreational activities, sports practice, enjoying nature close to an urban environment..., including the contribution to energy sustainability, due to its particular microclimate. They are ecosystems with their own dynamics, providing benefits to people and wildlife, and playing an important role in improving green infrastructure. Considering the methodology of the New Urban Agenda, these spaces are not just simple aesthetic features of the landscape, but drivers of development. The green infrastructure actions aim to carry out an urban planning strategy to undertake an adaptation to climate change based on urban forestry actions in the municipality of Lugo, Spain. Understanding of how these forests benefit people has grown to include different viewpoints, environmental, social, and economic.

The reciprocal dependence between climate change and biodiversity is evident. However, while the first is recognized by society, in general the level of concern about biodiversity loss remains poor. This may be because nature conservation is only associated with aspects such as the protection of species, leaving aside the key role that biodiversity must combat climate change. The effects that are already taking place on nature and ecosystems due to global warming, as well as the importance of contributing to reducing said effects, highlight the role that biodiversity plays in mitigating it. Despite this, many countries and development cooperation agencies still consider urban forestry solely as an activity for aesthetic purposes, the practice of which is desirable but not essential. Multi-purpose urban forestry is not recognized as an effective tool to maintain and conserve biodiversity.

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ADITIONAL PAPERS

EFFECTS OF HEAT AND BAGGING APPLICATIONS ON EARLY-RIPENING AND FRUIT QUALITY CHARACTERISTICS OF SOME NEW APRICOT CULTIVARS UNDER PROTECTED CULTIVATION

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Abstract

This study was carried out to determine the effects of heat and fruit bagging applications on early-ripening, fruit set, fruit yield, and quality characteristics of ‘Mikado’ and ‘Mogador’ apricot cultivars grown under protected cultivation, in the eastern Mediterranean region of Türkiye. Air cooling to apricot trees was applied between 06:00 pm and 07:00 am in December and January when the temperature was 10°C and the temperature was kept between 6.5°C and 7.2°C for the chilling requirements in the 2021-2022 season. In addition, the fruit of the cultivars was bagged for the fruit quality attributes about 30 days after the fruit set. The first blossoming in the ‘Mikado’ cultivar occurred on February 22, while the first bloom in the ‘Mogador’ cultivar occurred on February 25 under protected cultivation. The earliest fruit ripening was detected in the ‘Mogador’ cultivar (April 16). ‘Mogador’ also had the highest fruit weight (61.00 g). The yield per tree of the ‘Mogador’ cultivar (4.31 kg/tree) was highest compared to the ‘Mikado’ cultivar (3.84 kg/tree). The fruit bagging application mainly did not have a significant effect on the fruit quality of the cultivars. As a result, the controlled cooling application has positive effects especially on earliness in apricot cultivars, but it is necessary to continue working on the cooling application in a longer period for sufficient yield.

Keywords: *Apricot, cooling application, bagging, protected cultivation*.

Introduction

Türkiye is the main apricot producer and exporter in the world with a total production of 800,000 tons of apricots (TUİK, 2022). Türkiye has great potential for export of its early apricots because of its ecological advantages compared to France, Spain, and Greece. Recently, the production of fresh apricots for exports is rapidly increased mainly in the Mediterranean region of Türkiye (Tuzel and Oztekin, 2015; Çalışkan *et al*., 2021a).

Apricot fruits have a beneficial effect on human health because of antioxidants and anti-inflammatory and immune-stimulating functions that can be attributed to the content of many phenolic compounds and rich carotenoids (Hegedũs *et al*., 2010).

Liu (2018) indicated that economic developments and lifestyle changes increase the demand for safe fruit. Besides, early fruit production under the greenhouse not only provides economic benefits to the producers but also has the potential to respond to the demand for healthy fruit by significantly reducing the population of diseases and pests (Çalışkan *et al*., 2021b).

The countries of the Mediterranean region have important advantages for protected cultivation due to the mild winter that allows production under a simple plastic cover. Protected cultivation is mainly located on the south coast, where the ecological conditions are favorable for protected cultivation without additional heating in Türkiye. In these areas, some fruits such as strawberry, apricot, plum, and peach-nectarines are cultivated under-protected for earliness (Bayazıt *et al.*, 2021). Protected cultivation could also prevent apricot flowers from being damaged by late spring frosts. However, cultivars with low chilling requirements and high fruit quality should be preferred for protected cultivation (Layne *et al*., 2013). When apricots are planted in locations with insufficient winter chilling, irregular flowering, low fruit set, and yield loss are mainly occurred (Ledbetter and Krueger, 2017; Caliskan *et al*., 2019). Therefore, Demiral and Ülger (2019) showed that artificial chilling (30 days/720 hours at 5 °C) under plastic cover can be preferred for apricot cultivation in the pots. In addition, the chilling requirement is an important difference shown among the cultivars (Fadón *et al*., 2020). On the other hand, Ruiz *et al*. (2007) reported that there is a negative relationship between chilling requirements for breaking dormancy and heat requirements for flowering in apricots. In addition, Sun *et al*. (2010) indicated that the temperatures for apricot cultivation in the greenhouse should be maintained at 5-6℃ at night and about 26-28℃ during the day.

The fruit bagging can be used against pathogens, insect pests, physiological disorders, agrochemical residues, fruit abrasions, sunburn, and bird damage, and it further modifies the microenvironment for fruit development with its various beneficial effects on its external and internal quality (Buthelezi *et al.*, 2021). However, there is no more research on the effects of fruit bagging in apricots.

This study was carried out to determine the effects of heat and fruit bagging applications on early-ripening, fruit yield, and quality characteristics of ‘Mikado’ and ‘Mogador’ apricot cultivars grown under protected cultivation, in the eastern Mediterranean region of Türkiye.

Material and methods

The study was conducted at the Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, in the 2021-2022 season. ‘Mikado’ and ‘Mogador’ cultivars (PSB Producción Vegetal, Spain) were planted to cover area with 2.5 m x 3 m in June 2017. The cultivars were budded on Myrobolan 29C rootstock.

The side height of the plastic cover was 3.00 m and the highest point was 4.00 m. The fertigation system was used for irrigation and fertilization as a standard

The coverage was made 300 m-thick with UV+IR. Air-cooling for the artificial chilling was applied to apricot trees between 06.00 pm and 07.00 am between 13 January and 28 January when the temperature was 10°C and the temperature was kept between 6.5°C and 7.2°C. The cooling process was carried out with an air-forced cooling system. When the cooling application was terminated after 365 chill-hours had accumulated under protected, the heating application was started on 29 January. The heating application was arranged according to the temperature program specified according to the phenological periods for apricot cultivation under protected (Sun *et al*., 2010). In this application, the temperature inside the protected area was not lowered below 10°C from flowering to harvest. The plastic cover on sunny and hot days (above 30°C) was also opened from about 10:00 am to 4:00 pm.

The photosynthesis rates (umol/m^{-2}/s^{-1})

The photosynthesis of the apricot cultivars was determined using the mini Plant Photosynthesis Meter (miniPPM-300, Netherlands). Five leaves in full size were selected from each tree for the photosynthesis measurements. Data was taken in the daytime from 09:00 AM to 11:00 AM on each operational day.

The fruit of the ‘Mikado’ and ‘Mogador’ cultivars was bagged for the fruit quality attributes about 30 days (23 March) after the fruit set. The transparent fabric bag was used for fruit bagging application. The bags were removed 5 days before the expected date of harvesting. A total of 12 fruits on the tree were bagged.

The temperature values were recorded hourly with a data logger (Testo 174H). The quantification of the chill hours was calculated as the number of hours below 7.2°C and above 0°C.

Heat requirements were calculated as the accumulation of growing degree hours (GDH) based on hourly air temperatures above 4.5°C (Sawamura *et al*., 2017). Trees have no additional growth advantage at temperatures above 25°C. The GDH1 was calculated for 30 days after full flowering. The GDH2 was investigated from the full flowering to the harvest date. The days from full bloom to harvest (FBD; days) were also determined.

Phenological observations such as bud swelling, red calyx, first flowering, full flowering, end of flowering, and harvest time; fruit set rates such as bud drop rate, flowering rate, initial fruit set, and final fruit set rates; yield components such as yield per tree, yield per trunk cross-sectional area and yield per hectare; and pomological variables such as fruit weight, fruit diameter, fruit length, flesh/seed percentage, total soluble solids (TSS), pH, acidity, and TSS/acidity were investigated. A total of thirty fruits were used for pomological analysis.

Fruit skin and flesh color measurements of the cultivars were performed by Minolta color (CR-300) as L, a*, b*, Chroma (*C*), and hue angle (*h*°) values. The color values were measured on the two opposite skin or flesh surfaces of fruits. The L* value indicates darkness and the high L* values represent lightness. A negative a* value shows the green color and a positive a* value shows the red color. The low *C* value indicates color intensity. The *h*° is represented the angle value of the color (Caliskan *et al*., 2012). The trunk diameter was measured 10 cm above the budding union in December.

The study was carried out with 5 replications and one tree per replication in a completely randomized design. The data were analyzed by using SAS software and procedures (SAS, 2005). Analysis of variance tables was constructed with the T-test method at $p \leq 0.05$.

Results and discussion

In the study, a total of 365 chill hours were provided, including 170 hours before 13 January naturally and 195 hours of forced air-cooling (between 13 January and 28 January). In addition, while GHD1 was 7.427 and GDH2 was 15.272 in the ‘Mikado’ cultivar, this value was 7.445 for GDH1 and 13.940 for GDH2 in the ‘Mogador’ cultivar. These data showed that the total growth degree hours of the ‘Mogador’ cultivar was lower in the ‘Mikado’ cultivar (data not shown). During the controlled heating application, which started on January 28, the average relative humidity values under protected changed between 35% and 75%, and the average temperature values changed between 8°C and 24°C (Figure 1).

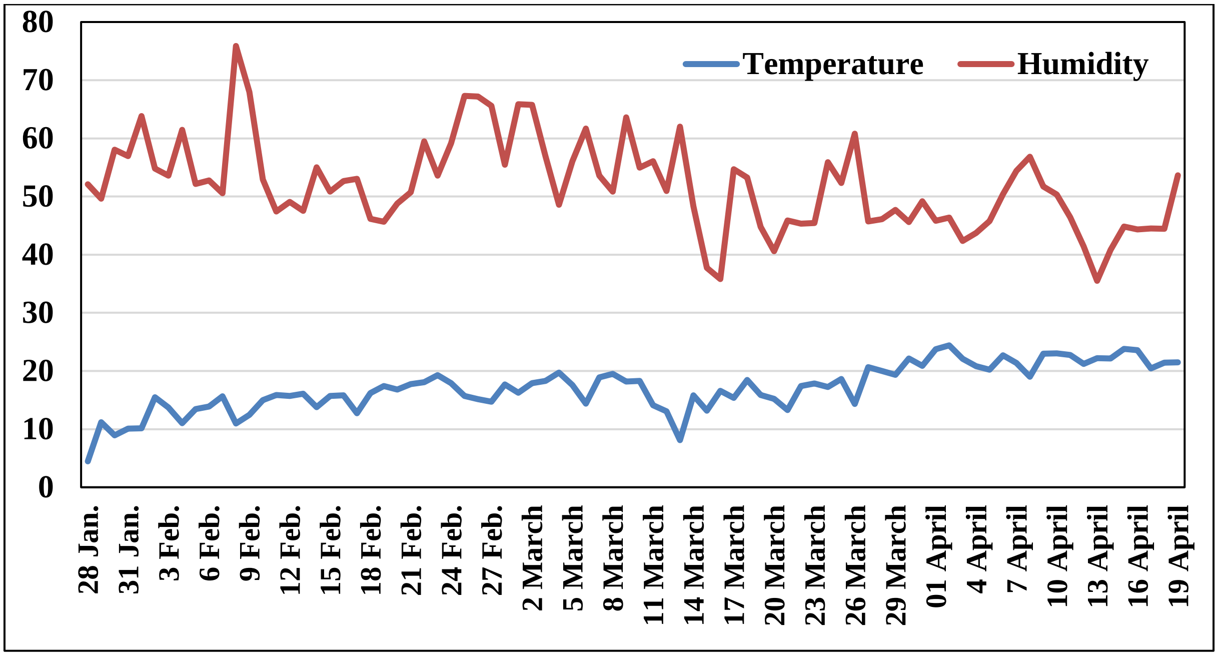

Figure 1. The mean temperature and humidity values under protected cultivation

Phenological observations

The phenological differences between the cultivars under artificial chilling and heating applications were shown in Table 1. Bud swelling of the 'Mikado' cultivar was observed on 15 February, while the bud swelling of the 'Mogador' cultivar was on 18 February. The earliest red calyx was observed in 'Mogador' (20 February). The flowering period of the cultivars was completed in 4 days under controlled heating. The flowering stages of the 'Mikado' cultivar were 1-2 days earlier than the 'Mogador' cultivar. However, the harvest date of the 'Mogador' cultivar (16 April) was 3 days earlier than the 'Mikado' cultivar (19 April). Caliskan *et al*. (2019) indicated that the flowering period of the 'Mogador' cultivar was between 25 February and 05 March and the cultivar was harvested on 26 April under protected cultivation in unheated conditions. Our results showed that heating application after artificial chilling in apricots grown under protected provided a more 10-day earliness in both flowering and harvest compared to unheated conditions Our results on fruit ripening date were earlier than the results of Demiral and Ülger (2019), who stated that harvesting of early apricot cultivars could be done in the first week of May under the plastic cover. The early harvest may be due to the earlier meeting of the growth degree temperatures within 30 days after full bloom (Lopez and Dejong, 2007) and the temperature totals from full bloom to harvest (Çalışkan *et al*., 2021a) due to the application of controlled heating. Similarly, Sikhandakasmita *et al*. (2021) reported that increasing air temperature in the days after flowering reduces the fruit ripening period and the 'KU-PP2' cultivar comes to harvest in 81 at 30°C, while it comes to harvest in 96 days at 20°C.

Table 10. Phenological characteristics of apricot cultivars under protected cultivation

Cultivar	Bud Swelling	Red Calyx	First Flowering	Full Flowering	End of Flowering	Harvest Date
Mikado	15 February	21 February	22 February	23 February	26 February	19 April
Mogador	18 February	20 February	23 February	25 February	27 February	16 April

Fruit set and yield parameters

Initial fruit set and final fruit set values of apricot cultivars were not statistically significant (Table 2). However, fruit set rates ranged from 17.23% ('Mogador') to 28.43% ('Mikado') and final fruit set rates ranged from 8.14% ('Mikado') to 8.65% ('Mogador'). These findings were lower than 20-25% (Westwood, 1993), which is the ideal rate for adequate yield in apricots. This may be due to bud drops up to 50% in both cultivars (unpublished data), especially indicating insufficient chilling time.

Yield parameters of apricot cultivars grown under controlled conditions in protected cultivation were presented in Table 3. The yield per tree and yield per hectare values of the 'Mogador' cultivar (4.31 kg tree^{-1} and 7.20 tons ha^{-1}) were higher than the 'Mikado' cultivar (3.84 kg tree $^{-1}$ and 7.20 tons ha^{-1}). The yield per trunk cross-sectional area value was not statistically significant between cultivars. Despite the chill accumulation of about 365 hours with a controlled air-cooling application under-protected, the yield characteristics of the 'Mikado' and 'Mogador' cultivars remained at a very low level.

Table 11. Fruit set and yield characteristics of the apricot cultivar under protected cultivation

Cultivar	Initial fruit set (%)	Final Fruit set (%)	Yield per tree (kg tree^{-1})	Yield per trunk cross-sectional area (kg cm^{-2})	Yield per hectare (tons ha^{-1})
Mikado	28.43	8.14	3.84 b	0.07	6.40 b
Mogador	17.25	8.65	4.31 a	0.06	7.20 a

Çalışkan *et al*. (2021a) reported that yield per tree values in the 3rd year after planting were over 10 kg/tree in the 'Mikado' and 25 kg/tree in the 'Mogador' under open area, Hatay conditions in the eastern Mediterranean region of Türkiye. In addition, Melgarejo *et al*. (2021) indicated that the yield per tree in the 4th year after planting was 29.80 kg/tree in the 'Mikado' cultivar, Murcia, Spain ecological conditions. These results showed that the controlled chilling duration applied 365 chilling hours under-protected was not sufficient for 'Mikado' and 'Mogador' apricots.

Fruit quality characteristic

'Mogador' cultivar had the highest fruit weight (61.00 g), whereas 'Mikado' had the highest flesh/seed ratio (18.07). The fruit diameter (mm), length (mm), height (mm), fruit firmness (kg-force), and seed weight (g) values were not statistically significant between cultivars. The findings of fruit physical properties in this study were higher than the values determined by Caliskan *et al*. (2019) and Caliskan *et al*. (2021a) on the same cultivars. This difference may be due to the chilling period differs depending on the growing seasons and the fruit load on the tree.

Table 12. Fruit physical characteristics of the apricot cultivars under protected cultivation

Cultivar	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit height (mm)	Firmness (kg-force)	Seed weight (g)	Flesh/seed ratio
Mikado	51.09 b	42.16	44.82	44.87	3.84	3.38	18.07 a
Mogador	61.00 a	42.91	46.71	45.31	3.60	3.21	14.11 b

The TSS and pH values of the ‘Mogador’ cultivar (12.97% and 3.73, respectively) were higher than the ‘Mikado’ cultivar (11.33% and 3.41, respectively) under protected cultivation. ‘Mikado’ had the highest fruit acidity (2.23%). Caliskan *et al*. (2019) indicated that TSS was 9.0%, pH was 3.22, acidity was 1.38% and TSS/acidity was 6.52 in the ‘Mogador’ cultivar grown under the unheated plastic cover. These differences may be due to the genetic capacity of the cultivar, the ecological conditions in the growing season, and the ripening stage of the fruit (Caliskan *et al*., 2019; Melgarejo *et al.*, 2021). Similar to these results, Caliskan *et al*. (2021b) reported that the high TSS content of peach-nectarine cultivars may occur due to the effect of partially controlled conditions under the plastic cover. In addition, we can say that the controlled heating application under-protected in this study has a positive effect on the TSS content of apricot cultivars.

Table 13. Fruit chemical characteristics of the apricot cultivars under protected cultivation

Cultivar	TSS (%)	pH	Acidity (%)	TSS/Acidity
Mikado	11.33 b	3.41 b	2.23 a	5.08
Mogador	12.97 a	3.73 a	1.90 b	6.83

Consumer acceptance of stone fruits has been related to soluble solid concentration, acidity, or soluble solid concentration/acidity ratio; however, the first attribute factor is fruit skin color (Crisosto and Costa, 2008). The fruit skin and flesh color a*, *C*, and *h*° values of the cultivars were not found statistically significant under-protected. The ‘Mogador’ cultivar had the highest fruit skin lightness (L) and b* values (66.95 and 52.14, respectively). Fruit flesh lightness (L) value was the highest in the ‘Mikado’ cultivar (65.81), whereas the fruit flesh b* value was the highest in the ‘Mogador’ cultivar (51.15) (Table 6). ‘Mikado’ and ‘Mogador’ cultivars had a low red-blushed color (4.88 and 5.69, respectively). These results were lower than those obtained by the other researchers, who indicated that for fruit skin a* value in the open area was 22.31 in the ‘Mikado’ (Melgarejo *et al.*, 2021) and 14.69 in the ‘Mogador’ (Çalışkan *et al*., 2021a). Similarly, Caliskan *et al*. (2019) reported that the ‘Mogador’ cultivar has a red-blushed fruit in the open area, whereas the fruits of the cultivar lose a red color when grown under the plastic cover. These results were also in agreement with those of Lang *et al*. (2014), who indicated that significant reductions in fruit skin color of apricots grown in high tunnels occurred.

Table 6. Fruit skin and flesh color characteristics of apricot cultivars under protected cultivation

Cultivar	Fruit skin color					Fruit flesh color				
	L	a*	b*	*C*	*h*°	L	a*	b*	*C*	*h*°
Mikado	69.45 b	4.88	49.29 b	49.49	84.40	65.81 a	4.11	49.34 b	49.53	85.27
Mogador	66.95 a	5.69	52.14 a	52.63	84.14	62.11 b	3.69	51.15 a	50.61	85.89

Photosynthesis ratio

Photosynthesis measurements were made about 1 month after full bloom in apricot cultivars (Figure 2). There was a difference between the photosynthesis values of both cultivars. Mikado

The photosynthesis rate in the 'Mikado' cultivar ranged from 37 to 76 umol m^{-2} s^{-1}, whereas it ranged from 52 to 101 umol m^{-2} s^{-1} in the 'Mogador' cultivar. The mean photosynthesis rate of the 'Mikado' cultivar grown under-protected is higher than the 'Mogador' cultivar. These findings may be an indication that the 'Mikado' cultivar has better adaptation to protected conditions than the 'Mogador' cultivar. Similar to these results, Wang *et al*. (2007) reported that the photosynthesis values of apricot cultivars under protected cultivation differ according to the genetic capacity of the cultivar.

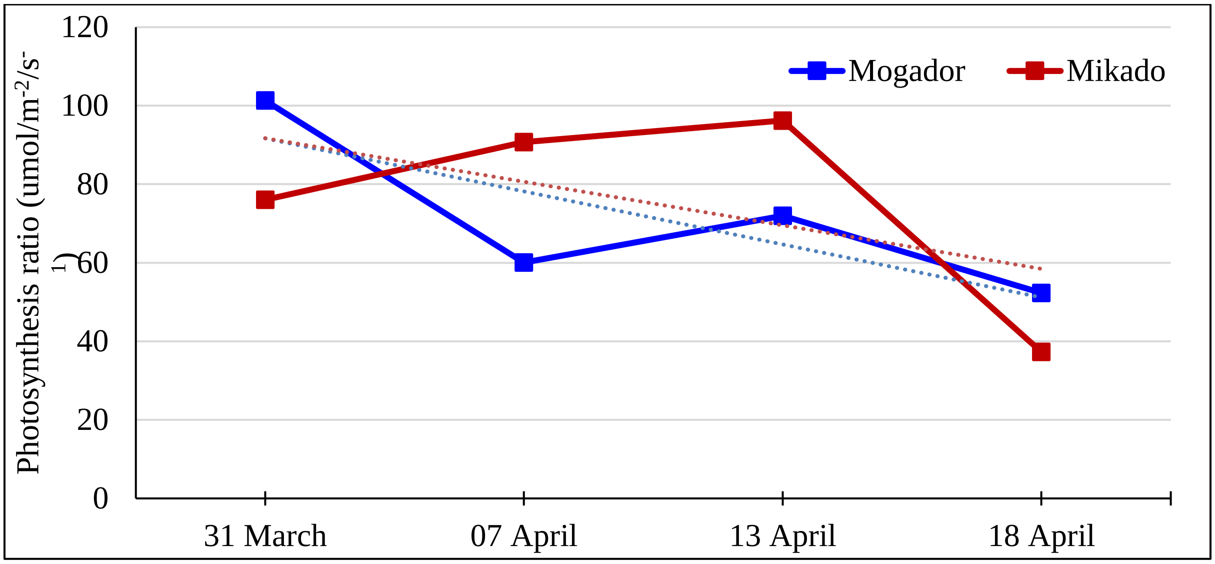

Figure 2. Changes in photosynthesis rates (umol m-2 s-1) in 'Mikado' and 'Mogador' apricot cultivars grown under protected cultivation.

Fruit bagging application

In this study, the effects of bagging application on the fruit quality of apricot cultivars grown under controlled conditions were also investigated. The effect of fruit bagging on the fruit quality characteristics in the 'Mikado' cultivar grown in the protected was not statistically significant. However, the fruit bagging application commonly reduces fruit size in the 'Mikado' cultivar (Table 7). The fruit bagging application reduced the fruit weight by 15% in the 'Mogador' cultivar. In the 'Mogador' cultivar, fruit bagging application decreased fruit weight, fruit length, and fruit height values (51.27 g, 43.73 mm, and 42.06 mm, respectively) compared to the control (61.00 g, 46.71 mm, and 45.31 mm, respectively). Fruit firmness of bagging fruits in the 'Mogador' cultivar (3.95 kg-force) was higher than in the control (3.60 kg-force). The seed weight of the 'Mogador' cultivar was also the lowest in fruit bagging application (2.77mm). Campbell *et al*. (2020) reported that although the application of bagging to fruit in peach caused reductions in fruit size, bagged fruit reduced the mechanical injury by 95%, fruit fly injury by 450%, and scab-like lesions by 810%.

Table 7. The effects of bagging application on fruit physical properties of apricot cultivars

Variable	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Fruit height (mm)	Firmness (kg-force)	Seed weight (g)	Flesh/seed ratio (%)
				‘Mikado’			
Control	51.09	42.16	44.82	44.87	3.84	3.38	14.11
Bagging	47.74	41.88	44.96	46.03	4.27	3.37	13.16
				‘Mogador’			
Control	61.00 a	42.91	46.71 a	45.31 a	3.60 b	3.21 a	18.08
Bagging	51.27 b	41.84	43.73 b	42.06 b	3.95 a	2.77 b	17.30

The fruit bagging application in the ‘Mikado’ cultivar decreased the acidity value (2.05%) compared to control fruits (2.23%) (Table 8). The effect of fruit bagging application on TSS and pH values was not statistically significant. However, the fruit bagging application reduced the TTS value in the ‘Mogador’ cultivar (11.30%). Besides, the pH and acidity values of fruit juice were not affected by fruit bagging application (Table 8).

Table 8. The effects of bagging application on fruit chemical properties of apricot cultivars

Variable	TSS (%)	pH	Acidity (%)
	‘Mikado’		
Control	11.33	3.41	2.23 a
Bagging	10.40	3.52	2.05 b
	‘Mogador’		
Control	12.97 a	3.73	1.90
Bagging	11.30 b	3.64	1.93

The effects of bagging on fruit skin and fruit flesh color values were shown in Table 9. The fruit bagging application mainly negatively affected fruit skin and flesh color (except for skin *h*° and flesh a* values) in the ‘Mogador’ cultivar, while the bagging application did not affect the fruit color properties in the ‘Mikado’ cultivar.

Table 14. The effect of fruit bagging on the fruit skin and flesh color traits of apricot cultivars

Variable	Fruit skin color					Fruit flesh color				
	L	a*	b*	*C*	*h*°	L	a*	b*	*C*	*h*°
					‘Mikado’					
Control	69.45	4.88	49.29	49.50	84.40	65.81	4.11	49.34	49.53	85.27
Bagging	68.22	3.23	49.05	49.28	84.37	68.01	4.50	48.61	48.90	84.92
					‘Mogador’					
Control	66.95 a	5.69 a	52.14 a	52.63 a	84.14	62.11 b	3.69	51.15 a	50.61 a	85.89
Bagging	64.83 b	2.66 b	49.10 b	49.51 b	84.59	64.33 a	4.32	47.58 b	47.82 b	84.81

The fruit skin lightness (L), a*, b*, and *C* values of the fruits with fruit bagging in the ‘Mogador’ cultivar were the highest in control (66.95, 5.69, 52.14, 52.63 respectively) compared to the bagging application (64.83, 2.66, 49.10, 49.51, respectively). The fruit flesh color L value in the bagging application of the ‘Mogador’ cultivar was higher (64.33) than the control (62.11). However, fruit flesh b* and *C* values had higher in control (51.15 and 50.61 respectively) than in the bagging application (47.58 and 47.82 respectively). These results were similar to those obtained by Xi *et al*. (2019), who displayed that fruit bagging significantly inhibited the transcript levels of PaMYB10 and the structural genes in the ‘Jianali’ apricot cultivar with blushed skin and blocked the red coloration and anthocyanin accumulation.

Conclusion

We investigated the effects of artificial cooling and heating application on earliness, yield, and fruit quality characteristics of ‘Mikado’ and ‘Mogador’ cultivars grown under-protected. The results showed that ‘Mogador’ provided precocity of 3 days (16 April) compared to ‘Mikado cultivar (19 April). The yield and fruit size, TSS, and TSS/acidity values of the ‘Mogador’ cultivar were higher than the ‘Mikado’ cultivar. However, although controlled air-cooling was applied, the yield values of both cultivars were found to be low. These findings revealed that insufficient chilling duration was the most serious problem for protected apricot cultivation. The results of the fruit bagging application varied depending on the apricot cultivars. While there was no bagging effect on fruit size and fruit skin and flesh coloration in the ‘Mikado’ cultivar, it decreased fruit size and fruit skin and flesh coloration in the ‘Mogador’ cultivar. In addition, we can say that the early harvest of apricots can occur in the first week of April with controlled cooling and heating application in the warm seasons of spring, in the eastern Mediterranean region of Türkiye. As a result, there is a need for long-term studies involving different fruit bagging materials and the effects of the controlled air-cooling and heating applications on the earliness and yield parameters for the development of protected apricot cultivation.

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QUALITY AND ENERGY VALUE OF FEED PEAS

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Abstract

Peas represent an important leguminous component in various cropping systems and are one of the higher-quality plants that can provide an answer to the global needs for protein in human and domestic animal nutrition. The great advantage of growing peas is that they are sown early and they make good use of winter moisture reserves and spring rainfall. In two years of research (2016 and 2017) on the experimental field of the Faculty of Agriculture in East Sarajevo with four varieties of spring field pea (NS Junior, Saša, Menhir and Dora), the faculty laboratory analyzed the quality and energy value of field pea (dry matter content; content of mineral substances; content of crude proteins; content of crude cellulose, content of crude fatty substances; proportion of nitrogen-free extractive substances), while the energy value (MJ kg-1 of dry matter) is calculated based on the results of chemical analyzes of the above-ground mass of the plant and is expressed as net energy for lactation (NEL), i.e. net energy for growth and meat production (NEM). The statistical processing of the obtained research results was done by two-factor analysis of variance (variety x year). Testing the significance of differences between individual and interaction means was performed with the LSD test. Analyzes of variance were conducted using the GenStat (2012) software package. The Dora variety had the highest content of dry matter, protein, cellulose and fat, while the Menhir (NEM) and NS Junior (NEL) varieties had the highest nutrient value. A lower crude protein content was achieved in 2017, which was less favorable for the cultivation of fodder peas compared to 2016. In 2016, there was a higher content of cellulose, BEM, as well as the energy value of nutrients (NEL and NEM), while in 2017, there was a higher content of dry matter, mineral matter and fat.

Key words: *peas, proteins, fats, cellulose, energy value, varieties.*

Introduction

Peas are used for human consumption (vegetable peas) and for feeding domestic animals in the form of grain and voluminous fodder (Maxted and Bennett, 2001; Mihailović et al., 2009). It is suitable for feeding domestic animals due to its high protein content (Corre- Hellou and Crozat, 2005; Naydenova et al., 2014). Fodder peas, in the form of fodder and protein peas, represent a cheap and high-quality component for the production of bulky, that is, concentrated fodder (Uzun et al., 2005). Winter and spring fodder pea varieties have the potential for fresh fodder yield of up to 60 t ha-1 and dry matter yield of up to 12 t ha-1, while varieties intended for grain production can achieve more than 6 t ha-1 of dry grain (Mikić et al., 2007; Mikić and Mihailović, 2014). Cultivation of annual fodder legumes in the production conditions of Republic of Srpska and B&H does not have a long tradition. With the appearance of haymaking machines, roller balers, the cultivation of these plant species intensified. In hilly and mountainous conditions,

fodder pea is widely used in the production of hay, less often silage, so that it is one of the most represented annual legumes in the sowing structure in our country. The use of fodder and peas in the daily ration of domestic animals significantly reduces the consumption of other nutrients and increases the production of milk and meat thanks to the high protein content.
The aim of the work is to determine the energy value of spring fodder pea varieties of different origins in the production conditions that prevail in the location of East Sarajevo.

Material and Methods

Research was carried out during 2016 and 2017 on the experimental field of the Faculty of Agriculture in East Ilidža municipality (Entity of Republic of Srpska, Bosnia and Hezegovina)). This locality is at 550 m above sea level (43°49'01" N and 18°20'57" E) and is located in a mountainous region. The basic tillage of the soil was done in late autumn, to a depth of 25 cm, and pre-sowing soil preparation in the spring to a depth of 8 to 10 cm. The field experiment was set up according to a randomized block system in four replications. The size of the basic plot is 5 m2. Sowing (04/07/2016, i.e. 04/04/2017) was done manually in rows with a distance of 12.5 cm between the rows and a distance of 8 cm between the plants in the row. During the growing season, standard crop care measures were applied, where special attention was paid to protecting crops from weeds, diseases and pests. Four varieties of spring field pea (NS Junior, Saša, Menhir and Dora) were used for research.
Analyzes of the aerial part of the plant were carried out in the laboratory of the Faculty of Agriculture in East Sarajevo, where the following was analyzed:

- Dry matter content;
- Content of mineral substances, by annealing at 550^{o}C;
- Crude protein content, according to the micro-Kjeldahl modification method according to Bermner (1960);
- Raw cellulose content, Henberg-Stohman method;
- Content of raw fatty substances, according to Soxlet;
- The share of nitrogen-free extractive substances (BEM) was calculated based on the results of the chemical composition of dry matter.
- The energy value (MJ kg-1 of dry matter) was calculated based on the results of chemical analyzes of the above-ground mass of the plant (dry matter; mineral matter; crude protein; crude fat and nitrogen-free extractive matter), and is expressed as net energy for lactation (NEL), i.e. net energy for growth and meat production (NEM).

The statistical processing of the obtained research results was done by two-factor analysis of variance (variety x year). Testing the significance of differences between individual and interaction means was performed with the LSD test. Analyzes of variance were conducted using the GenStat (2012) software package.
Soil conditions. The soil on the experimental field of the Faculty of Agriculture in East Sarajevo has a neutral reaction. It is moderately supplied with humus, while the provision of nitrogen (0.27%) is good. Provision of the arable soil layer with easily accessible phosphorus (40.40 mg 100 g-1 of soil) and potassium (36.41 mg 100 g-1) is very good.
Meteorological conditions. The average temperature for the vegetation period (IV-VII) in 2016 was 16.9°C, and in 2017 it was 16.7°C, while the sum of precipitation was 343.5 mm, and in 2017 it was 327, 7 mm. During the growing season in 2016, March and April were warmer than the multi-year average. In 2017, during April and May, the average temperatures were lower

than the multi-year average, while in June and July significantly higher temperatures were recorded compared to the multi-year average. During the vegetation period of 2016, only in April (60.5 mm) a lower amount of precipitation was recorded compared to the multi-year average. During the fodder pea vegetation all other months had more precipitation than the multi-year average (1961-1990). During the vegetation period in April 2017, 132.4 mm of precipitation was recorded, which was 58.8 mm more than the multi-year average. Such high amounts of precipitation at the beginning of the growing season adversely affected the cultivation of fodder peas. By analyzing the climate parameters, it can be concluded that 2017 was less favorable for the cultivation of fodder peas.

Table 1. Average monthly air temperatures (°C), monthly precipitation amounts (mm) and multi-year averages for East Sarajevo

Year \ Month		III	IV	V	VI	VII
2016.	°C	7.1	12.9	13.9	19.5	21.1
	mm	131.7	60.5	82.1	96.4	104.5
2017.	°C	8.5	9.2	15.3	20.3	21.8
	mm	43.6	132.4	73.8	55	66.5
1961.-1990.	°C	5.1	9.4	14.1	17	18.9
	mm	70.3	73.6	81.6	91	80.2

Results and Discussion

The chemical composition of the whole cowpea plant remains constant during growth and development, with the exception of the transformation of soluble sugars to starch and cellulose (Salawu et al., 2001). Ruminants prefer to consume legumes than grasses, regardless of the fact that they are characterized by similar digestibility of dry matter (Salawu et al., 2002; Bertilsson and Murphy, 2003). The dry matter of field peas in full bloom contains about 17.6% of crude protein; 21.7% of raw cellulose; 2.0% of crude fat and 10.1% of crude ash (Mišković, 1986).

The dry matter content of fodder peas was highly influenced by the examined cultivars, the year in which the research was carried out, as well as the interaction of cultivar x year (Table 2). The Dora variety had the highest dry matter content, and the Menhir variety had the lowest. In 2017, peas had a higher content of dry matter. The Dora variety had the highest dry matter content in 2016 and the lowest in 2017, while the NS Junior variety had the lowest dry matter content in 2016 and the highest in 2017.

The content of mineral substances in the dry matter of peas was influenced by the variety, while the influence of year and the interaction of variety x year had no significance. Varieties NS Junior and Saša had significantly more mineral substances compared to the variety Menhir.

The variety has a significant influence on the content of crude proteins in dry matter, which is in agreement with the results of Čupić et al. (2013). The variety Dora had the most and the variety Saša the least protein. These differences were highly significant, as were the differences between the variety Dora and the variety NS Junior, and significant differences were found between the varieties Menhir and Saša. Site conditions, especially unfavorable conditions at the time of sowing and sprouting of peas, can affect the dry matter yield and crude protein content. In 2016, peas had significantly more protein compared to 2017.

Variety, year and the interaction of variety x year had a significant effect on the cellulose content in the dry matter of fodder pea. The variety Dora had more cellulose compared to the varieties Saša and Menher. In 2016, the Saša variety had the least and in 2017 the most cellulose, while the Dora variety had the most cellulose in 2016. The variety Menhir had the lowest cellulose content in 2017.
Variety and year significantly influenced the fat content in dry matter. The Dora variety had significantly more fat, compared to the Saša variety. In 2017, the dry matter of peas had more fat, compared to 2016.
BEM content was affected by variety, year and the interaction of variety x year. The variety Saša had the most BEM in dry matter, and Dora the least. There were more BEMs in 2016, and compared to 2017. In 2016, the Dora variety had the most BEM, and in 2017 the least BEM, while in 2016, the NS Junior variety had the lowest BEM content, and the Saša variety had the most in 2017. Mihailović et al. (2003) state that the quality of forage dry matter was greatly influenced by the phenological phase in which mowing was performed. The same authors also pointed out that in pea forage, the highest content of crude protein (25.12%) and ash (9.86%) was determined in the budonization phase, and raw cellulose (20.86%) in the pod formation phase. At the same time, they found the lowest content of crude protein (17.50%) and ash (6.88%) in forage that was cut in the phase of pod formation, while the lowest content of crude cellulose (14.38%) was in forage that was cut in the phase of tree growth. According to research by Göhl (1982) and Ćupina (1993), hay contained 89.3% of dry matter, 14.9% of crude protein, 31.5% of crude cellulose, 8.3% of crude ash, 2.7% of crude fatty matter and 42.7% BEM, while in the results of this research, forage peas had 85.95% of dry matter, 20.59% of crude protein, 19.10% of crude cellulose, 8.96% of ash, 2.17% of crude fat and 35.11% BEM. The content of dry matter, crude cellulose, crude fatty substances and BEM in the dry matter of fodder peas, in these studies, was somewhat lower compared to the results reported by Göhl (1982) and Ćupina (1993), while the content of crude protein and crude ash was bigger.
The quality of the dry matter of fodder peas depends on numerous factors, such as the stage of growth, the development of the plants at the time of mowing, the characteristics of the variety, etc. The above-mentioned more important factors affect the chemical composition of dry matter, and thus the energy value of feed, expressed as net energy for lactation (NEL) and net energy for growth, maintenance and fattening (NEM). The content of NEM and NEL was highly influenced by variety, year and the interaction of variety x year. During the two-year tests of the energy value of dry matter, the average value of net energy for meat production (NEM) in fodder pea varieties was from 4,196 MJ kg-1 DM (Saša) to 4,542 MJ kg-1 DM (Menhir), (Table 2). Unlike the first year of testing, in the second year (2017) a lower energy value of the dry matter of fodder peas was achieved. When analyzing fodder pea varieties, it was determined that the Menhir variety had higher NEM values in the years of testing, and the Saša variety had the lowest values in 2016, while the NS Junior variety had the lowest value in 2017. During the two-year trials, the average value of net energy for lactation was from 4,259 MJ kg-1 SM (Dora) to 4,527 MJ kg-1 SM (NS Junior).

Table 2. Quality and energy value of fodder pea hay

Variety (A)	Dry matter (%)			Mineral substances (%)			Proteins (%)			Cellulose (%)		
	Year (B)		Average	Year (B)		Average	Year (B)		Average	Year (B)		Average
	2016.	2017.		2016.	2017.		2016.	2017.		2016.	2017.	
NS Junior	85.88	85.96	85.92	9.14	9.18	9.16	20.75	20.14	20.45	19.19	19.01	19.10
Saša	85.99	85.80	85.90	9.11	9.13	9.12	20.32	19.98	20.15	19.00	19.01	19.01
Menhir	86.23	85.36	85.80	8.65	8.75	8.70	20.78	20.56	20.67	19.17	18.98	19.08
Dora	87.45	84.92	86.19	8.87	8.87	8.87	21.00	21.17	21.09	19.45	19.00	19.23
Average	85.51	86.39	85.95	8.94	8.98	8.96	20.71	20.46	20.59	19.20	19.00	19.10
	A	B	A x B	A	B	A x B	A	B	A x B	A	B	A x B
F	50.36**	5.27**	19.60**	8,98*	0.16нз	1.42нз	46.56**	4.86*	3.02нз	5.12*	4.87*	4.93*
$LSD_{0,05}$	0.168	0.102	0.984	0.321	0.130	0,526	0.313	0.232	0.715	0.143	0,107	0.265
$LSD_{0,01}$	0.363	0.340	1.322	0.502	0.175	0.703	0.555	0.452	0.961	0,272	0,215	0.481

Variety (A)	Fat (%)			BEM (%)			NEM (MJ kg^{-1})			NEL (MJ kg^{-1})		
	Year (B)		Average	Year (B)		Average	Year (B)		Average	Year (B)		Average
	2016.	2017.		2016.	2017.		2016.	2017.		2016.	2017.	
NS Junior	2.10	2.16	2.13	34.71	35.47	35.09	4.403	4.134	4.269	4.660	4.394	4.527
Saša	2.07	2.15	2.11	35.49	35.53	35.51	4.255	4.136	4.196	4.512	4.394	4.453
Menhir	2.14	2.25	2.20	35.46	34.76	35.11	4.679	4.405	4.542	4.417	4.142	4.280
Dora	2.15	2.35	2.25	35.93	33.51	34.72	4.664	4.389	4.527	4.399	4.119	4.259
Average	2.12	2.23	2.17	35.40	34.82	35.11	4.500	4.266	4.383	4.497	4.262	4.380
	A	B	A x B	A	B	A x B	A	B	A x B	A	B	A x B
F	8.30*	5.91*	2.07нз	12.74*	9.24*	12.31*	67.68**	8.20**	46.68**	94.66**	6.67**	68.36**
$LSD_{0,05}$	0.121	0.089	0.332	0.542	0.397	0.948	0.0367	0.0149	0.0519	0.0311	0.0140	0.0439
$LSD_{0,01}$	0.224	0.195	0.457	1.025	0.783	1.328	0.0493	0.0200	0.0698	0.0417	0.0188	0.0590

The NS Junior variety had the highest value of net energy for lactation in the years of testing, then Saša, and the smallest variety Dora. In these experiments, the energy value of peas was lower than the values reported by other authors (Stevović et al., 2006; Sinclair et al., 2009), and the average NEL was 4,380 MJ kg-1 DM.

Conclusion

The quality of the dry matter largely depended on the weather conditions, the variety, the stage of growth and development of the fodder pea plants at the time of use. The Dora variety had the highest content of dry matter, protein, cellulose and fat, while the Menhir (NEM) and NS Junior (NEL) varieties had the highest nutrient value. The highest content of crude proteins in the dry matter of peas is in the stage of full flowering - formation of the first pods. By mowing the green mass at this stage, a balance is achieved between the yield and the quality of the fodder. The yield of dry matter as well as the crude protein content were greatly influenced by variety and year. Site conditions, especially unfavorable conditions at the time of sowing and sprouting of peas, can affect the dry matter yield and crude protein content. A lower crude protein content was achieved in 2017, which was less favorable for the cultivation of fodder peas compared to 2016. In 2016, there was a higher content of cellulose, BEM as well as the energy value of feed, expressed as net energy for lactation (NEL) and net energy for growth, maintenance and fattening (NEM), while in 2017 there were higher contents of dry matter, mineral matter and fat.

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INFLUENCE OF LOCATION AND YEAR ON GRAIN, PROTEIN AND FAT YIELD OF SELECTED FORAGE PEA VARIETIES

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Abstract

Fodder peas represent a cheap and high-quality component for the production of bulky, that is, concentrated fodder. Cultivation of one-year forage legumes in the production conditions of Republic of Srpska and Bosnia and Herzegovina does not have a long tradition, which is why two-year research (2016 and 2017) was conducted in two locations (East Sarajevo and Banja Luka) with five varieties of spring fodder pea, intended for grain production (NS Javor, Baccara and NS Dukat) and the combined method of exploitation (NS Junior and Saša). Basic tillage of the soil at both locations was done in late autumn, to a depth of 25 cm, and pre-sowing soil preparation in the spring to a depth of 8 to 10 cm. A combination of mineral fertilizer N8P24K24 in the amount of 350 kg ha-1 was used for nutrition. The field experiment was set up according to a randomized block system in four replications. Among the traits, the following were monitored: grain yield per unit area (kg ha-1), grain protein yield (kg ha-1) and grain fat yield (kg ha-1). The statistical processing of the obtained research results was done using a three-factor analysis of variance (variety x locality x year). In the years of testing, the highest grain yield was in the NS Javor variety, followed by the NS Dukat variety, and in third place was the Baccara variety. Varieties for combined purposes NS Junior and Saša had a significantly lower grain yield compared to varieties for grain production, which was also reflected in the total yield of raw proteins in pea grains. The fat yield of fodder peas varies depending on the variety, the year and the region where it is grown. Given that such research was carried out for the first time in the areas of Banja Luka and East Sarajevo, it is possible to apply the obtained results in practice.

Keywords*: fodder pea, yield, proteins, fats, variety, locality.*

Introduction

Pea (Pisum sativum L.) is an annual legume, winter or spring type, which is grown from 20° to 67° north latitude. Peas are used for human consumption and for feeding domestic animals in the form of grains and bulky fodder (Mihailović et al., 2009). It is suitable for feeding domestic animals due to its high protein content (Naydenova et al., 2014). Fodder peas, in the form of fodder, and protein peas represent a cheap and high-quality component for the production of bulky, that is, concentrated fodder (Uzun et al., 2005). Winter and spring fodder pea varieties have the potential for fresh fodder yield of up to 60 t ha-1 and dry matter yield of up to 12 t ha-1, while varieties intended for grain production can achieve more than 6 t ha-1 of dry grain (Mikić et al., 2007; Mikić and Mihailović, 2014). Pea proteins are characterized by a good balance of essential amino acids. Peas can successfully supplement or replace soy proteins and be the main source of vegetable proteins. Pea for grain is an economically important agricultural plant species that is primarily grown for its high protein content in the grain. In most modern varieties of protein pea, the crude protein content of the dry matter of the grain is between 250 g kg-1 and 260 g kg-1 (Mihailović et al., 2007). Cultivation of annual fodder legumes in the production conditions of Republic of Srpska and B&H does not have a long tradition. The aim of paper is to determine the yield of grain, protein and fat of spring fodder pea varieties of different origins under the production conditions that prevail in the locations of East Sarajevo and Banja Luka.

Material And Methods

Research was carried out during 2016 and 2017 at two locations located in cities of East Sarajevo and Banja Luka. The field experiment in the area of the City of East Sarajevo was set up on the experimental field of the Faculty of Agriculture in the territory of the municipality of East Ilidža, at 550 m above sea level (43°49'01" SS and 18°20'57" IGD) and is located in a mountainous area. The second site is located in the area of the City of Banja Luka, and is located on the experimental field of the Agricultural Institute of Republic of Srpska, in the village of Delibašina, at an altitude of 163 m (44°48'13"SGŠ and 17°4912'19" IGD) and is located in the lowland region. For research, five varieties of spring fodder peas intended for grain production (NS Javor, Baccara and NS Dukat) and a combined method of utilization (NS Junior and Saša) were used. The basic cultivation of the land in both localities was done in late autumn, to a depth of 25 cm, and pre-sowing land preparation in the spring to a depth of 8 to 10 cm. A combination of mineral fertilizer $N_8P_{24}K_{24}$ in the amount of 350 kg ha-1 was used for nutrition. The field experiment was set up according to a randomized block system in four replications. The size of the basic plot was 5 m2. Sowing was done by hand in rows with a distance of 12.5 cm between rows and a spacing of 8 cm between plants in a row. During the growing season, standard crop care measures were applied, where special attention was paid to protecting crops from weeds, diseases and pests.

Before setting up field experiments, soil samples were taken and chemical analyzes of soil fertility were performed in the laboratory of the Faculty of Agriculture in East Sarajevo. Soil pH value in H2O and KCl, humus content (%), readily available phosphorus content (mg P2O5 100 g-1 soil) and readily available potassium content (mg K2O 100 g-1 soil) were analyzed using standard methods. In the laboratory of the Faculty, the content of crude proteins in pea grains was adjusted according to the method of micro-Kjeldahl modifications according to Bermner (1960), as well as the content of crude fatty substances according to Soxlet.

Among the traits, the following were monitored: grain yield per unit area (kg ha-1), grain protein yield (kg ha-1) (calculated on the basis of grain yield and % crude protein in grain) and grain fat content (kg ha-1) (by calculation based on grain yield and % fat in grain).

The statistical processing of the obtained research results was done using a three-factor analysis of variance (variety x locality x year). Testing the significance of differences between individual and interaction means was performed with the LSD test. Analyses of variance were conducted using the GenStat (2012) software package.

Soil conditions. Optimum soil pH values for growing peas differ among individual authors. Brkić et al. (2004) in research carried out in the area of eastern Croatia found higher pea seed yields at pH 7.12 than at pH 6.62, while the minimum pH value for growing peas was 5.52 (Mahler and McDole, 1987). Different species, as well as varieties within the same species, in unfavorable conditions of soil reaction exhibit different ability to absorb nutrients (Bukvić et al., 1998). In the experimental field of the Agricultural Institute of Republic of Srpska, the soil is of alkaline reaction, with a low humus content (1.9%). The content of readily available phosphorus is good (20.3 mg in 100 g-1), while the availability of potassium in the soil is average (16.1 mg in 100 g-1). The soil on the experimental field of the Faculty of Agriculture in East Sarajevo has a neutral reaction, it is moderately supplied with humus, while the provision of nitrogen (0.27%) is good. Provision of the arable soil layer with easily accessible phosphorus (40.40 mg 100 g-1 of soil) and potassium (36.41 mg 100 g-1) is very good. The land in both localities is suitable for growing fodder peas.

Meteorological conditions. The total amount of heat for peas is from 1300 to 2800°C, and it depends on the length of the vegetation. When analyzing temperature, in addition to daily averages, extremes are very important, that is, the lowest and highest temperatures in certain periods of growth and development of peas. Optimum mean daytime temperatures in the vegetative stage are from 12 to 16°C, and during pod formation from 16 to 22°C, and for good pea metabolism, the optimal difference between day and night temperatures is 6 to 10°C. The average temperature for the vegetation period (III-VII) in 2016 in the Banja Luka area was 16.5°C, while the sum of precipitation was 344.8 mm. The average temperature for the vegetation period (III-VII) in 2017 was 17.2°C, while the sum of precipitation was 437.6 mm.

Table 1. Average monthly air temperatures (°C), monthly precipitation amounts (mm) and multi-year averages for Banja Luka and East Sarajevo

Month Year			III	IV	V	VI	VII
Banja Luka	2016	°C	8.0	13.5	16.2	21.5	23.3
		mm	122.2	0.5	100.6	117.8	125.9
	2017	°C	9.7	11.7	17.5	22.9	24.4
		mm	124.0	148.4	92.1	35.1	38
	1961-1991	°C	6.1	10.9	15.7	18.9	20.5
		mm	79.1	86.9	97.8	110.8	94.8
East Sarajevo	2016	°C	7.1	12.9	13.9	19.5	21.1
		mm	131.7	60.5	82.1	96.4	104.5
	2017.	°C	8.5	9.2	15.3	20.3	21.8
		mm	43.6	132.4	73.8	55	66.5
	1961-1990	°C	5.1	9.4	14.1	17	18.9
		mm	70.3	73.6	81.6	91	80.2

In the location of East Sarajevo, the average temperature for the vegetation period (IV-VII) in 2016 was 16.9°C, and in 2017 it was 16.7°C, while the sum of precipitation was 343.5 mm in 2016, and 327.7 mm in 2017.

Results and Discussion

Grain yield of fodder pea varieties is characterized by great variability, caused by habitat conditions and meteorological conditions during growth and development (Annicchiarico and Iannucci, 2008). In two-year tests (2016-2017), the yield of pea grains was affected by variety, locality and year, as well as the interaction of variety x locality (tab. 2), which was in agreement with the results of Makashev (1983). The average yield of peas was 4032 kg ha-1 (tab. 3). The highest grain yield was in the variety NS Javor (4673 kg ha-1), and the lowest was in the variety NS Junior (2569 kg ha-1). The determined differences were significant, as well as the differences of all tested varieties compared to the NS Junior variety, which was in agreement with the results of Mihailović and Mikić (2004), while in the experiments conducted in Banja Luka, the Baccara variety had a grain yield of 4,979 .35 kg ha-1, and for the Saša variety grain yield was 4,302.75 kg ha-1 (Lakić et al., 2017), which were slightly higher yields compared to those studies. The average yield of peas in East Sarajevo was significantly higher compared to the yield in Banja Luka.

Table 2. Analysis of variance

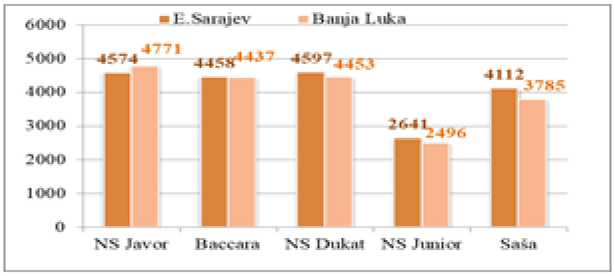
	d.f.	Grain yield	Protein yield	Fat yield
		s.s.	s.s.	s.s.
Repetition	3	71318	8180	160.18
Sort	4	47588937**	2475902**	39566.17**
Location	1	155155*	12127*	889.86**
Year	1	277318*	18482*	173.58*
Sort*location	4	595677**	67337**	2844.23**
Sort*year	4	213450$^{n.z.}$	15048 $^{n.z.}$	405.57*
Location*year	1	14739 $^{n.z.}$	863 $^{n.z.}$	252.70*
Sort*location*year	4	93596 $^{n.z.}$	7242 $^{n.z.}$	1304.69**
Rest	57	1446698	98659	1817.36
Total	79	50456889	2703839	47414,34

Table 3. The influence of location and year on grain yield, protein yield and fat yield of some fodder pea varieties (kg ha-1)

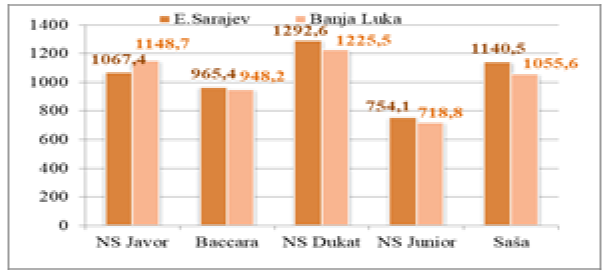
Trait	Grain yield	Protein yield	Fat yield
Variety			
NS Javor	4673	1108.1	72.33
Baccara	4447	956.8	73.89
NS Dukat	4525	1259.1	120.88
NS Junior	2569	736.4	53.85
Saša	3948	1098.0	75.04
Average for sort	4032	1031.7	79.20
Location			
East Sarajevo	4076	1044.0	82.53
Banja Luka	3988	1019.4	75.86
Year			
2016	4091	1046.9	80.67
2017	3973	1016.5	77.73

In 2016, the average yield was significantly higher, compared to 2017. Higher temperatures and less precipitation in the yield formation phase affected lower yields in 2017. What is the influence of location and year on yield and yield components was confirmed in their experiments by Acikgoz et al. (2009), Fikere et al. (2009) and Tolessa et al. (2013). The tested varieties had higher yields on the experimental field in East Sarajevo, the exception being the NS Javor variety, which was more suited to the agroecological conditions of the experimental field in Banja Luka (Graph 1).

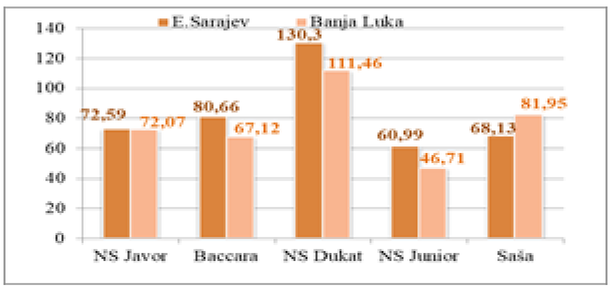
The protein content of peas varies depending on the variety, year and region where it is grown. Forage pea protein yield was influenced by variety, locality and year. The variety x locality interaction affected the protein yield of fodder pea (Tab. 2). These results are in agreement with the results of Lhuillier-Soundélé et al. (1999) who determined that the protein content in pea kernels depends on the variety and factors of the external environment during the pouring of the kernels. The variety NS Dukat had the highest protein yield, which was in agreement with the results obtained by Mihailović et al. (2010). The NS Junior variety had the lowest protein yield. Peas produced in East Sarajevo had a higher yield of protein, compared to Banja Luka. In 2016, a higher yield was achieved compared to the production of peas in 2017. Research by Santalla et al. showed that there is a significant interaction between variety and environment (2001) and Wang and Daun (2004). The variety x locality interaction significantly influenced the protein yield of cowpea. The NS Javor variety had a significantly higher protein yield in the Banja Luka location, and the NS Dukat and Saša varieties in the East Sarajevo location (Graph. 2).



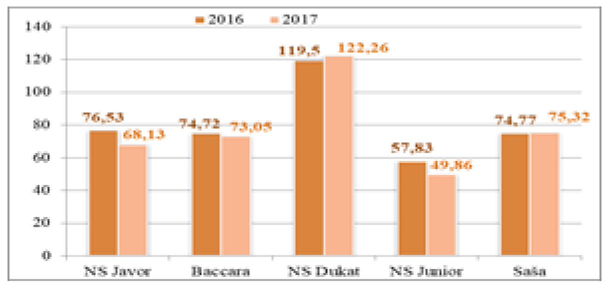
Graph. 1 Influence of variety x locality interaction on pea grain yield



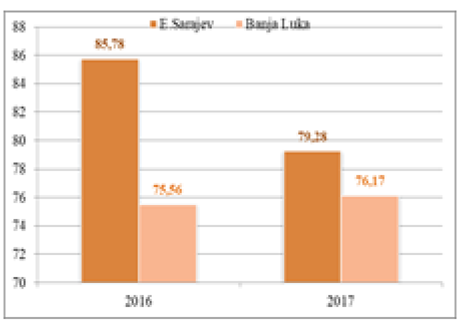
Graph. 2 Effect of variety x locality interaction on protein yield



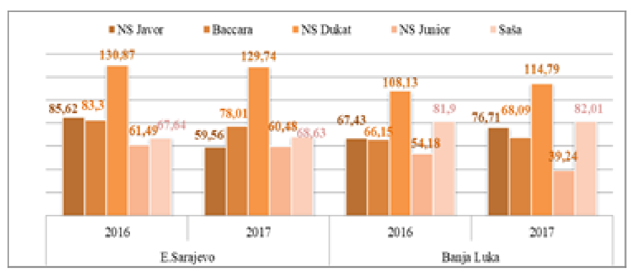
Graph. 3 Effect of variety x locality interaction on fat yield



Graph. 4 Effect of variety x year interaction on fat yield



Graph. 5 The influence of the site x year interaction on fat yield



Graph. 6 The influence of the variety x locality x year interaction on the yield

The fat yield of field peas was influenced by variety, locality and year as well as interactions (variety x locality; variety x year; locality x year and variety x locality x year). The variety NS Dukat had the highest and the variety NS Junior the lowest fat yield. The determined differences were significant, as well as the differences in the fat yield of the other tested varieties and the NS Junior variety. The influence of variety on the fat content of peas was determined by Hendawey and Younes (2013), and the research of Księżak et al. (2018) did not determine the influence of variety on fat content in pea grains, but a significant influence of location was determined. The fat content of legume grains is influenced by weather conditions, which is in agreement with the results of Pisulewska et al. (1996). The fat yield was higher in the location of East Sarajevo, compared to Banja Luka. Peas had a significantly higher fat yield in 2016 (Tab. 3). All tested cultivars had a higher fat yield in the East Sarajevo location, the exception being the Saša variety, which had a significantly higher fat yield in the Banja Luka location (Graph 3). Varieties NS Javor and NS Dukat had higher fat yields in 2016, and NS Dukat in 2017 (Graph 4). In the East Sarajevo location, there was a higher yield of fat in the years of testing, compared to the Banja Luka location. The East Sarajevo locality had a higher fat yield in 2016, while the Banja Luka locality had a higher fat yield in 2017 (Graph 5). The variety NS Dukat had the highest fat yield in both locations and in both years of testing. The variety NS Javor at the location of East Sarajevo did not suit the meteorological conditions in 2017, so it had the lowest yield of fat, and the variety NS Junior had the lowest yield at the location of Banja Luka in the years of testing and at the location of East Sarajevo in 2016 (Graph 6).

Conclusion

In the years of testing, the highest grain yield was in the NS Javor variety, followed by the NS Dukat variety, and the Baccara variety was in the third place. The forage pea varieties intended for the combined method of utilization had a significantly lower average grain yield. Protein yield in pea grain depended on variety, location, year and variety x location interaction. In addition to the yield of pea grains, the protein yield is also influenced by the crude protein content of the grain itself. Varieties for combined purposes NS Junior and Saša had a significantly lower grain yield compared to varieties for grain production, which was also reflected in the total yield of raw proteins in pea grains. The fat yield of fodder peas varies depending on the variety, the year and the region where it is grown. Fat yield depended on variety, year, locality and their interactions. Given that such research was carried out for the first time in the areas of Banja Luka and East Sarajevo, it is possible to apply the obtained results in practice. Since spring fodder peas in our country have found the greatest application in hilly and mountainous areas, the obtained results can be used to achieve high yields of fodder and pea grains and thus affect a significant reduction in the consumption of other nutrients and increase the production of milk and meat thanks to the high protein content.

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AUTHOR INDEX

ERRATA CORRIGE

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Pag. 1427: Added words „ADDITIONAL PAPERS“.

Pp. 1428-1438: Added full paper entitled „EFFECTS OF HEAT AND BAGGING APPLICATIONS ON EARLY-RIPENING AND FRUIT QUALITY CHARACTERISTICS OF SOME NEW APRICOT CULTIVARS UNDER PROTECTED CULTIVATION“ of authors: Derya KILIÇ, Oğuzhan ÇALIŞKAN.

Pp. 1439-1445: Added full paper entitled „QUALITY AND ENERGY VALUE OF FEED PEAS“ of authors: Igor ĐURĐIĆ, Tatjana KRAJIŠNIK, Branka GOVEDARICA, Tanja MIJATOVIĆ, Vesna MILIĆ, Željko LAKIĆ.

Pp. 1446-1454: Added full paper entitled „INFLUENCE OF LOCATION AND YEAR ON GRAIN, PROTEIN AND FAT YIELD OF SELECTED FORAGE PEA VARIETIES“ of authors: Igor ĐURĐIĆ, Tatjana KRAJIŠNIK, Branka GOVEDARICA, Tanja MIJATOVIĆ, Nikola DEMONJIĆ, Vesna MILIĆ.

Pag. 1427: from this page all remaining pages starting from „INDEX OF AUTHORS“ are moved forward by the sum number of pages of the three inserted papers.